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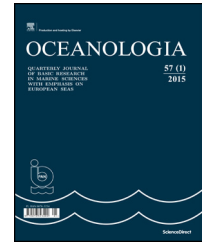
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ORIGINAL RESEARCH ARTICLE

Life in sympatry: coexistence of native *Eurytemora affinis* and invasive *Eurytemora carolleeae* in the Gulf of Finland (Baltic Sea)

Natalia Sukhikh ^{a,*}, Anissa Souissi ^b, Sami Souissi ^b, Anne-Catherine Holl ^c, Nikolaos V. Schizas ^d, Victor Alekseev ^a

^a Zoological Institute, Russian Academy of Science, Saint Petersburg, Russia

^b Lille University, CNRS, ULCO, LOG, Wimereux, France

^c Lille University of Science and Technology, CNRS, Villeneuve d'Ascq Cedex, France

^d Department of Marine Sciences, University of Puerto Rico, Mayagüez, Puerto Rico

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Summary The invasion of exotic species into native ecosystems is becoming a crucial issue in global biology. Over the last ten years, at least 45 invasions of aquatic species have been reported in the eastern part of the Gulf of Finland; the majority of them were introduced through ballast water. Recently, invasion of the estuarine calanoid copepod *Eurytemora carolleeae* (Temoridae), originating from North America, has been reported in several European estuaries and particularly in the Gulf of Finland. This species is morphologically very similar to the native *Eurytemora affinis*, but it is easily discriminated by molecular markers. In this study, we monitored the distribution area of the invasive copepod species in European waters, as well as the population structure of (native) *E. affinis* and (invasive) *E. carolleeae*, from 2006 to 2018 in the Gulf of Finland. The population density of *E. affinis* was significantly higher, compared to *E. carolleeae*, during most of the study period. The only exception was Neva Bay in 2010, wherein the invasive species dominated possibly due to high temperatures and differences in the levels of fish predation. The reproductive performance of *E. carolleeae* was also higher than that of *E. affinis*. These results show different population dynamics between the two species. It was revealed that invasive *E. carolleeae* develops in some of the very

* Corresponding author. Universitetskaya emb., 1, St.-Petersburg, 199034, Russia. Tel.: +7 812 328 13 11; fax: +7 812 328 02 21.

E-mail addresses: Sukikh1@mail.ru (N. Sukhikh), anissa.ben-radhia@univ-lille1.fr (A. Souissi), Sami.Souissi@univ-lille1.fr (S. Souissi), Anne-Catherine.Holl@univ-lille1.fr (A.-C. Holl), nschizas@gmail.com (N.V. Schizas), alekseev@zin.ru (V. Alekseev).

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same habitats as native *E. affinis*, thereby potentially becoming a significant component of the zooplankton in the studied area. Moreover, invader has the potential to displace native *E. affinis*. © 2018 Institute of Oceanology of the Polish Academy of Sciences. Production and hosting by Elsevier Sp. z o.o. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

An estimated 140–171 aquatic invasions have been reported in the Baltic Sea during the last two centuries (www.stateofthebalticsea.helcom.fi; www.corpi.ku.lt). The geologically young ecosystem of the Baltic Sea, in combination with salinity gradients, has resulted in many new ecological niches. These factors have been hypothesized to provide the key necessary conditions for the spread of new invasive species and their naturalization in the area (Leppäkoski et al., 2002a,b). Previous and ongoing intensive maritime traffic, however, results in the displacement of million of tons of ballast water from site to site (www.helcom.fi/Lists/Publications). These transfers are impacting the Baltic's flora and fauna, and they may be a major factor in the multiple invasions recorded in the region during the last century (Ojaveer and Kotta, 2015).

The Gulf of Finland is one of the most dense maritime traffic areas in the Baltic Sea; it includes several active international shipping routes and large ports (Pollumäe and Valjätaga, 2004). Consequently, more than 40 alien species have been found during the last ten years in only the eastern part of the Gulf of Finland, most of which were invertebrates (Lehtiniemi et al., 2016). Most of these species were introduced through ballast water (Berezina et al., 2011; Katajisto et al., 2013; Lehtiniemi et al., 2016; Panov et al., 2003; www.helcom.fi/Lists/Publications; www.stateofthebalticsea.helcom.fi), including: *Cercopagis pengoi* (Ostroumov, 1891) (Crustacea: Cladocera), *Mytilopsis leucophaea* (Conrad, 1831), (Mollusca: Bivalvia), *Palaemon serratus* (Pennant, 1777) (Crustacea: Decapoda), *Eriocheir sinensis* (Milne-Edwards, 1853) (Crustacea: Decapoda), *Palaemon elegans* (Martin Rathke, 1837) (Crustacea: Decapoda), *Neogobius melanostomus* (Pallas, 1814) (Fish).

