Mar Biol (2011) 158:1841–1856
DOI 10.1007/s00227-011-1696-5

ORIGINAL PAPER

Genetic heterogeneity among *Eurytemora affinis* populations in Western Europe

Gesche Winkler · Sami Souissi · Céline Poux · Vincent Castric

Received: 6 July 2010 / Accepted: 6 April 2011 / Published online: 10 May 2011

Abstract Evolutionary diversification of the broadly distributed copepod sibling species complex *Eurytemora affinis* has been documented in the northern hemisphere. However, the fine scale geographic distribution, levels of genetic subdivision, evolutionary, and demographic histories of European populations have been less explored. To gain information on genetic subdivision and to evaluate heterogeneity among European populations, we analyzed samples from 8 locations from 58° to 45°N and 0° to 23°E, using 549 base pairs of the mitochondrial cytochrome oxidase subunit I (COI) gene. We discovered three distinct lineages of *E. affinis* in Western Europe, namely the East Atlantic lineage, the North Sea/English Channel (NSEC) lineage, and the Baltic lineage. These geographically separated lineages showed sequences divergence of 1.7–2.1%, dating back 1.9 million years (CI: 0.9–3.0 My) with no indication of isolation by distance. Genetic divergence in Europe was much lower than among North American lineages. Interestingly, genetic structure varied distinctively among the three lineages: the East Atlantic lineage was divided between the Gironde and the Loire populations, the NSEC lineage comprised one single population unit spanning the Seine, Scheldt and Elbe rivers and the third lineage was restricted to the Baltic Proper (Sweden). We revealed high haplotype diversity in the East Atlantic and the Baltic lineages, whereas in the NSEC lineage haplotype diversity was comparatively low. All three lineages showed signs of at least one demographic expansion event during Pleistocene glaciations that marked their genetic structure. These results provide a preliminary overview of the genetic structure of *E. affinis* in Europe.

Communicated by T. Reusch.

Electronic supplementary material The online version of this article (doi:10.1007/s00227-011-1696-5) contains supplementary material, which is available to authorized users.

G. Winkler (✉)
Institut des Sciences de la Mer de Rimouski, Québec-Océan, Université de Québec à Rimouski, 310 Allée des Ursulines, Rimouski, QC G5L 3A1, Canada
e-mail: gesche_winkler@uqar.qc.ca

S. Souissi · C. Poux · V. Castric
Université Lille Nord de France, 59000 Lille, France

S. Souissi
USTL, LOG, Station Marine de Wimereux, 28 av. Foch, 62930 Wimereux, France

S. Souissi
CNRS-UMR 8187, LOG, 62930 Wimereux, France

C. Poux · V. Castric
Université des Sciences et Technologies de Lille-Lille 1, UMR CNRS 8016, UFR de Biologie, 59655 Villeneuve d'Ascq Cedex, France

Introduction

In spite of the dispersive habitat they inhabit, sharp genetic discontinuities at large geographic scales have been described in numerous coastal marine organisms (Peijnenburg et al. 2004; Roman and Palumbi 2004; Sotka et al. 2004), eventually resulting in sibling species (Knowlton 2000; Bilton et al. 2002). These sibling species, though not easily distinguishable by morphology, may exhibit distinct physiological characteristics, habitat preferences, behavior, genetic structure, or life history. These distinct properties could strongly influence evolutionary trajectories and ecological dynamics of the sibling species. The growing evidence for the preponderance of sibling species

complexes has encouraged research on how isolation, divergence and finally speciation take place in the marine realm (Knowlton 1993, 2000; Schluter 2001; Bilton et al. 2002). Relatively few studies have examined the functional consequences of genetic and demographic subdivision among sibling species (or populations; Lee and Gelembiuk 2008). Both intrinsic (genetic) and extrinsic (environmental) factors influence spatial–temporal distribution patterns and population dynamics that might affect in return ecosystem functioning and productivity. These factors have important consequences for biodiversity, biogeography, conservation, and fisheries management (Knowlton 1993; Lecomte et al. 2000; Lee 2000, 2002; Gelembiuk et al. 2006; Jolly et al. 2006). Consequently, knowledge of phylogeography and population genetic structure of sibling species can help formulate hypotheses explaining phenotypic variations across geographic scales (Beyrend-Dur et al. 2009).

Estuaries represent spatially discrete ecosystems isolated by physical barriers, such as salinity, temperature, and currents, so that eco-physiological boundaries may promote isolation resulting in specialized estuarine species (Lee et al. 2003). A number of studies have shown that estuarine species are composed of geographically isolated and genetically distinct populations (Caudill and Bucklin 2004; Chen and Hare 2008; Winkler et al. 2008). The more upstream the distribution of estuarine fauna within an estuary, the stronger might be the barriers to natural dispersal via the ocean due to geographic distance and/or physiological tolerance (for review, see Bilton et al. 2002 and reference therein). In copepods, estuarine taxa showed population differentiation, whereas high dispersal potential might have prevented small-scale genetic differentiation among marine taxa such as *Calanus* spp. (Bucklin et al. 2000). Highly divergent clades of estuarine copepods, *Acartia tonsa* and *Eurytemora affinis*, were found among estuaries and fjords of the north Atlantic coasts (Lee 1999a, b; Caudill and Bucklin 2004) and within the large estuarine systems of Chesapeake Bay (Chen and Hare 2008) and the St. Lawrence System (Winkler et al. 2008).

In this study, we focused on the broadly distributed copepod *E. affinis*. It is an important component in terms of abundance and/or biomass of the copepod community in various habitats of the northern hemisphere, ranging from stable brackish environments, estuaries, to highly variable estuarine salt marsh tidal ponds as well as freshwater lakes and reservoirs (Lee 1999b, 2000). *E. affinis* has a central position in the food web of several estuarine transition zones, channeling carbon from protists to higher trophic levels and ultimately supporting an important nursery zone for fish, shrimp, and mysids (e.g., Mouny et al. 1998; Sirois and Dodson 2000; North and Houde 2003; Winkler et al. 2003; David et al. 2006; Winkler et al. 2007). Six highly divergent clades of *E. affinis* have been described until now in the northern hemisphere: four in America, one in Europe and one in Asia (Lee 1999b, 2000). High genetic divergences and differences of life history traits and salinity tolerance were found within and among clades in North America as well as between clades of North America and Europe (Lee 1999b; Beyrend-Dur et al. 2009), indicating separate evolutionary histories and possibly suggesting adaptation to specific environments. Within the North American continent, four clades are highly divergent, to the point that they show reproductive isolation. Their distributions, however, overlap due to secondary contacts following speciation events (Lee 2000; Winkler et al. 2008). At a smaller geographical scale, Winkler et al. (2008) found evidence of genetic subdivision among and within clades as well as habitat partitioning in the contact zone of the St. Lawrence system. Survival and developmental time varied significantly between clades in response to salinity and food conditions, reflecting differences in their physiological performance related to the contrasted microhabitats they inhabit (Skelly et al. in revision).

While the studies on the North American clades covered a large number of locations and at the scale of the St. Lawrence system a large number of individuals (Lee 1999b, 2000, Winkler et al. 2008), the fine scale geographic distribution, levels of genetic subdivision, evolutionary, and demographic histories of European populations remain poorly studied. This is problematic, since knowledge of population genetic structure may help to define reasonable entities for research, resource management, and conservation. In fact, as a consequence of the absence of clear identification of genetic structure within the European populations of *E. affinis,* most geographic entities have been studied independently: the Baltic Sea (e.g., Vuorinen and Ranta 1987; Hansson et al. 1990; Hirche 1992), the North Sea estuaries (e.g., Burkill and Kendrall 1982; Peitsch 1993; Escaravage and Soetaert 1995; Köpcke and Kausch 1996; Tackx et al. 2004; Mialet et al. 2010), the English Channel estuaries (e.g., Mouny and Dauvin 2002; Devreker et al. 2004, 2007, 2008, 2009; Dur et al. 2009; Michalec et al. 2010; Souissi et al. 2010), and the East Atlantic estuaries (e.g., Castel 1995; David et al. 2005; Irigoien et al. 1993). Only few studies have compared population dynamics and life cycle strategies among European populations (Tackx et al. 1995, Sautour and Castel 1995, Gasparini et al. 1999, Tackx et al. 2003, Beyrend-Dur et al. 2009). Before comparing results of studies from various environments, one should first study *E. affinis* population genetic structure throughout Western Europe.

The objective of this study was to analyze the population genetic structure of the broadly distributed sibling species complex *E. affinis* in Western Europe from the Baltic Sea to the East Atlantic coast in France in order to

(1) test whether highly divergent clades as found on the North American continent also exist in Europe, (2) describe the phylogeographic structure and the demographic and evolutionary history of this species in Western Europe. We analyzed 249 individuals from 8 locations from 58° to 45°N latitude and 0° to 23°E longitude, using 549 base pairs of the mitochondrial cytochrome oxidase subunit I (COI) gene.