The invasive species list includes several copepod species, among which there is a report of a subtle invasion in 2007 of the estuarine North American copepod *Eurytemora carolleeae* Alekseev and Souissi, 2011 in the eastern part of the Gulf of Finland (Alekseev et al., 2009; Sukhikh et al., 2013). Later, this species was also detected in the Gulf of Riga and in the Amsterdam channels (Sukhikh et al., 2013), as well as in additional locations (Wasmund et al., 2013), namely: Kiel Bight, Mecklenburg Bight, Arkona Sea, Bornholm Sea, and in Eastern Gotland Sea.

It is interesting that, according to pictures and descriptions of *Eurytemora* species in English waters (Gurney, 1931), *E. carolleeae* already inhabited this area of water at the beginning of 20th century. Possibly, it was an invasion through ship ballast water, similar to the case of *Eurytemora americana* Williams, 1906, which was originally discovered in 1933 in the same area (Sukhikh et al., 2016a). Recent genetic studies of *Eurytemora* populations have not revealed the

presence of *E. carolleeae* in English waters (Lee, 2000; Sukhikh et al., 2016b; Winkler et al., 2011). However, genetic studies targeted few crustacean specimens, and it is likely that they missed *E. carolleeae*. In addition, early morphological studies may have misidentified this species as *Eurytemora affinis* (Poppe, 1880).

The *E. affinis* species complex is a group of species inhabiting the Holarctic (Sukhikh et al., 2013). The species complex is currently represented by three species: *E. affinis* with Palearctic distribution; North American *E. carolleeae*; and Asian *Eurytemora caspica* Sukhikh and Alekseev, 2013. All of these species inhabit estuaries and freshwater reservoirs where they are the dominant pelagic species and constitute the main food source for animals at higher trophic levels (e.g. Devreker et al., 2008, 2010; Dur et al., 2009; Lee, 2000).

The *E. affinis* species complex has been well studied (Devreker et al., 2008, 2010; Dur et al., 2009; Hirche, 1992; Knatz, 1978; Lajus et al., 2015; Lloyd et al., 2013). Experimental studies comparing the reproductive traits (development time, clutch size and longevity) of *E. affinis* (from the Seine estuary, France) and *E. carolleeae* (from St. Lawrence salt marshes, Canada; and Chesapeake Bay, USA) have confirmed the higher fitness of the North American population (Beyrend-Dur et al., 2009; Devreker et al., 2012) compared to the European one (Devreker et al., 2009, 2012). In addition, field measurements have suggested that, in both populations, egg production decreased when temperatures rose above 18°C (Lloyd et al., 2013; Pierson et al., 2016). This corroborates results from laboratory experiments (Devreker et al., 2012).

In this paper, we investigated the coexistence of these two *Eurytemora* species in the Gulf of Finland. The presence of both species in the Baltic Sea is the result of secondary contact. Historically, only *E. affinis* inhabited the studied region, whereas the native habitat of *E. carolleeae* was the North American Atlantic coast. *E. affinis* and *E. carolleeae* diverged approximately 5.1 million years ago, dating to the time of the Miocene/Pliocene boundary (Lee, 2000). They have a mean sequence divergence of 15% in part of the mitochondrial cytochrome c oxidase I (COI) gene.

The detection of these related species in Baltic waters is likely the result of recent invasion by *E. carolleeae* via the ballast water of ships (Alekseev et al., 2009; Sukhikh et al., 2013). The most likely source of this invasion is the Atlantic coast of the United States (Alekseev et al., 2009; Sukhikh et al., 2013).

E. carolleeae and *E. affinis* are very similar morphologically and it appears as though they occupy, more or less, the same ecological niches. Like other invasive species, however, displacements can be detrimental to ecosystem stability. At the beginning of the invasion, sibling species cause unidentifiable changes in biological diversity, followed by rearran-

gement of the aquatic communities (Gelembiuk et al., 2006). In fact, such species can exhibit distinct habitat preferences defined by depth, salinity, or exposure. Successional differences between sibling species, reflecting temporal partitioning of resources in response to seasonal change or disturbance, have also been documented (Knowlton, 1993). This may be the result of different physiologies. Moreover, hybridization experiments, between these North American and European species, have shown reproductive incompatibility among them (S. Souissi, unpublished). For example, six *Tubifex tubifex* (oligochaetes) lineages living sympatrically differed in their tolerance to cadmium (Sturm-bauer et al., 1999) and in their resistance to infection by *Myxobolus cerebralis* (Beauchamp et al., 2001).