Materials and methods

Population collection

We sampled *E. affinis* from 8 locations in Western Europe in 2006 (Table 1; Fig. 1). The most northern locations were in the Baltic Sea, including the Gulf of Riga (location 1, two stations: St. 163 and St. 165 according to S. Strake (pers. com.)) and the Swedish coast (location 2, two stations B1 and H4 according to Gorokhova et al. (2004)). These two Baltic Sea locations are characterized by brackish water without tidal influence. All other samples came from estuarine locations. In the North Sea region, we sampled the Elbe estuary (location 3, two stations: Este [~90 km from river mouth] and Brunsbüttel [~30 km from river mouth]) and the Scheldt estuary (location 4, three stations: 1 [58 km from river mouth], 4 [79 km from river mouth], 9 [111 km from river mouth], according to Tackx et al. 2004 and Mialet et al. 2010). In the English Channel, estuarine populations from the Seine estuary (location 5, one station: Tancarville, ~28 km from river mouth, Cailleaud et al. 2007) and the Tamar estuary (location 6: one station, ~8 km from river mouth, according to P. Licandro [pers. com.]) were analyzed. The most southern locations were along the East Atlantic Coast situated in the Loire estuary (location 7, ~17 km from river mouth, one station: Paimboeuf) and the Gironde estuary (location 8; three stations: 2 [~12 km from river mouth], K [~48 km from river mouth], and E [~70 km from river mouth] according to David et al. 2005). The Gironde estuary is close to the southern distribution limit of *E. affinis* in Europe (estuaries near Bilbao, Spain; Albaina

Table 1 Locations sampled in Western Europe for populations of *E. affinis*

Sampling locations (km from river mouth)	Sampling date	Sample size[a]	Tidal state	Salinity (PSU)[b]	Temperature (°C)[b]	Latitude	Longitude
Gulf of Riga (1)							
St. 163, 0–9 m	01/06/06	1	No	ND	ND	57°N	23°E
St. 165, 0–8 m	01/06/06	4	No	ND	ND	57°N	23°E
Swedish coast (2)							
B1	07/2006	9	No	6–7	ND	58°44′28 N	17°37′60 E
H4	07/2006	22	No	6–7	ND	58°59′07 N	17°43′60 E
Elbe estuary (3)							
Este (~90 km)	17/03/06	32	High	0.5	2.2	53°32′06 N	09°47′31 E
Brunsbüttel (~30 km)	18/03/06	8	High	1.7	2.9	53°53′24 N	09°08′44 E
Scheldt estuary (4)							
1 (58 km)	04/04/06	27	High	10	9.0	51°21′06 N	04°14′58 E
4 (79 km)	04/04/06	5	Ebb	1.5	10.3	51°13′42 N	04°23′86 E
9 (111 km)	04/04/06	14	Ebb	0.5	11.5	51°05′31 N	04°10′54 E
Seine estuary (5)							
Tancarville (~28 km)	23/05/06	38	ND	2	16	49°28′33 N	00°27′54 W
Tamar estuary (6)							
1 (~8 km)	07/06	5	ND	ND	ND	50°N	04°E
Loire estuary (7)							
Paimboeuf (~17 km)	18/04/06	22	Ebb	ND	3.7	47°17′23 N	02°01′52 W
Gironde estuary (8)							
2 (~12 km)	17/04/06	28	Low	11.55	13.2	45°31′00 N	01°57′00 W
E (~48 km)	20/04/06	5	Flood	2.87	14.9	45°14′80 N	00°43′50 W
K (~70 km)	19/04/06	29	Flood	0.26	13.4	45°04′10 N	00°38′30 W

ND not determined

[a] Number of individuals sequenced per location

[b] As recorded at time of sampling

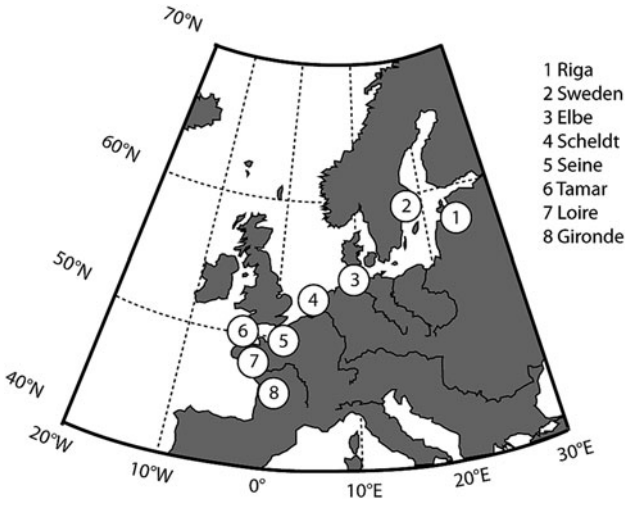

Fig. 1 Map of sampling locations indicated from north east to south west; *1* Gulf of Riga, *2* Baltic Proper (Sweden), *3* Elbe estuary, *4* Scheldt estuary, *5* Seine estuary, *6* Tamar estuary, *7* Loire estuary, and *8* Gironde estuary

et al. 2009). Samples were collected with a 230-µm plankton net from the surface layer (1–2 m deep) by boat (locations 1, 2, 4, 5, 6, and 8), or from shore (locations 3 and 7) and preserved in 95% ethanol. These sampling locations differed in salinity and tidal influence (Table 1). At estuarine locations with more than one sampling station, we covered habitats with different salinities in a range from 0.3 to 12 psu.

DNA sequencing

DNA sequence data were obtained for individual copepods from 8 locations (sample sizes are shown in Table 1; Fig. 1). For each individual, 549 bp of the mitochondrial cytochrome oxidase subunit I (COI) gene was sequenced. DNA was extracted from ethanol-preserved individual copepods using a cell lysis buffer with Proteinase-K protocol modified from Hoelzel and Green (1992) (Lee and Frost 2002). After we encountered problems with PCR amplification with the commonly used Primers COIH 2198 and COIL 1490 (Folmer et al. 1994), we designed two new specific primers:

EuF1: 5′-CGTATGGAGTTGGGACAAGC-3′ and
EuR2: 5′-CAAAATAAGTGTTGGTATAAAATTGGA-3′.

The PCR profile taken from Lee (2000) was modified for our PCR amplification: starting with denaturation at 95°C for 30 s, followed by 5 cycles of 30 s at 90°C, 60 s at 55°C, 90 s at 72°C, followed by 27 cycles of 30 s at 90°C, 45 s at 55°C, 60 s at 72°C ending with 5 min at 72°C. The PCR product was purified using a Qiagen extraction kit (Qiagen, Inc., Valencia, CA). The purified PCR product was sequenced on both strands using the ABI Big-Dye chemistry and run on a capillary Applied Biosystems Inc. 3100 sequencer. All sequences have been deposited in the GenBank database (GenBank [accession numbers JF727310-JF727558]).

Haplotype diversity and networks

Genetic diversity was compared across samples using estimators implemented in DnaSP version 4.0 (Rozas et al. 2003), including haplotype diversity (Hd, probability that two randomly chosen haplotypes are different in the sample; Nei 1987) and nucleotide diversity (π, average number of nucleotide differences per location between two sequences; Nei 1987). A statistical parsimony haplotype network was constructed to show genetic relatedness among extant haplotypes using the software package TCS 1.21 (Clement et al. 2000).

Analysis of molecular covariance

The hierarchical distribution of molecular variance was assessed by performing an analysis of molecular variance (AMOVA) using the software package ARLEQUIN version 3.01 (Excoffier et al. 2005). Regions were defined a priori according to geographic region from north to south. In the Baltic Sea, we separated the Gulf of Riga from the Baltic Proper location (Sweden) due to the absence of shared haplotypes between these locations. The AMOVA was performed both among all regions (Gulf of Riga, Baltic Proper, North Sea/English Channel, and East Atlantic coast) and between all pairwise regions. According to the isolation by distance hypothesis (Wright 1943), we would expect that the highest differentiation occurs between populations separated by the greatest geographic distances. Significance of the observed values of the Φ_{ST} statistic was tested using a random permutation procedure available in ARLEQUIN, under the null hypothesis of no population structure.

Global and pairwise fixation indices (F_{ST}) among populations from all locations were also computed by treating mtDNA haplotypes as allelic data at a single locus. The Raymond and Rousset exact test of population differentiation was used to test the null hypothesis of identity of haplotype distribution across populations (Raymond and Rousset 1995). For comparison of genetic structure between both continents, sequence data from North American populations (Ile Verte [$n = 67$], Montmagny [$n = 34$], Berthier [$n = 26$], La Pocatière [$n = 20$], St. Jean Port Joli [$n = 22$], and Cap Brulé [$n = 28$]) were taken from Winkler et al. (2008) and aligned with the European sequences to get similar sequences of 549 bp.

Demographic analyses

The pairwise haplotype mismatch analysis was performed for populations in the following regions (A) the Baltic Proper (location 2), (B) the North Sea/English Channel estuaries (locations 3–6), and the East Atlantic estuaries, (C) the Loire estuary (location 7), and (D) the Gironde estuary (location 8) using DNASP 4.10 (Rozas et al. 2003). We did not analyze pairwise haplotype mismatch for the Gulf of Riga due to low sample size ($n = 5$). To test for deviations from a neutral Wright–Fisher model consistent with population expansion, Fu's *Fs*, Fu and Li's *F**, Fu and Li's *D**, and Tajimas's *D* statistic were calculated using DNASP 4.10 (Rozas et al. 2003).

The pairwise mismatch distribution was used to estimate the time since demographic expansion, τ ($=2\mu t$, where $\mu =$ mutation rate per generation and $t =$ time in generations), as well as initial and final θ, under a model of sudden demographic expansion, using the software package ARLEQUIN version 3.01 (Excoffier et al. 2005). A generalized least-squares approach was employed for parameter fitting to the pairwise mismatch distribution. Confidence intervals for the mismatch parameters, τ and initial and final θ, were calculated using 1,000 permutations. A goodness of fit test was performed to test the validity of the sudden expansion model, using a parametric bootstrap approach based on the sum of square deviations (SSD) between the observed and the expected mismatch distributions (Schneider and Excoffier 1999). In order to estimate effective population size and timing of population expansion (in years), a mutation rate of 0.7% per million years (My) was used based on rates of COI sequence divergence in snapping shrimps (Knowlton and Weigt 1998). This rate estimate was used because a calibrated molecular clock does not exist for mtDNA in *E. affinis*. Generation time used was based on six generations per year, a conservative estimate for *E. affinis* in the European estuaries.

Tests for population growth were performed by coalescent modeling of sequence evolution using the software package LAMARC version 2.1 (Kuhner 2006). Maximum-likelihood estimates were obtained for the joint likelihood surface for θ_1 ($=2Nf\mu$ for mtDNA, where $\mu =$ mutation rate, $Nf =$ female effective population size) and population growth rate g under a model of exponential growth. The θ_1 computed here is the final θ following population growth. A Metropolis–Hastings Markov chain Monte Carlo algorithm was used, with 10 Markov chains each with 10,000 steps and two final Markov chains each with 200,000 steps. The relationship between θ_0, θ_1, time (t), and population growth rate (g) is $\theta_0 = \theta_1 e^{-gt}$ (Kuhner 2006). A positive value of g indicates population growth, whereas a negative value indicates diminishing population size. Tests were performed for 15 randomly chosen individuals from the Baltic (location 2), the NSEC estuaries (locations 3–5), and the two separate populations (location 7 and 8) from the East Atlantic estuaries (Table 6).

Phylogenetic analyses

Phylogenetic analyses of the European *E. affinis* populations were conducted both on the complete dataset (249 sequences) and on a reduced one containing only the 12 most frequent haplotypes (haplotypes represented by at least 3 individuals). As outgroup, six sequences of the American clades (three from the Atlantic clade and three from the North Atlantic clade) were taken from Winkler et al. (2008). Phylogenetic reconstructions were performed by maximum-likelihood (ML) with PHYML v3.0 (Guindon et al. 2010) and by Bayesian analyses with BEAST version 1.6 (Drummond and Rambaut 2007). The best fitting model under the ML criterion was selected from the "Bayesian Information Criterion" (BIC) output of jMODELTEST v0.1.1 (Posada 2008). The ML analyses were conducted using a TN93 model without gamma rate distribution (Γ) nor invariable sites (I), the best tree topology was searched using NNI and SPR methods and node stability was estimated by 100 nonparametric bootstrap replicates (Felsenstein 1985). With the Bayesian reconstruction, both the phylogenetic relationships and the molecular dating were determined. A major advantage of Bayesian phylogenetic inference is the possibility of partitioning the data, giving each partition its own best fitting model of sequence evolution. As the second codon position was uninformative, it was removed from the analyses. For the first codon positions, the TN93 model without Γ nor I was used, and for the third position, the HKY model without Γ nor I was chosen. Based on the COI substitution rate described in snapping shrimps (Knowlton and Weigt 1998), a uniform strict molecular clock with lower and upper values of 0.7 and 1.4% per My was specified. Two independent runs of 10 million generations each were performed. Analyses were undertaken by sampling every 1,000th generation. Tracer 1.5 was used to check for convergence of the model likelihood and parameters between each run until reaching stationarity using 10% of burnin. The resulting log and tree files were then combined using LogCombiner. Results were considered reliable once the effective sampling size (ESSs) of all parameters was above 200.

Results

Population structure

The haplotype network revealed the existence of three distinct genetic entities in Europe (Fig. 2). Each lineage

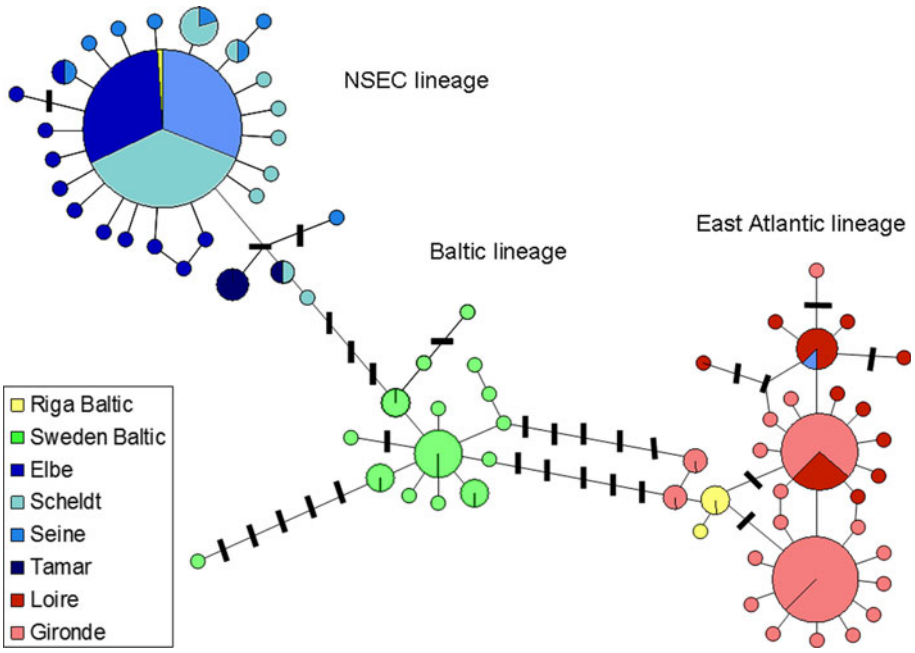

Fig. 2 Parsimony mtDNA haplotype network for the European *E. affinis* populations. *Circle size* is proportional to haplotype frequency. The *smallest* and the *biggest circle* represent one and 93 individuals, respectively. *Black bars* represent missing haplotypes. *Colors* indicate the geographic origin of haplotypes, according to Fig. 1

was largely correlated with a specific geographic region: (1) Baltic Proper, including samples from location 2 (Sweden), (2) North Sea/English Channel (NSEC), including samples from locations 3–6 (Elbe, Scheldt, Seine, and Tamar), and (3) East Atlantic, including samples from locations 7 and 8 (Loire and Gironde). Two genetically distinct regions were clearly distinguishable within the Baltic Sea as the Gulf of Riga shared no haplotype with the Baltic Proper population but instead was composed of a mixture of haplotypes from both NSEC and East Atlantic lineages. In addition, one Seine haplotype belonged to the East Atlantic rather than NSEC lineage. The three lineages were separated by three and five mutational steps (Baltic–NSEC and Baltic–East Atlantic, respectively, Fig. 3). Interestingly, the relative position of the three lineages in the haplotype network did not reflect their relative geographic position, the Baltic lineage being situated in the center of the network between the NSEC and the East Atlantic lineages. Furthermore, the three lineages had sharply contrasted topologies. Haplotypes of NSEC populations formed a star-like pattern centered around a single dominant haplotype (Fig. 2). In contrast, haplotypes from Swedish populations (Baltic lineage) were more dispersed with no clearly defined phylogenetic structure except one haplotype separated by five mutational steps. Two closely related dominant haplotypes were observed in East Atlantic populations (Fig. 2).

Patterns of genetic heterogeneity within the European clade

We found 73 distinct haplotypes among the 249 individuals sequenced. Exact tests revealed no evidence for differences in haplotype frequencies among the different samples collected within estuaries ($P > 0.05$); therefore, different stations within each estuary were pooled for further analyses (Table 1). Most of the haplotypes (66) were private, and only 7 were shared among locations (Figs. 2, 3). The maximum number of singletons was found in the Gironde Estuary population (15 haplotypes; Table 2; Fig. 2, 3).

Haplotype diversity (Hd) was higher in the two Baltic locations and East Atlantic region with overall Hd = 0.700 ± 0.218 (Riga), Hd = 0.908 ± 0.034 (Sweden), and Hd = 0.828 ± 0.030 (East Atlantic), respectively, than in the NSEC populations (Hd = 0.450 ± 0.057, Table 2). Nucleotide diversity showed similar patterns, with higher values in the Baltic Proper and East Atlantic regions ($\pi = 0.0041 \pm 0.0008$ [Sweden] and $\pi = 0.0030 \pm 0.0003$ [East Atlantic]) compared with the NSEC region ($\pi = 0.0015 \pm 0.0004$). The haplotypes from the Riga location cluster either with NSEC or with the East Atlantic lineages; therefore, this location displays a high π value ($\pi = 0.0984 \pm 0.0049$),

The AMOVA analyses revealed that most of the genetic variance (87.10%) was distributed among regions with only 2.51% of total genetic variance among populations within regions (Table 3a), indicating a strong genetic subdivision between regions. The pairwise AMOVA analyses (Table 3b–g) showed no evidence for isolation by distance, as already apparent from the central position of the Baltic lineage in the haplotype network. Strikingly, the Baltic (Riga and Sweden) and East Atlantic populations (separated by the greatest geographical distance) showed lower differentiation (75.00%) than the geographically closer NSEC and East Atlantic populations (90.49%).

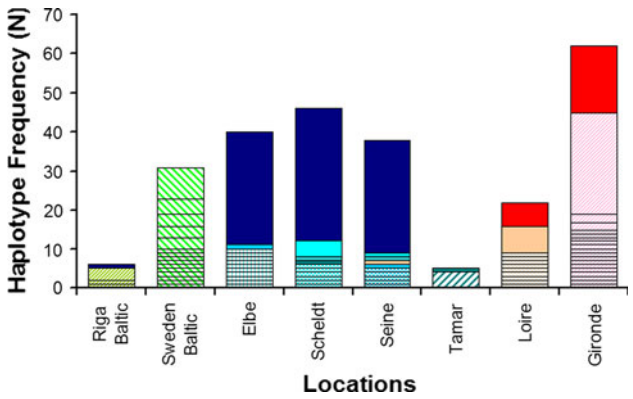

Fig. 3 Distribution of COI haplotypes frequencies (*N*) for *E. affinis* from 8 brackish-water locations in Western Europe

In addition, the exact test suggested significant genetic subdivision in Western Europe, as confirmed by the high pairwise genetic distances between populations (F_{ST}; Table 4). The northern populations in the Baltic (Sweden and Riga) were genetically distant from each other ($F_{ST} = 0.658$, $P < 0.0001$), as well as from all other locations. In contrast, three of the NSEC populations (Elbe, Scheldt and Seine) showed no evidence of genetic subdivision as indicated by low and nonsignificant global F_{ST} ($F_{ST} = 0.006$, $P = 0.067$), suggesting one panmictic population. The westernmost NSEC population (Tamar) showed high and significant F_{ST} values with the three other populations from this lineage (average pairwise $F_{ST} = 0.654$, $P < 0.0001$), suggesting another gene pool in this region. However, low sampling size ($n = 5$) in the Tamar estuary might have biased our estimation of F_{ST}. The two locations from the East Atlantic lineage (Loire and Gironde) showed intermediate significant divergence ($F_{ST} = 0.284$, $P < 0.0001$).