Previous data on the region's zooplankton community is rather limited and has been published mainly in Russian. The zooplankton community of the eastern part of the Gulf of Finland is represented mainly by freshwater species. The bulk of zooplankton, by mass, usually consists of Cladocera (Pollumae and Kotta, 2007; Uitto et al., 1999), while copepods dominate numerically (Ogorodnikova and Volkhonskaya, 2006; Ostov, 1971; Ryabova and Pogrebov, 1991). In general, zooplankton in the Russian Gulf of Finland are distributed irregularly, and the areas of highest zooplankton abundance are located in the southern and eastern regions (Ostov, 1971). Depending on the year of the study, zooplankton biomasses have varied from 140 to 1000 mg m⁻³ (Antsulevich et al., 1995; Basova, 1983; Lavrentieva and Finogenova, 1999). As a result, Luga Bay and Neva Bay (both situated in the south-eastern Gulf of Finland) serve as the main areas for fish feeding and breeding (Golubkov, 2009). The main consumers of zooplankton in the Gulf of Finland are Baltic herring. Since the mid-1990s, however, Sprat (*Sprattus sprattus* (Linnaeus)), which is recovering from overfishing in the 1970s, has also begun to play a significant role as a zooplankton predator (Alimov et al., 2004).

Zooplankton aggregations are represented by both brackish and freshwater species in Luga Bay. Studies (Lavrentieva and Finogenova, 1999; Ogorodnikova and Volkhonskaya, 2006; Ryabova and Pogrebov, 1991; Sergeev et al., 1971) have shown that different species have dominated aggregations in different years: *Keratella quadrata* (Muller); *Keratella cochlearis* (Gosse); *Synchaeta baltica* Ehrenberg; *Synchaeta oblonga* Ehrenberg; *Bosmina obtusirostris* Sars; *Acartia clausi* Giesbrecht; *Daphnia cristata* Sars; *Daphnia cucullata* Sars; and *Eurytemora* spp. Generally speaking, these dominant species occur in others parts of the Gulf of Finland as well (Pollumae and Kotta, 2007; Uitto et al., 1999). *Eurytemora* spp. are invariably present in these species lists (Uitto et al., 1999). It is one of the dominant members in the Gulf of Finland (<https://www.st.nmfs.noaa.gov/copepod/time-series/fi-30103/>), and they reach up to 50% of all zooplankton biomass in the study area (Sukhikh, unpublished data). *Eurytemora* spp. consist up to 45% of all stomachs of cyprinid species and are abundantly found in the stomachs of sticklebacks (Demchuk et al., 2017).

Little is known about local *Eurytemora* spp. populations and even less is known about the new invasive species, *E. carolleeae*, in the Baltic Sea. This is the first study of the population structure and reproductive traits of two related species living together in the Baltic Sea: native *E. affinis* and invasive *E. carolleeae* (of western Atlantic origin). We have

used genetic markers to examine the potential for hybridization between these two closely related species (*E. affinis* and *E. carolleeae*) which are living in sympatry.

As the invasion of *E. carolleeae* seems to be a recent and rapid process, we hypothesize here that it has the potential to displace native *E. affinis* in the Gulf of Finland ecosystem and possibly in the entire Baltic Sea. Such an outcome is especially possible under certain conditions, such as force majeure events that cause profound environmental changes. We seek to clarify spatial and temporal differences in their distributions that are related to, or dependent on, environmental parameters in order to gain a better understanding of the potential for native *E. affinis* to be displaced by invasive *E. carolleeae*.

2. Material and methods

2.1. Sampling

In order to reveal the distribution of invasive *E. carolleeae* in European waters, copepods were collected from 11 European sites between 2004 and 2017: channels in Amsterdam; the Elbe, Seine, Schelde, Loire, and Gironde estuaries; the Lake of the Bois de Boulogne (Paris); Umeå Seaport (Sweden); Vistula Lagoon; the Gulfs of Riga and Finland (the Baltic Sea); and the Northern Dvina River (Fig. 1, Table 1). Three sites were analyzed in the Gulf of Finland: the Gulf of Vyborg, Neva Bay and Luga Bay.