Population expansion events

In light of the previous results, we examined the mismatch distributions (Fig. 4a–d) for the Baltic Proper populations (location 2), the NSEC estuarine populations (locations 3–6), the Loire population (location 7), and the Gironde population (location 8) of the East Atlantic lineage separately. All four populations showed evidence of a relatively recent expansion based on a significant deviation from the neutral Wright–Fisher model (Table 5), the mismatch distributions analyses (Fig. 4) and positive values for g (Table 6). The pairwise mismatch distributions of the Baltic Proper showed a dominant peak in frequency at one and two pairwise differences, consistent with population growth (Fig. 4a). A second much smaller peak at eight pairwise differences was consistent with one satellite haplotype in the Baltic lineage haplotype network (Fig. 2). The East Atlantic populations were unimodal with a peak in frequency at one pairwise difference, consistent with recent population growth (Fig 4c, d). The mismatch distribution of the NSEC estuarine populations fits best the expected pairwise mismatch distribution under a Wright–Fisher model (with highest frequency at 0 pairwise differences), suggesting a stable population with constant population size or a very recent expansion which is not well represented in the figure (Fig. 4b). The latter hypothesis seems more likely regarding other statistical tests (Fu and Li's $F^* = -5.097$, $P < 0.02$; Fu and Li's $D^* = -5.366$, $P < 0.02$, Tajimas's $D = -2.581$, $P < 0.0001$) and the haplotype network (Fig. 2) suggesting population expansion (Table 5). High growth values of g were also consistent with population expansion ($g = 7{,}504$, Table 6). Mismatch distribution analyses under a sudden expansion model (Table 7) suggested that population expansion events occurred earlier in the Baltic

Table 2 Patterns of mtDNA haplotype variation of *E. affinis* populations at each location

Region	Sampling locations	*N*	# Haplotypes	# Singelton haplotypes	Haplotype diversity (Hd) ± SE	Nucleotide diversity (π) ± SE	Overall haplotype diversity (Hd) ± SE	Overall nucleotide diversity (π) ± SE
Baltic	Riga (1)	5	3	1	0.700 ± 0.218	0.0984 ± 0.0049		
	Sweden (2)	31	15	11	0.908 ± 0.034	0.0041 ± 0.0008		
North Sea/ English Channel	Elbe (3)	40	12	10	0.479 ± 0.099	0.0012 ± 0.0003	0.450 ± 0.057	0.0015 ± 0.0005
	Scheldt (4)	46	10	6	0.452 ± 0.091	0.0012 ± 0.0003		
	Seine (5)	38	10	5	0.422 ± 0.102	0.0022 ± 0.0011		
	Tamar (6)	5	2	0	0.400 ± 0.237	0.0015 ± 0.0009		
East	Loire (7)	22	11	9	0.844 ± 0.058	0.0030 ± 0.0006	0.828 ± 0.030	0.0030 ± 0.0003
Atlantic	Gironde (8)	62	19	15	0.755 ± 0.044	0.0025 ± 0.0003		

Table 3 Analysis of molecular covariance (AMOVA, Excoffier et al. 2005; a) among and within regions, (b) between Sweden and North Sea/English Channel (NSEC) populations, (c) between Riga and NSEC populations, (d) between and within Sweden and East Atlantic populations, (e) between and within Riga and East Atlantic populations, (f) between and within Sweden and Riga populations, and (g) between and within NSEC and East Atlantic populations. Φ_{CT} is defined as the variance among groups divided by total variance, Φ_{SC} is the variance among populations divided by the variance among and within populations, and Φ_{ST} is the variance among groups and among populations divided by total variance (Excoffier et al. 2005)

Source of variance	*df*	Covariance	% Total	Fixation indices		*P* value
(a) Sweden vs. Riga vs. NSEC vs. East Atlantic populations						
Among regions (σ_a^2)	3	4.997	87.10	$\Phi_{CT} = \sigma_a^2/\sigma_T^2$	0.87	<0.0001
Among populations within regions (σ_b^2)	11	0.144	2.51	$\Phi_{SC} = \sigma_b^2/(\sigma_b^2 + \sigma_c^2)$	0.20	<0.0001
Within populations (σ_c^2)	235	0.596	10.39	$\Phi_{ST} = (\sigma_a^2 + \sigma_b^2)/\sigma_T^2$	0.90	<0.0001
Total	249	5.718				
(b) Sweden vs. NSEC populations						
Between regions (σ_a^2)	1	3.645	85.78	Φ_{CT}	0.86	0.025
Among populations within regions (σ_b^2)	7	0.066	1.55	Φ_{SC}	0.28	<0.0001
Within populations (σ_c^2)	152	0.538	10.26	Φ_{ST}	0.90	<0.0001
Total	160	4.249				
(c) Riga vs. NSEC populations						
Between regions (σ_a^2)	1	3.394	85.83	Φ_{CT}	0.86	<0.0001
Among populations within regions (σ_b^2)	7	0.155	3.91	Φ_{SC}	0.11	<0.0001
Within populations (σ_c^2)	126	0.406	12.67	Φ_{ST}	0.87	<0.0001
Total	134	3.955				
(d) Sweden vs. East Atlantic populations						
Between regions (σ_a^2)	1	4.480	81.23	Φ_{CT}	0.82	0.071
Among populations within regions (σ_b^2)	9	0.135	2.67	Φ_{SC}	0.14	<0.0001
Within populations (σ_c^2)	109	0.816	16.10	Φ_{ST}	0.81	<0.0001
Total	114	5.069				
(e) Riga vs. East Atlantic populations						
Between regions (σ_a^2)	1	0.920	48.00	Φ_{CT}	0.48	0.067
Among populations within regions (σ_b^2)	4	0.294	15.35	Φ_{SC}	0.30	<0.0001
Within populations (σ_c^2)	83	0.703	36.65	Φ_{ST}	0.63	<0.0001
Total	88	1.917				
(f) Riga vs Sweden						
Between regions (σ_a^2)	1	2.166	56.45	Φ_{CT}	0.57	0.067
Among populations within regions (σ_b^2)	2	0.619	16.13	Φ_{SC}	0.37	<0.0001
Within populations (σ_c^2)	32	1.052	27.43	Φ_{ST}	0.73	<0.0001
(g) NSEC vs. East Atlantic populations						
Between regions (σ_a^2)	1	6.092	90.49	Φ_{CT}	0.91	<0.0001
Among populations within regions (σ_b^2)	10	0.081	1.20	Φ_{SC}	0.13	<0.0001
Within populations (σ_c^2)	264	0.559	8.31	Φ_{ST}	0.92	<0.0001
Total	275	6.732				

lineage ($\tau = 1.902$) compared with the East Atlantic lineage ($\tau = 1.594$) and the NSEC lineage ($\tau = 0.926$). This was confirmed by dating population expansions estimated (with large confidence intervals) around ~247,000 BP for the Baltic, ~207,000 BP for the East Atlantic lineages, and ~121,000 BP for the NSEC lineage (Table 7). Timing of population expansions could also correspond to the last selective sweep in each of these populations (Bazin et al. 2006). Under the assumption of purely demographic expansion, populations of the Baltic and East Atlantic lineage were inferred to have expanded from very small to huge effective female population sizes ($>7 \times 10^{10}$, Table 7). However, the effective population sizes in the NSEC lineage were inferred to have expanded moderately (3×10^4 to 9×10^5, Table 7).

Table 4 Pairwise genetic distances between populations from the European lineages and the North American lineages

European lineages	Riga (1)	Baltic Sweden (2)	NSEC Elbe (3)	Scheldt (4)	Seine (5)	Tamar (6)	East Atlantic Loire (7)	Gironde (8)
Riga (1)		0.0010	0.0047	0.0073	0.0035	0.0286	0.0052	<0.0001
Sweden (2)	0.658***		<0.0001	<0.0001	<0.0001	0.0022	<0.0001	<0.0001
Elbe (3)	0.870***	0.848***		0.0899	1	<0.0001	<0.0001	<0.0001
Scheldt (4)	0.872***	0.853***	0.017*		0.6351	<0.0001	<0.0001	<0.0001
Seine (5)	0.805***	0.813***	0.008	−0.004[a]		<0.0001	<0.0001	<0.0001
Tamar (6)	0.631*	0.764***	0.710***	0.701***	0.550***		0.0025	0.0041
Loire (7)	0.540***	0.809***	0.924***	0.925***	0.892***	0.867***		<0.0001
Gironde (8)	0.562***	0.837***	0.923***	0.923***	0.903***	0.889***	0.284***	

North American lineages[b]	Atlantic			North Atlantic		
	Ile Verte	Montmagny	Berthier	La Pocatière	St. Jean Port Joli	Cap Brulé
Ile Verte		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Montmagny	0.154*		0.351	<0.0001	<0.0001	<0.0001
Berthier	0.127*	0.009		<0.0001	<0.0001	<0.0001
La Pocatière	0.934***	0.963***	0.957***		0.647	0.534
St. Jean Port Joli	0.936***	0.965***	0.960***	−0.028[a]		0.800
Cap Brulé	0.939***	0.966***	0.961***	−0.021[a]	−0.019[a]	

Estimated pairwise F_{ST} values are indicated in the lower triangular matrix, with asterisks indicating significance level: * $P < 0.05$ and *** $P < 0.001$. Values in the upper triangular matrix are estimated P-values of the Raymond and Rousset (1995) exact test of significance (10,000 steps in the Markov chain)

[a] Because parameter estimates will often deviate from the true value, a small negative estimate of F_{ST} could be obtained if the true parameter value is zero

[b] Sequence data used for this analysis were extracted from Winkler et al. (2008). Sequences were shortened to 549 bp of COI to fit our data set

Comparison of divergence pattern between the European and the North American lineages

As expected from Lee (1999b, 2000), the haplotype phylogeny (Fig. 5) confirmed the large sequence divergence at COI between America and Europe, with an average of 16.8% divergence (Tamura–Nei 93 model) across continents. In comparison, sequence divergence among European lineages was much lower (2.1% between the NSEC and the East Atlantic lineages, followed by 1.9% between the East Atlantic and the Swedish lineages and 1.7% between NSEC and Sweden). Based on COI substitution rates in snapping shrimps, 0.7–1.4% per million years (Knowlton and Weigt 1998), European lineages would have diverged from one another about 1.9 millions years ago (My; 95% CI: 0.9–3.0 My, Fig. 5). In contrast, the American lineages (North Atlantic and Atlantic clades) would have diverged significantly more anciently, about 11.5 My (95% CI: 6.8–17.7 My, Fig. 5).