Monitoring of invasive species has been carried out periodically since 2004 in Neva Bay and since 2006 in Luga Bay. To estimate the relative percentage of the two *Eurytemora* species in Neva and Luga Bays, samplings were performed once per year, usually during August when high population densities are observed; Luga Bay sampling in 2010 was an exception and occurred in September. The total number of studied specimens ranged from 15 to 181 per site (the number of specimens obtained in three replicates, three nets in each).

Seasonal monitoring of adult population densities (*E. affinis* and *E. carolleeae*) in Luga Bay (Gulf of Finland) was carried out from 10.06 to 27.08 in 2006, from 19.04 to 17.09 in 2008 and from 16.06 to 27.09 in 2015. Sampling was performed at the mouth of Luga River, every ten days in 2006 and in 2008, and every twenty days in 2015. Water salinity and temperature at the mouth of the Luga River were measured using a COM-100 waterproof combination meter (HM Digital, USA).

Samples were collected with 100 µm or 230 µm mesh plankton nets by vertical tows from depth to surface in three replicates and preserved in 96% ethanol or in 4% formalin solution (sampling information is given in Table 1).

2.2. Species identification

Identification of adult *E. affinis* and *E. carolleeae* copepods was accomplished by following published taxonomical keys (Alekseev and Souissi, 2011; Sukhikh and Alekseev, 2013). Morphological analysis of adult copepods was performed under an SZX2 dissection microscope (Olympus) with a 5 µm resolution ocular micrometer. *E. carolleeae* type material from the Russian Academy of Sciences Zoological Insti-

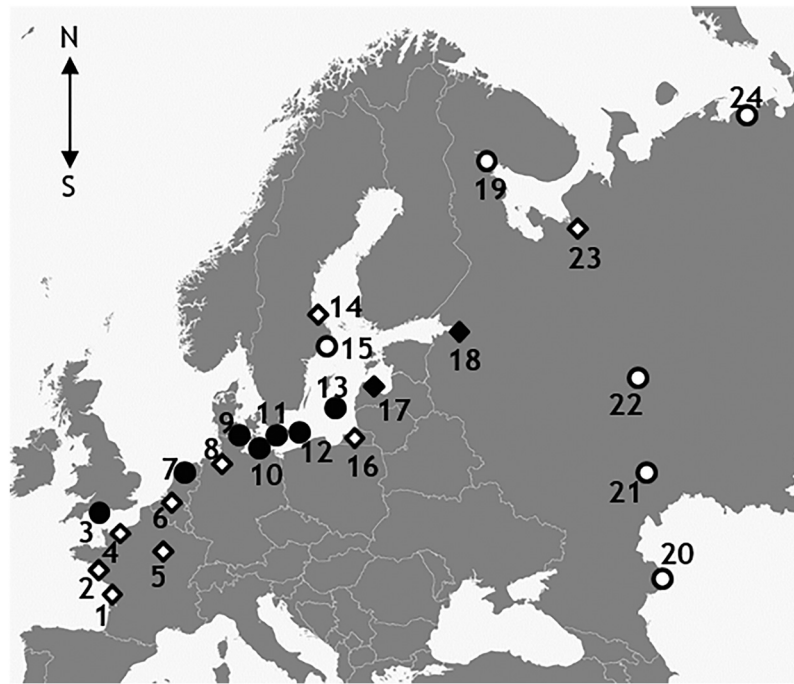


Figure 1 Sampling locations analyzed by the authors (rhombi) and literature data on the distribution of invasive *Eurytemora carolleeae* in Europe (circles). Black figures represent the presence of invasive *Eurytemora carolleeae* in studied area. 1 – Gironde Estuary; 2 – Loire Estuary; 3 – Chelson Meadow, Plymouth, British waters (Gurney, 1931); 4 – Seine Estuary; 5 – Lake in Bois de Boulogne (Paris); 6 – Scheldt Estuary; 7 – Amsterdam channels (Sukhikh et al., 2013); 8 – Elbe Estuary; 9–13: 9 – Kiel Bight, 10 – Mecklenburg Bight, 11 – Arkona Sea, 12 – Bornholm Sea and 13 – Eastern Gotland Sea (Wasmund et al., 2013); 14 – Gulf of Bothnia, Umeå; 15 – Stockholm (Gorokhova et al., 2013); 16 – Vistula lagoon; 17 – Gulf of Riga; 18 – Gulf of Finland; 19 – the White Sea (Sukhikh et al., 2016a,b and pers. comm. of Polyakova N.V.); 20–22 – the Caspian Sea and the drainage basin of Volga River (Lazareva et al., 2018; Sukhikh et al., 2018); 23 – Northern Dvina River; 24 – Pechora Estuary (Cherevichko, 2017; Fefilova, 2015).

tute collection was used for reference in this study (type collection #55052-55054). Identification of specimens from the Schelde River, Seine estuary, Gulf of Riga, Gulf of Finland, Vistula Lagoon, Loire estuary, Lake in the Bois de Boulogne, and Northern Dvina River was also supported by DNA sequencing of a portion of the mitochondrial cytochrome oxidase subunit 1 gene (COI, see below). In studying and estimating population densities in Luga Bay, only adult stages of *E. affinis* and *E. carolleeae* were analyzed as there are no clear morphological features distinguishing the juvenile stages (nauplii and copepodites) of these closely related species. Moreover, an additional *Eurytemora* species, *Eurytemora lacustris* (Poppe, 1887), was present in the zooplankton community of the sampled area. The juvenile stages of *E. lacustris* are also indistinguishable from those of *E. affinis* and *E. carolleeae*. As a result, it was impossible for us to separately distinguish or estimate nauplii and copepodites densities for these three *Eurytemora* species.

2.3. Morphological and reproductive traits measurements

For measurement of reproductive parameters, 20 *E. carolleeae* females and 23 *E. affinis* females were randomly selected from the same sample collected in July 2015 in Luga Bay (water temperature 17.3°C). The number of eggs per clutch and the egg diameters of 5–10 eggs from each

clutch were calculated for each female of both species. In addition, the lengths and widths of the prosome and the egg sac were measured under a dissection microscope (as above).

2.4. Statistical analysis

Differences between the species, in terms of reproductive parameters as well as in the lengths and widths of prosomes and egg sacs, were quantified using the nonparametric Kruskal–Wallis test as implemented in the Statistica 7 software package. The relationships between female prosome length and clutch size, in both studied species, were shown by linear regression analysis (Statistica 7). The significance limit was set at $p < 0.05$.

2.5. Material used for genetic analysis

The nuclear ribosomal 18S gene, ITS regions (including 5.8S), and one mitochondrial (COI) gene were analyzed in the present study. Specimens used for genetic analysis were obtained from: Neva Bay (Russia), July 2014 (*E. affinis*, *E. carolleeae*); the Loire and Seine Rivers (France), April 2011 (*E. affinis*); the Saint-Lawrence estuary (France), September 2014 (*E. carolleeae*); and a laboratory collection (*E. carolleeae*), originally from Chesapeake Bay (U.S.A.). A total of 18 *E. affinis* individuals and 23 *E. carolleeae* individuals were analyzed with genetic tools.

Table 1 Sampling locations of populations of *Eurytemora affinis* and *Eurytemora carolleeae*.

Sampling locations	Sampling date	Sample size for genetic analysis ^a	Sample size for morphological analysis ^b	Latitude	Longitude
Elbe estuary	March 2006		50	53°53'24N	09°08'44E
Scheldt River	April 2011	7	15	51°13'42N	04°23'86E
Antwerp	April 2011	1	15	51°N	04°E
Duaene					
Seine estuary	April 2011	37	10	49°28'33N	00°27'54W
	May 2008		8	49°28'33N	00°27'54W
	July 2008		9	49° N	00°W
Gulf of Riga	Aug. 2008	14	29	57° 04'44N	23°04'44E
City Port					
Gulf of Finland:	Sep. 2007	35	10	60°23'39N	28°26'74E
Gulf of Vyborg	Aug. 2009	30	110	59°32'36N	29°28'17E
Neva R. estuary	Aug. 2010	30	227	59°24'13N	28°11'06E
Luga R. estuary	Aug. 2004, 2007, 2010–12, 2014–15				
	Aug. 2006–09, 2011, 2015				
	Sep. 2010				
Vistula Lagoon	Oct. 2007	5	30	54°65'02N	20°23'37E
	Jun. 2015		30		
Northern Dvina River	Aug. 2015	5	10	64°33'00N	40°32'00E
Gulf of Bothnia, Umeå	May 2010		10	63°49'30"N	20°15'50"E
Loire estuary	April 2011	52	10	47°17'23N	02°01'52W
St.1	July 2009		9		
St.2					
Gironde estuary	May 2005		10	45°04'10N	00°38'30W
St.1	July 2009		4		
St.3					
Lake in the Bois de Boulogne (Paris)	July 2010	3		48°51'48N	2°15'07E
Saint-Lawrence Estuary	Sep. 2014	4		48°1'1N	69°20'8W
Chesapeake Bay	Feb. 2013	3		38°36'15N	76°4'54W

^a Number of individuals sequenced per location.^b Number of individuals analyzed with morphological method.