Discussion

In this study, we analyzed 249 individuals of the sibling species complex *Eurytemora affinis* in Western Europe to determine their genetic biodiversity, phylogeographic structure, and demographic history. The results showed (1) shallow but significant genetic structure into three distinct lineages geographically well separated, (2) moderate differences in timing of population expansion and effective population size across lineages, and (3) contrasting patterns of diversity among lineages, ranging from complete panmixia to no haplotype sharing across similar geographic extents.

Genetic structure in *Eurytemora affinis* in Western Europe

Using a more comprehensive sampling scheme within Europe than in previous studies (Lee 1999b, 2000), our results, although concordant with previous results (Lee 1999b, 2000), more fully elucidate the patterns of differentiation among the European lineages. European haplotypes sequenced by Lee (1999b, 2000) clustered according to geographic sampling, differentiating the Gironde location from more northern populations. Our study revealed shallow but significant genetic structure, dividing European populations into three separate lineages, which remained in general geographically isolated. Only two exceptions were found, with one East Atlantic haplotype found in the Seine

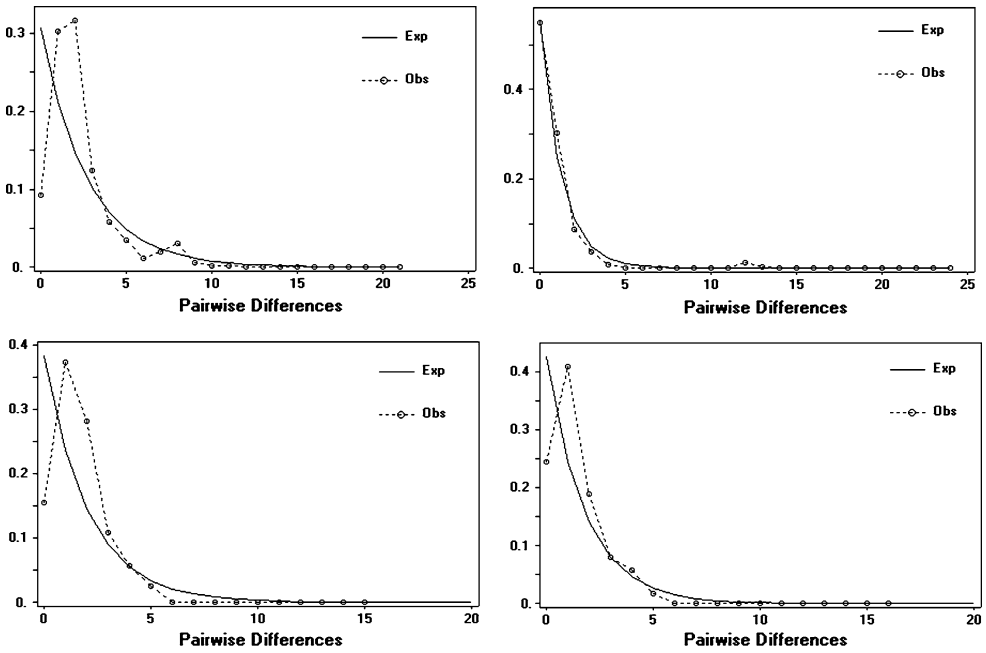

Fig. 4 Pairwise haplotype mismatch distributions for 4 *E. affinis* European populations. The *solid line* represents the expected pairwise mismatch distribution under a Wright–Fisher model, while the *dashed lines* are the observed frequencies. *Graphs* show populations from **a** the Baltic Proper (location 2), **b** the NSEC estuaries (locations 3–6), **c** the Loire estuary (locations 7), and **d** the Gironde estuary (location 8)

Table 5 Statistical tests of deviation from the standard neutral model, used to test for population growth

Region: populations (locations)	Fu's F_S	Fu and Li's F^*	Fu and Li's D^*	Tajimas's D
Sweden (2)	−8.805	−2.825 (<0.05)	−2.597 (>0.05)	−1.985 (<0.05)
North Sea and English Channel (3–6)	−33.779	−4.867 (<0.02)	−5.097 (<0.02)	−2.509 (<0.001)
East Atlantic (7)	−6.878	−3.359 (<0.02)	−3.285 (<0.02)	−1.938 (<0.05)
East Atlantic (8)	−16.585	−4.115 (<0.02)	−4.207 (<0.02)	−2.108 (<0.05)

Significance values are shown in parentheses. We did not correct for multiple testing

Table 6 Estimates of the population genetic parameter θ ($2N_f\mu$), based on number of segregating site locations (Watterson 1975) and on coalescent modeling of sequence evolution in LAMARC (Kuhner et al. 2006)

Populations (locations)	Watterson's $\theta_{\text{per site}} \pm$ SD	LAMARC $\theta_{\text{per site}}$ (95% CI)	g (95% CI)
Baltic (2)	0.00957 ± 0.00352	1.562 (0.111 to >1,500)	2,072 (1,030–4,796)
North Sea and English Channel (3–5)	0.01092 ± 0.00312	144.519 (2.402 to >1,500)	7,504 (4,706–9,375)
East Atlantic (7)	0.00650 ± 0.00273	190.192 (0.46 to >1,500)	9,981 (4,061–11,849)
East Atlantic (8)	0.00776 ± 0.00263	37.363 (0.22 to >1,500)	4,954 (2,354–7,236)

Population growth rate (g) was computed under a model of exponential growth using LAMARC

Estuary, and several haplotypes from the NSEC and the East Atlantic lineages found in the Gulf of Riga.

The parsimony mtDNA haplotype network demonstrated that all three lineages had one (NSEC) or several (Baltic, East Atlantic) prevalent haplotypes. Rare haplotypes of each clade were in general one to three mutational steps apart from the prevalent haplotypes. In the NSEC lineage, the star-like pattern of the haplotype network was associated with low haplotype and nucleotide diversity. In contrast, the haplotype networks of the Baltic and the East Atlantic lineages showed more dispersed patterns and were associated with twice higher haplotype and nucleotide diversity than the NSEC lineage. Low haplotype and nucleotide diversity can be attributed to a recent population bottleneck or founder event and high haplotype and low nucleotide diversity typically indicate rapid population growth after a period of low effective population size (Avise et al. 1984, Grant and Bowen 1998, Dodson et al. 2007). This interpretation is in line with the population genetic parameters estimated from the pairwise haplotype mismatch analyses for all three lineages, suggesting drastic increases in female effective population size in the Baltic and the East Atlantic lineages and more stable moderate effective population sizes in the NSEC lineage (Table 7).

Table 7 Population genetic parameters estimated by pairwise haplotype mismatch analysis under a sudden expansion model

Based on the population genetic parameters, timing of population expansion, and effective population size (females) before (N_{ef0}) and after expansion (N_{ef1}) were calculated, assuming 6 generations per year and a mutation rate of 0.7% million/years

[a] Significance from a goodness of fit test of a sudden expansion model, where a small P value would reject the model

	Baltic lineage: (location 2)	NSEC lineage: (locations 3–6)	East Atlantic lineage: (locations 7–8)
Number of individuals	31	129	84
τ	1.902	0.926	1.594
θ_0	0.002	0.039	0
θ_1	>99,999	1.155	>99,999
$P_{(\text{Sim. SSD} >= \text{Obs. SSD})}$[a]	0.204	0.950	0.287
Timing of population expansion (years BP)	2.47×10^5	1.21×10^5	2.07×10^5
CI 5%	1.29×10^5	0	1.43×10^5
CI 95%	3.68×10^5	4.67×10^5	2.72×10^5
N_{ef0}	0.561	3.05×10^4	0
CI 5%	0	0	0
CI 95%	4.79×10^5	7.42×10^4	2.08×10^5
N_{ef1}	$>7.81 \times 10^{10}$	9.01×10^5	$>7.81 \times 10^{10}$
CI 5%	3.96×10^6	3.91×10^5	4.37×10^6
CI 95%	$>7.81 \times 10^{10}$	$>7.81 \times 10^{10}$	$>7.81 \times 10^{10}$

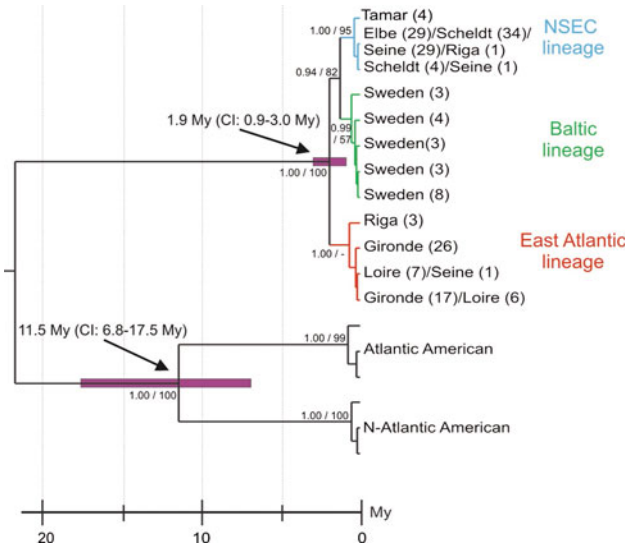

Fig. 5 Chronogram reconstructed from COI mtDNA sequences (549 bp) as inferred by Bayesian analysis on the 12 most frequent haplotypes. The ML analysis gave sensibly the same result, but the East Atlantic lineage was not recovered as monophyletic. Sequences from the Atlantic and North Atlantic clade of the North American continent were taken from Winkler et al. (2008). *Bracketed numerals* indicate the frequency of this particular haplotype in the samples. For each node above population level, values of the Bayesian Posterior Probability (*PP*) and the Bootstrap Percentage (*BP*) are given, respectively. The 95% confidence intervals on calculated ages are indicated by *bars* for the two compared nodes (divergence time of the European and American clades). The time scale is expressed in million years (*My*). *Numbers* in the *parentheses* next to the locations indicate the number of individuals found with that haplotype at that location