2.6. DNA extraction, amplification, and sequencing

Genomic DNA was extracted from single adult copepods preserved in 96% ethanol using a standard method described by Aljanabi and Martinez (1997) or using a cell lysis buffer with Proteinase-K protocol modified from Hoelzel and Green (1992) and Lee (2000). Polymerase chain reaction (PCR), in order to achieve cytochrome oxidase subunit 1 (COI) amplification, utilized both universal (COIH, COIL) and specific (EuF1, EuR2) primers. Their sequences are: COIH 2198 (5'-TAAACTTCAGGGTGACCAAAAATCA-3'); COIL 1490 (5'-GGTCAACAAATCATAAAGATATTGG-3'; Folmer et al., 1994); EuF1 (5'-CGTATGGAGTTGGGACAAGC-3'); and EuR2 (5'-CAAATAAGTGTGGTATAAAATTGGA-3'; Winkler et al., 2011). Two thermocycling programs, modified from Lee (2000), were used for PCR amplification. The first was 5 cycles of 90°C (30 s), 45°C (60 s), 72°C (90 s); followed by 27 cycles of 90°C (30 s), 55°C (45 s), 72°C (60 s); and ending with 5 min at 72°C. The second program featured an initial denaturation at 95°C for 30 s; followed by 5 cycles of 90°C (30 s), 55°C (60 s), 72°C (90 s); followed by 27 cycles of 90°C (30 s), 55°C

(45 s), 72°C (60 s); and ending with 5 min at 72°C. These conditions and methods were used in our previous work (Sukhikh et al., 2016a,b).

Complete 18S rDNAs were amplified using the primer pair 18A1 mod (5'-CTGGTTGATCCTGCCAGTCATATGC-3') and 1800 mod (5'-GATCCTTCCGAGGTTCCACTACG-3') (Raupach et al., 2009). The ITS-4 and ITS-5 universal nITS (nuclear ribosomal DNA internal transcribed spacer) primers (White et al., 1990) were used for amplification of the ITS1-5.8S rRNA-ITS2 region. PCR conditions for both sets of primers (18S rRNA and nITS) were: initial denaturation at 95°C for 30 s; followed by 38 cycles of 95°C (30 s), annealing (50°C for nITS or 55°C for 18S rRNA) for 30 s, 72°C (70 s); and a final extension at 72°C for 7 min.

Amplified products were purified with a QIAquick PCR purification kit (Qiagen, Valencia, CA, USA) and sequenced using an ABI 3100 or 3130 automated sequencer (Applied Biosystems Inc., Foster City, CA, USA). Both DNA strands were sequenced to confirm the accuracy of each sample sequence.

Sequences were aligned using the CLUSTAL W algorithm (Thompson et al., 1994) implemented in BIOEDIT v.7.2 (Hall, 1999) with manual editing of ambiguous sites. The number of

polymorphic sites was estimated using DNASP v6 (Librado and Rozas, 2009). The level of nucleotide differences between the species was calculated using the Tamura-Nei 93 model with the MEGA 6.06 software package (Tamura et al., 2013).

3. Results

3.1. Distribution of invasive species in European waters

Apart from the Gulf of Finland, the presence of invasive American *Eurytemora* species was monitored at 11 sampling locations (Table 1) over the last 12 years. As a result, *E. carolleeae* was detected in Riga Bay and in Amsterdam channels. The density of American *Eurytemora* in Riga Bay did not exceed 2% of total density (both *Eurytemora* species). In contrast, *E. carolleeae* was more prevalent in Amsterdam channels with a total of about 30% of the combined *Eurytemora* density. *E. carolleeae* was absent from all samples from the Schelde, Seine, Loire, and Gironde estuaries, and also absent from the Bois de Boulogne (Paris), Vistula Lagoon, the Gulf of Bothnia (the Baltic Sea), and Northern Dvina River.