Significant separation in the three European lineages concord with 2 biogeographic genetic breaks, one between East Atlantic and NSEC populations (2.1% sequence divergence) and the second one between NSEC and Baltic Proper populations (1.7% sequence divergence), but no isolation by distance. The genetic break between the East Atlantic and NSEC lineages was sharp, with very restricted overlap (one single East Atlantic haplotype found in the Seine estuary). This genetic break has already been described in several coastal invertebrates such as the polychaete, *Pectinaria koreni*, with 16% sequence divergence (Jolly et al. 2005) and the bivalve, *Macoma balthica* with 1–2% sequence divergence (Luttikhuizen et al. 2003). The genetic break between North Sea and Baltic Sea populations was also observed in numerous algae, invertebrates, and fish taxa (Johannesson and Andre 2006). A meta-analysis based on 29 species, mostly marine, indeed proposed that this break between North and Baltic seas may be the result of a genetic cline from marine (North Sea) to brackish (Baltic Sea) environments, typically resulting in regions of overlap of different haplotypes and diminished genetic diversity toward the innermost parts of the Baltic Sea (Johannesson and Andre 2006). Our study did not reveal a genetic cline from the NSEC to the Baltic Proper, as no haplotypes were shared among these populations from the NSEC and the Baltic Proper. However, this might be due to restricted sampling in the Baltic Proper only, missing the potential overlap region in the Kattegat and Skagerrak. Our results contradict general findings on diminished genetic diversity in the Baltic compared with the North Sea (Johannesson and Andre 2006). Interestingly, haplotype and nucleotide diversity were at least two times higher in the Swedish population (Hd = 0.908; $\pi = 0.0041$) compared with the NSEC populations (Hd = 0.450; $\pi = 0.0015$), and similar to the East Atlantic populations (Hd = 0.828; $\pi = 0.0030$), suggesting different evolutionary histories.

Evolutionary history of the European lineages

The phylogenetic analyses, the level of sequence divergence, the mismatch analyses, the timing of population expansion, and the absence of geographic overlap among lineages suggest that subdivision occurred long before the Last Glacial Maximum (LGM; ~18,000 BP) around the early Pleistocene or late Pliocene (1.9 My, 95% CI: 0.9–3.0 My). As the Pleistocene was characterized by major climatic changes, which had great influence on the biogeographical distribution of aquatic and terrestrial organisms (Avise 1998), it is likely that *E. affinis* populations expanded and contracted on several occasions, structuring the population genetic structure of the species. The most recent population expansion of the Baltic and the East Atlantic lineage, detected by mismatch analyses, was inferred to be situated ~250,000 BP and ~207,000 BP, respectively, which coincides with the interglacial of the Saal/Riss glaciations, starting 270,000–200,000 BP (Dawson 1992). Comparable timing of population expansion was found for estuarine populations of the Atlantic clade in the St. Lawrence estuary (North America; Winkler et al. 2008). Later expansion was inferred for the NSEC lineage approximately 120,000 years ago in an interglacial warmer period of the Weichsel/Würm glaciation (Dawson 1992). These findings suggest that reduction expansion events in *E. affinis* during the most recent glaciations did not leave important imprints on the genetic structure.

However, as the ice sheet covered the Baltic Sea, reaching as far south as the British Isles and the permafrost region extended to 47°N during the LGM, the Baltic population, and the NSEC populations must have retreated into glacial refugia. An ice-free corridor of dry land in the central North Sea separated the Scandinavian and the British ice sheets due to much lower sea levels (Dawson 1992). Therefore, it was suggested that marine organisms retreated into glacial refugia located either south of the permanent ice shields (Luttikhuizen et al. 2003; Jolly et al. 2005; Remerie et al. 2009) or in the Irish Sea, around Scotland and in the English Channel (Roman and Palumbi 2004; Provan et al. 2005). High genetic diversity within the East Atlantic lineage supports that this lineage may have survived in the proposed southern glacial refugium. Furthermore, only one single haplotype was shared between the East Atlantic and the NSEC region, suggesting very restricted participation of the East Atlantic lineage in the most recent range expansion to northern areas after the LGM. A similar pattern was suggested for the estuarine mysid shrimp species *Neomysis integer* by Remerie et al. (2009). In contrast, variable degrees of admixture among lineages in the different regions were found for marine bivalves, possibly as a result of colonization from different glacial refugia after the LGM (Luttikhuizen et al. 2003; Nikula et al. 2007). Further North, a glacial brackish lake existed in the southern North Sea that was proposed as a refugium for salmon and sand goby (*Pomatoschistus minutus*; Verspoor et al. 1999; Gysels et al. 2004). Possibly the NSEC lineage may have been preserved in this northern glacial refugium, given that no haplotype from this lineage was found further south. This refugium may have represented adequate environmental conditions (salinity around 8 PSU) for a broadly distributed species such as *E. affinis* (Dawson 1992). The Baltic lineage probably survived also several recent glaciations separate from the two other lineages. A potential postglacial recolonization route might have been eastward from the Atlantic and the North Sea, but it seems unlikely that the Baltic lineage shared a refugium with the NSEC lineage in the above proposed brackish lake of the southern North Sea, as no haplotype was shared in the recent distribution. Alternatively, glacial refugia east of the Baltic Sea may have existed as suggested for salmon and trout (Osinov and Bernatchez 1996; Koljonen et al. 1999). A more complete comprehension of re-colonization routes of *E. affinis* will now require extended sampling especially in regions of potential ancient glacial refugia.

Contrasting patterns of genetic structure across lineages

The degree of divergence among populations within each of the three lineages ranged from completely connected to partly isolated, at comparable geographic scales. In the NSEC lineage, haplotypes were homogenously distributed between the Elbe, Scheldt, and Seine estuaries. The lack of genetic subdivision within the NSEC lineage was consistent with panmixia. The relative short time span (<18,000 BP) since separation of populations during the last re-colonization after the LGM into separate estuaries, might not have allowed for sufficient time for genetic drift to create detectable differences in haplotype frequencies across the three estuaries. In addition, gene flow through migrants can not be excluded as particular hydrodynamics of theses estuaries may have favored occasional water exchange, resulting in the transport of *E. affinis* individuals, particularly at early life stages. Events of high freshwater discharge such as spring floods may shift the oligo and mesohaline section of the estuaries downstream into the mouth regions, possibly allowing for the transport of individuals via the coastal current from south to north east (Delhez 1996, Delhez and Deleersnijder 2002). Young life stages (Nauplii) with low swimming capacity and high survival in fluctuating salinity (Devreker et al. 2004) may be particularly vulnerable to transport. Recent time distribution of *E. affinis* showed that maximum population abundance in the Seine, Western Scheldt, and Ems estuaries occurred in the mesohaline zone in the lower part of

the estuary (Sautour and Castel 1995; Mouny et al. 1998; Peitsch et al. 2000; Devreker et al. 2008), increasing the probability of advection and dispersal along the English Channel and North Sea under high discharge conditions. This is consistent with findings in the Elbe estuary where the position of *E. affinis* is related to river discharge, showing *E. affinis* in the lower part of the estuary at high river runoff (Peitsch et al. 2000). Since the industrialization, we should also consider human altered transport (e.g., in ballast water of ships) as a potential vector of dispersal between populations, especially in zones of intensive shipping activity such as the NSEC estuaries.

In contrast, significant genetic subdivision was evident within the East Atlantic lineage between the Loire and the Gironde populations (Table 4, pairwise F_{ST}, Raymond and Rousset exact test), suggesting either a long period of restricted gene flow or independent founder events. Similar genetic subdivision was found in the Atlantic clade in the St. Lawrence estuary between populations from two distinct habitat types, the salt-marsh ponds and the oligohaline zone of the estuary (Winkler et al. 2008). This study proposed that gene flow was probably restricted through competitive exclusion in the central portion of the St. Lawrence Middle estuary, as two congeners, the North Atlantic clade and *E. herdmani* were present (Winkler et al. 2008). No congeners of the East Atlantic lineage were found in Gironde and Loire estuary who could have acted as a barrier against dispersal. On the other hand, *E. affinis* in the Gironde is only found in the oligohaline, upstream part of the estuary (Sautour and Castel 1995), so opportunities for natural dispersal via the Atlantic ocean between the Loire and the Gironde estuaries may be reduced.

A haplotype found earlier in the Gulf of Finland, Gulf of Bothnia, Baltic Proper (7 individuals from M. Viitasalo in Lee 1999b, 2000) is consistent with the haplotypes of the Baltic lineage of this study. Surprisingly, we did not find any Baltic lineage haplotype in the Gulf of Riga. This may be the result of the enclosed situation of the Gulf of Riga, due to reduced water exchange with the Baltic Proper. Interestingly, haplotypes from the Gulf of Riga belonged to both NSEC and East Atlantic lineages. Keeping in mind that this result has to be interpreted with caution due to small sampling size of the Gulf of Riga population ($n = 5$), we suggest secondary contact in this zone. Natural dispersal even over geological time scales seems unlikely, as we did not find any of NSEC and East Atlantic haplotypes in the Baltic Proper. We propose that secondary contact is more likely a result of introduction by ballast water of commercial ships. Riga's Port has a large international container terminal with important commercial routes from Rotterdam and overseas (Riga Port Authority). Similar introductions have been found around St. Petersburg (Russia), where *E. affinis* from the North American clade was found (Alekseev et al. 2009). In contrast, the sampling stations in the Swedish Baltic Proper were not in the vicinity of an important commercial port, so that occurrence of invaded *E. affinis* lineages was less likely. Further sampling will now be needed to determine the relative frequencies and potential hybridization of NSEC and East Atlantic lineages in the Gulf of Riga.

Consequences on ecological and management questions

Estuaries are now recognized as highly ecologically valuable in terms of biological productivity (Simenstad et al. 1990; Mallin and Paerl 1994). However, European estuaries are heavily impacted by anthropogenic activities, so that they become important monitoring sites to detect environmental and anthropogenic changes (Mouny and Dauvin 2002; Kimmel and Roman 2004; Tackx et al. 2004; Kimmerer 2005; Kimmel et al. 2006; Cailleaud et al. 2009; Mialet et al. 2010). In order to reduce the pollution in these environments and improve water quality, the European Water Directive has been designed a few years ago. This directive represents an adequate framework to promote different ecological indicators. For pelagic ecosystems, several recent studies suggested the great potential of copepods to develop new indicators of water quality. Among these species the copepod *E. affinis* shows a great potential and is promoted in several estuaries, including the Baltic Sea (Jeppesen et al. 2007), the Seine estuary (Cailleaud et al. 2007), the Scheldt estuary (Appeltans et al. 2003; Mialet et al. 2010), the Gironde estuary (David et al. 2007), some Basque estuaries (Albaina et al. 2009), and the San Francisco Bay (Ger et al. 2009). The relevance of these ecological studies based on a complex biological model requires a detailed study of the genetic patterns of this species complex.