3.2. Coexistence of native and invasive *Eurytemora* species in the Gulf of Finland

During the entire study period, *E. affinis* numerically dominated the *Eurytemora* species assemblage in the Gulf of Finland (Fig. 3a, b). *Eurytemora carolleeae* accounted for 2–30% in Luga Bay and from 0% to 100% in Neva Bay. During the whole study period, *E. carolleeae* occurred in fewer numbers than *E. affinis* in Neva and Luga Bay regions in the Gulf of Finland. The maximum *E. carolleeae* density percentages were observed in 2010 and 2015 (Fig. 3a). At the same time, the densities of *E. carolleeae* adult females during the unusual temperature conditions in 2010 and 2015, were similar to those seen during the thermally normal year 2011, in which *E. affinis* was prevalent (631 ± 259 ind m^{-3}). Indeed, the density of *E. carolleeae* adult females in mid September 2010 in Neva Bay was 24 ± 11 ind m^{-3} . In July 2011, the density of *E. carolleeae* adult females was about 16 ± 10 ind m^{-3} . In mid August 2015, the density of *E. carolleeae* adult females was 24 ± 8 ind m^{-3} . *E. affinis* adult females densities in 2010 and 2015 were low: 108 ± 51 ind m^{-3} and 1 ± 1 ind m^{-3} , respectively.

3.3. Salinity and temperature conditions in Luga Bay, Gulf of Finland

Water salinity in the studied area at the mouth of the Luga River changed from 0.67 to 2.31 psu during the monitoring period. Temperatures during the summers of 2006, 2008, 2015, and 2017 ranged from a minimum of 12.8°C (in June 2015) to a maximum of 23.2°C (in July 2006) (Fig. 2). In this region of the Gulf of Finland, mean water temperature in July is usually between 18 and 20°C (<http://weatherarchive.ru>). During 2010, 2015, and 2017, however, water temperatures were unexpectedly different. In 2010, for example, 23.5°C was recorded. Yet, the summers of 2015 and 2017, by contrast, were rather cool and mean July temperatures were 17.1°C and 15.8°C, respectively (<http://weatherarchive.ru>).

3.4. Density changes in adult *E. affinis* and *E. carolleeae* populations

The average density of the zooplankton community (represented mainly by Rotifera, Cladocera, and Copepoda) was about 10^5 ind m^{-3} in all studied periods. The Order Copepoda dominated the summer zooplankton community ($\sim 50,000$ ind m^{-3}). The predominant zooplankton species were the rotifer *Keratella quadrata*, the calanoid copepod *E. affinis*, and the cladoceran *Bosmina longispina*. *E. carolleeae* was present in all of the Gulf of Finland study locations. Seasonal monitoring of *E. carolleeae* and *E. affinis* in Luga Bay showed that both species exhibited two summer population density peaks (in years 2015 and in 2008) and one strong peak in 2006 (Fig. 4). In 2006, the major peak for both species was observed in the end-of-June to beginning-of-July time frame, yet with an almost five fold higher density for *E. affinis* than for *E. carolleeae*. A minor peak was noted for *E. affinis* at the beginning of August, as well as a slight increase at the end of the month (Fig. 4a).

In 2008, the highest density was observed between mid-June and the beginning of July for *E. affinis*, and a second peak was recorded at the end of August. At the same times, two peaks of density were also observed for *E. carolleeae* but of smaller magnitude (Fig. 4b).

In 2015, the first density peak, for both species, was recorded during mid-June and the beginning of July, and the second one was observed at the beginning of September (Fig. 4c). Neva Bay sampling in summer 2017 (24.07.17) did not detect any *Eurytemora* specimens.

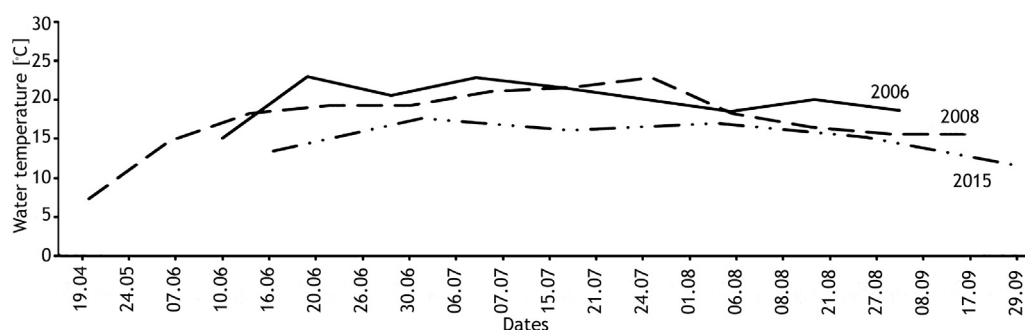


Figure 2 Mean changes in Luga Bay, Gulf of Finland, surface water temperature (°C) during spring, summer, and early autumn in the years: 2006 (full line); 2008 (dotted line); and 2015 (dashed line).