In this paper, we showed significant patterns of genetic structure of *E. affinis* in Western Europe shaped by a combination of historic and contemporary factors that should be taken into account in the definition of *E. affinis* as biomarker. In particular, even though the percentage of divergence was relatively low (1.7–2%) compared with the deep divergence among the four North American clades (up to 15%, Lee 1999b, 2000), almost no overlap in haplotypes existed among the different regions. These findings strongly suggest that the population genetic structure of *E. affinis* in Europe might have ecological consequences to the species itself as well as for the ecosystem they live in, which should be determined in the future to define the capacity of *E. affinis* species complex as bioindicator for estuarine and brackish-water environments.

Acknowledgments Funding for this research was provided by Seine-Aval, 2 conjoint projects: Fond France-Canada for Research

and France-Québec #Program Samuel-De Champlain # *62.108* and the Research Federation FR1818. CP was funded by a postdoctoral grant from the University of Lille 1. We thank gratefully H. Gorokhova and S. Strake for providing samples from the Swedish Baltic and the Gulf of Riga, respectively. Special thanks are extended to S. Oesman, A. Cugat; P. Meire, Tom Maris, and M. Tackx; D. Devreker; B. Sautour and V. David for their help in sampling the Elbe, the Scheldt, the Seine, and the Gironde estuaries, respectively. We thank J. Cuguen to have generously accepted a zooplankton post doc (GW) in his botanical laboratory and P. Saumitou-Laprade for fruitful discussions. A. Courseaux and C. Godé provided excellent technical help in the laboratory. We thank C. E. Lee for providing *E. affinis* sequences for comparison and discussion purposes. We thank 2 anonymous reviewers for their greatly appreciated and helpful comments to improve this paper. Computational resources were provided by CRI-Lille 1 supported by the CNRS and Lille 1 University—Science and Technology. This paper is a contribution to the transversal action "genetics of copepods" within the Multidisciplinary Research Institute for Environemental Sciences (*IRePSE*) of Lille 1 University and Québec-Océan.

References

Albaina A, Villate F, Uriarte I (2009) Zooplankton communities in two contrasting Basque estuaries (1999–2001): reporting changes associated with ecosystem health. J Plankton Res 31:739–752

Alekseev V, Abramson N, Sukhikh N (2009) Introduction of sibling species to the ecosystem of the Baltic Sea. Doklady Biol Sci 429:544–547

Appeltans W, Hannouti A, Van Damme S, Soetaert K, Vanthomme R, Tackx M (2003) Zooplankton in the Schelde estuary (Belgium The Netherlands). The distribution of *Eurytemora affinis*: effect of oxygen? J Plankton Res 25:1441–1445

Avise JC (1998) Conservation genetics in the marine realm. J Hered 89:377–382

Avise JC, Neigel JE, Arnold J (1984) Demographic influences on mitochondrial-DNA lineage survivorship in animal populations. J Mol Evol 20:99–105

Beyrend-Dur D, Souissi S, Devreker D, Winkler G, Hwang JS (2009) Life cycle traits of two transatlantic populations of *Eurytemora affinis* (Copepoda: Calanoida): salinity effects. J Plankton Res 31:713–728

Bilton DT, Paula J, Bishop JDD (2002) Dispersal, genetic differentiation and speciation in estuarine organisms. Estuar Coast Shelf Sci 55:937–952

Bucklin A, Kaartvedt S, Guarnieri M, Goswami U (2000) Population genetics of drifting (*Calanus* spp.) and resident (*Acartia clausi*) plankton in Norwegian fjords. J Plankton Res 22:1237–1251

Burkill PH, Kendrall TF (1982) Production of the copepod *Eurytemora affinis* in the Bristol Channel. Mar Ecol Progr Ser 7:21–31

Cailleaud K, Maillet G, Budzinski H, Souissi S, Forget-Leray J (2007) Effects of salinity and temperature on the expression of enzymatic biomarkers in *Eurytemora affinis* (Calanoida, Copepoda). Comp Biochem Physiol Part A Mol Integr Physiol 147:841–849

Cailleaud K, Forget-Leray J, Peuhiet L et al (2009) Tidal influence on the distribution of hydrophobic organic contaminants in the Seine Estuary and biomarker responses on the copepod *Eurytemora affinis*. Environ Pollut 157:64–71

Castel J (1995) Long-term changes in the population of *Eurytemora affinis* (Copepoda, Calanoida) in the Gironde estuary (1978–1992). Hydrobiologia 311:85–101

Caudill CC, Bucklin A (2004) Molecular phylogeography and evolutionary history of the estuarine copepod, *Acartia tonsa*, on the Northwest Atlantic coast. Hydrobiologia 511:91–102

Chen G, Hare MP (2008) Cryptic ecological diversification of a planktonic estuarine copepod, *Acartia tonsa*. Mol Ecol 17:1451–1468

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657–1659

David V, Sautour B, Chardy P, Leconte M (2005) Long-term changes of the zooplankton variability in a turbid environment: the Gironde estuary (France). Estuar Coast Shelf Sci 64:171–184

David V, Chardy P, Sautour B (2006) Fitting a predator-prey model to zooplankton time-series data in the Gironde estuary (France): Ecological significance of the parameters. Estuar Coast Shelf Sci 67:605–617

David V, Sautour B, Chardy P (2007) The paradox between the long-term decrease of egg mass size of the calanoid copepod *Eurytemora affinis* and its long-term constant abundance in a highly turbid estuary (Gironde estuary, France). J Plankton Res 29:377–389

Dawson AG (1992) Ice age earth: late quaternary geology and climate. Routledge, London

Delhez EJM (1996) Modelling the general circulation on the north-western European continental shelf in the perspective of interdisciplinary environmental studies. Bull Soc Roy Sci Liège 65:51–54

Delhez EJM, Deleersnijder E (2002) The concept of age in marine modelling: 2. Concentration distribution function in the English Channel and the North Sea. J Mar Syst 31:279–297

Devreker D, Souissi S, Seuront L (2004) Development and mortality of the first naupliar stages of *Eurytemora affinis* (Copepoda, Calanoida) under different conditions of salinity and temperature. J Exp Mar Biol Ecol 303:31–46

Devreker D, Souissi S, Forget-Leray J, Leboulenger F (2007) Effects of salinity and temperature on the post-embryonic development of *Eurytemora affinis* (Copepoda; Calanoida) from the Seine estuary: a laboratory study. J Plankton Res 29:117–133

Devreker D, Souissi S, Molinero JC, Nkubito F (2008) Trade-offs of the copepod *Eurytemora affinis* in mega-tidal estuaries: insights from high frequency sampling in the Seine estuary. J Plankton Res 30:1329–1342

Devreker D, Souissi S, Winkler G, Forget-Leray J, Leboulenger F (2009) Effects of salinity, temperature and individual variability on the reproduction of *Eurytemora affinis* (Copepoda; Calanoida) from the Seine estuary: a laboratory study. J Exp Mar Biol Ecol 368:113–123

Dodson JJ, Tremblay S, Colombani F, Carscadden JE, Lecomte F (2007) Trans-Arctic dispersals and the evolution of a circumpolar marine fish species complex, the capelin (*Mallotus villosus*). Mol Ecol 16:5030–5043

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7:214

Dur G, Souissi S, Devreker D, Ginot V, Schmitt FG, Hwang JS (2009) An individual-based model to study the reproduction of egg bearing copepods: application to *Eurytemora affinis* (Copepoda Calanoida) from the Seine estuary, France. Ecol Model 220:1073–1089

Escaravage V, Soetaert K (1995) Secondary production of the brackish copepod communities and their contribution to the carbon fluxes in the Westernscheldt estuary (The Netherlands). Hydrobiologia 311:103–114

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinforma 1:47–50

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech 3:294–299

Gasparini S, Castel J, Irigoien X (1999) Impact of suspended particulate matter on egg production of the estuarine copepod, *Eurytemora affinis*. J Mar Syst 22:195–205

Gelembiuk GW, May GE, Lee CE (2006) Phylogeography and systematics of zebra mussels and related species. Mol Ecol 15:1033–1050

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/

Ger KA, Teh SJ, Goldman CR (2009) Microcystin-LR toxicity on dominant copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi* of the upper San Francisco Estuary. Sci Tot Environ 407:4852–4857

Gorokhova E, Fagerberg T, Hansson S (2004) Predation by herring (*Clupea harengus*) and sprat (*Sprattus sprattus*) on *Cercopagis pengoi* in a western Baltic Sea bay. Ices J Mar Sci 61:959–965

Grant WS, Bowen BW (1998) Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. J Hered 89:415–426

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. System Biol 59:307–321

Gysels ES, Hellemans B, Pampoulie C, Volckaert FAM (2004) Phylogeography of the common goby, *Pomatoschistus microps*, with particular emphasis on the colonization of the Mediterranean and the North Sea. Mol Ecol 13:403–417

Hansson S, Larsson U, Johansson S (1990) Selective predation by herring and mysids, and zooplankton community structure in a Baltic Sea coastal area. J Plankton Res 12:1099–1116

Hirche HJ (1992) Egg production of *Eurytemora affinis*—effect of k-strategy. Estuar Coast Shelf Sci 35:395–407

Hoelzel AR, Green A (1992) Analysis of population-level variation by sequencing PCR-amplified DNA. In: Hoelzel AR (ed) Molecular genetic analysis of populations: a practical approach. Oxford University Press, New York

Irigoien X, Castel J, Sautour B, Heip C (1993) In situ grazing activity of planktonic copepods in the Gironde Estuary. Cah Biol Mar 34:225–237

Jeppesen E, Sondergaard M, Pedersen AR et al (2007) Salinity induced regime shift in shallow brackish lagoons. Ecosystems 10:47–57

Johannesson K, Andre C (2006) Life on the margin: genetic isolation and diversity loss in a peripheral marine ecosystem, the Baltic Sea. Mol Ecol 15:2013–2029

Jolly MT, Jollivet D, Gentil F, Thiebaut E, Viard F (2005) Sharp genetic break between Atlantic and English Channel populations of the polychaete *Pectinaria koreni*, along the North coast of France. Heredity 94:23–32

Jolly MT, Viard F, Gentil F, Thiebaut E, Jollivet D (2006) Comparative phylogeography of two coastal polychaete tubeworms in the Northeast Atlantic supports shared history and vicariant events. Mol Ecol 15:1841–1855

Kimmel DG, Roman MR (2004) Long-term trends in mesozooplankton abundance in Chesapeake Bay, USA: influence of freshwater input. Mar Ecol Progr Ser 267:71–83

Kimmel DG, Miller WD, Roman MR (2006) Regional scale climate forcing of mesozooplankton dynamics in Chesapeake Bay. Estuaries Coasts 29:375–387

Kimmerer W (2005) Long-term changes in apparent uptake of silica in the San Francisco estuary. Limnol Oceanogr 50:793–798

Knowlton N (1993) Sibling species in the sea. Ann Rev Ecol System 24:189–216

Knowlton N (2000) Molecular genetic analyses of species boundaries in the sea. Hydrobiologia 420:73–90

Knowlton N, Weigt LA (1998) New dates and new rates for divergence across the Isthmus of Panama. Proc Roy Soc Lond Ser B Biol Sci 265:2257–2263

Koljonen ML, Jansson H, Paaver T, Vasin O, Koskiniemi J (1999) Phylogeographic lineages and differentiation pattern of Atlantic salmon (*Salmo salar*) in the Baltic Sea with management implications. Can J Fish Aquat Sci 56:1766–1780

Köpcke B, Kausch H (1996) Distribution and varibility in abundance of *Neomysis integer* and *Mesopodopsis slabberi* in relation to environmental factors in the Elbe Estuary. Arch Hydrobiol Suppl 110:263–282

Kuhner MK (2006) lamarc 2.0: maximum likelihood and Bayesian estimation of population parameters. Bioinformatics 22:768–770

Lecomte F, Dodson JJ, Georges S (2000) Impact de la pêcherie commerciale de Charlevoix sur les populations d'éperlans de l'estuaire moyen du Saint-Laurent, p. 47. Société de la Faune et des Parcs du Québec, Direction régionale de Québec, Québec

Lee CE (1999a) Independent invasions of fresh water: comparison of sodium pumping ability among lineages of the copepod *Eurytemora affinis*. Am Zool 39:93

Lee CE (1999b) Rapid and repeated invasions of fresh water by the copepod *Eurytemora affinis*. Evolution 53:1423–1434

Lee CE (2000) Global phylogeography of a cryptic copepod species complex and reproductive isolation between genetically proximate "populations". Evolution 54:2014–2027

Lee CE (2002) Evolutionary genetics of invasive species. Trends Ecol Evol 17:386–391

Lee CE, Frost BW (2002) Morphological stasis in the *Eurytemora affinis* species complex (Copepoda : Temoridae). Hydrobiologia 480:111–128

Lee CE, Gelembiuk GW (2008) Evolutionary origins of invasive populations. Evol Appl 1:427–448

Lee CE, Remfert JL, Gelembiuk GW (2003) Evolution of physiological tolerance and performance during freshwater invasions. Integr Comp Biol 43:439–449

Luttikhuizen PC, Drent J, Baker AJ (2003) Disjunct distribution of highly diverged mitochondrial lineage clade and population subdivision in a marine bivalve with pelagic larval dispersal. Mol Ecol 12:2215–2229

Mallin MA, Paerl HW (1994) Planktonic trophic transfer in an estuary—seasonal, diel, and community structure effects. Ecology 75:2168–2184

Mialet B, Azemar F, Maris T, Sossou C, Ruiz P, Lionard M, Van Damme S, Lecerf A, Muylaert K, Toumi N, Meire P, Tackx M (2010) Spatial spring distribution of the copepod *Eurytemora affinis* (Copepoda, Calanoida) in a restoring estuary, the Scheldt (Belgium). Estuar Coast Shelf Sci 88:116–124

Michalec F-G, Souissi S, Dur G, Mahjoub M-S, Schmitt FG, Hwang J-S (2010) Differences in behavioral responses of *Eurytemora affinis* (Copepoda, Calanoida) reproductive stages to salinity variations. J Plankton Res 32:805–813

Mouny P, Dauvin JC (2002) Environmental control of mesozooplankton community structure in the Seine estuary (English Channel). Oceanol Acta 25:13–22

Mouny P, Dauvin JC, Bessineton C, Elkaim B, Simon S (1998) Biological components from the Seine estuary: first results. Hydrobiologia 374:333–347

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Nikula R, Strelkov P, Vainola R (2007) Diversity and trans-Arctic invasion history of mitochondrial lineages in the North Atlantic *Macoma balthica* complex (Bivalvia : Tellinidae). Evolution 61:928–941

North EW, Houde ED (2003) Linking ETM physics, zooplankton prey, and fish early-life histories to striped bass *Morone saxatilis* and white perch *M. americana* recruitment. Mar Ecol Progr Ser 260:219–236

Osinov A, Bernatchez L (1996) Atlantic and Danubean phylogenetic groupings of brown trout (*Salmo trutta* L.) complex: genetic divergence, evolution, and conservation. J Ichthyol 36:762–786

Peijnenburg K, Breeuwer JAJ, Pierrot-Bults AC, Menken SBJ (2004) Phylogeography of the planktonic chaetognath *Sagitta setosa* reveals isolation in European seas. Evolution 58:1472–1487

Peitsch A (1993) Difficulties in estimating mortality rates of *Eurytemora affinis* in the brackish water region of the Elbe estuary. Cah Biol Mar 34:215–224

Peitsch A, Koepcke B, Bernat N (2000) Long-term investigation of the distribution of *Eurytemora affinis* (Calanoida; Copepoda) in the Elbe Estuary. Limnologica 30:175–182

Posada D (2008) jModelTest: phylogenetic model averaging. Mol Biol Evol 25:1253–1256

Provan J, Wattier RA, Maggs CA (2005) Phylogeographic analysis of the red seaweed *Palmaria palmata* reveals a Pleistocene marine glacial refugium in the English Channel. Mol Ecol 14:793–803

Raymond M, Rousset F (1995) GENEPOP Version 1.2: population genetic software for exact test and ecumenism. J Hered 86:248–249

Remerie T, Vierstraete A, Weekers PHH, Vanfleteren JR, Vanreusel A (2009) Phylogeography of an estuarine mysid, *Neomysis integer* (Crustacea, Mysida), along the north-east Atlantic coasts. J Biogeogr 36:39–54

Roman J, Palumbi SR (2004) A global invader at home: population structure of the green crab, *Carcinus maenas*, in Europe. Mol Ecol 13:2891–2898

Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497

Sautour B, Castel J (1995) Comparative spring distribution of zooplankton in three macrotidal European estuaries. Hydrobiologia 311:1–3

Schluter D (2001) Ecology and the origin of species. Trends Ecol Evol 16:372–380

Schneider S, Excoffier L (1999) Estimation of demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. Genetics 152:1079–1089

Simenstad C, Small L, McIntire C (1990) Consumption processes and food web structure in the Columbia River estuary. Progr Oceanogr 25:1–4

Sirois P, Dodson J (2000) Influence of turbidity, food density and parasites on the ingestion and growth of larval rainbow smelt *Osmerus mordax* in an estuarine turbidity maximum. Mar Ecol Progr Ser 193:167–179

Skelly DA, Chau K, Chang YM, Winkler G, Lee CE (in revision) Limits to range expansions into freshwater habitats: Physiological contrasts between sympatric invasive and noninvasive populations of the copepod *Eurytemora affinis*

Sotka EE, Wares JP, Barth JA, Grosberg RK, Palumbi SR (2004) Strong genetic clines and geographical variation in gene flow in the rocky intertidal barnacle *Balanus glandula*. Mol Ecol 13:2143–2156

Souissi A, Souissi S, Devreker D, Hwang JS (2010) Occurence of intersexuality in a laboratory culture of the copepod *Eurytemora affinis* from the Seine estuary (France). Mar Biol 157:851–861

Tackx M, Irigoien X, Daro N et al (1995) Copepod feeding in the Western-Scheldt and the Gironde. Hydrobiologia 311:1–3

Tackx MLM, Herman PJM, Gasparini S, Irigoien X, Billiones R, Daro MH (2003) Selective feeding of *Eurytemora affinis* (Copepoda, Calanoida) in temperate estuaries: model and field observations. Estuar Coast Shelf Sci 56:305–311

Tackx MLM, De Pauw N, Van Mieghem R et al (2004) Zooplankton in the Scheldt estuary, Belgium and the Netherlands. Spatial and temporal patterns. J Plankton Res 26:133–141

Verspoor E, McCarthy EM, Knox D (1999) The phylogeography of European Atlantic salmon (*Salmo salar* L.) based on RFLP analysis of the ND1/16sRNA region of the mtDNA. Biol J Linn Soc 68:129–146

Vuorinen I, Ranta E (1987) Dynamics of marine meso-zooplankton at Seili, northern Baltic sea, in 1967–1975. Ophelia 28:31–48

Winkler G, Dodson JJ, Bertrand N, Thivierge D, Vincent WF (2003) Trophic coupling across the St. Lawrence River estuarine transition zone. Mar Ecol Progr Ser 251:59–73

Winkler G, Martineau C, Dodson JJ, Vincent WF, Johnson LE (2007) Trophic dynamics of two sympatric mysid species in an estuarine transition zone. Mar Ecol Progr Ser 332:171–187

Winkler G, Dodson JJ, Lee CE (2008) Heterogeneity within the native range: population genetic analyses of sympatric invasive and noninvasive clades of the freshwater invading copepod *Eurytemora affinis*. Mol Ecol 17:415–430

Wright S (1943) Isolation by distance. Genetics 28:114–139