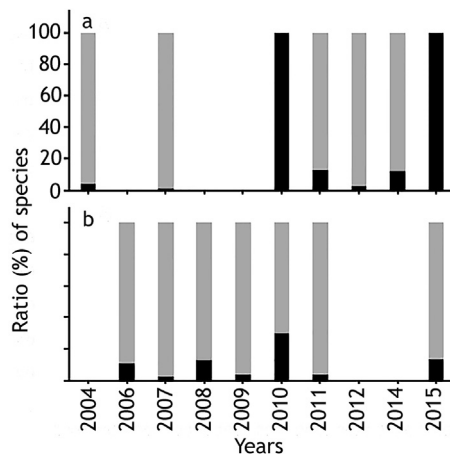


Figure 3 Percent ratio of invasive *Eurytemora carolleeae* (black sector) and native *Eurytemora affinis* (gray sector), during the last 10 years, in Neva (a) and Luga Bays (b), Gulf of Finland.

Overall, *E. affinis* population densities were generally several times higher than those of *E. carolleeae*. The maximal densities were observed for both species in 2006, namely 1295 ind m⁻³ for *E. affinis*, and 201 ind m⁻³ for *E. carolleeae*. The minimal population densities were observed during the summer of 2015 in which no more than 117 ind m⁻³ were observed for *E. affinis* and 24 ind m⁻³ for *E. carolleeae*.

In Fig. 5, the detailed densities of males and females, of both species, are shown. During 2006 and 2008, there were more males than females in both *E. affinis* (Fig. 5a, b) and *E. carolleeae* populations (Fig. 5d, e). However, during 2015 (Fig. 5c, f), the opposite occurred, and the sex ratio was generally in favor of females except for one date (beginning of July, *E. affinis*) (Fig. 5c).

3.5. Reproductive parameters of *Eurytemora* females

The two *Eurytemora* species studied in the Gulf of Finland were significantly different ($p < 0.05$) in their morphological (prosoma length and width, egg sac width) and reproductive

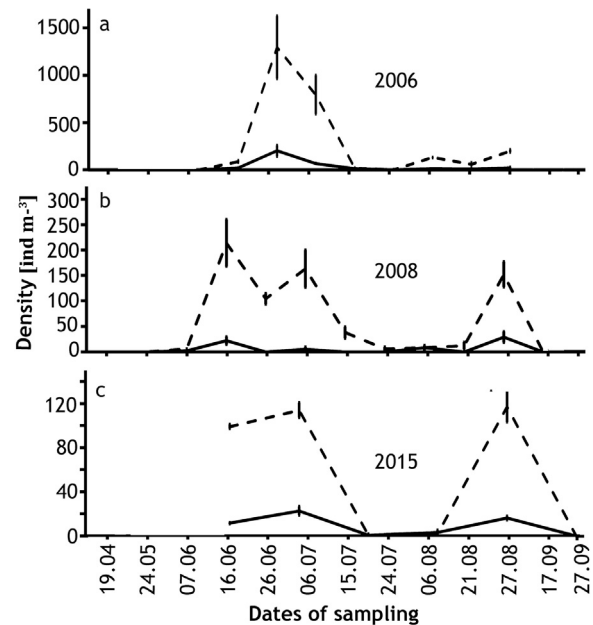


Figure 4 Population density changes in adult *Eurytemora affinis* (dotted line) and *Eurytemora carolleeae* (solid line) during the 2006 (a), 2008 (b), and 2015 (c) summer seasons.

(clutch size) characteristics (Table 2). The respective prosome lengths and widths were $830.2 \pm 7.0 \mu\text{m}$ and $310.1 \pm 6.4 \mu\text{m}$ in *E. carolleeae* and $744.0 \pm 15.5 \mu\text{m}$ and $247.9 \pm 5.3 \mu\text{m}$ in *E. affinis*.

Clutch size was almost two times larger in *E. carolleeae* than in *E. affinis*: 61.7 ± 2.4 and 34.0 ± 1.4 , respectively. Analysis of egg size and egg sac length did not reveal substantial differences between the two species. The difference in egg sac width between the two species ($252.3 \pm 11.5 \mu\text{m}$ in *E. carolleeae* versus $226.9 \pm 5.1 \mu\text{m}$ in *E. affinis*) reflects differences in the shape of the sac, which is more rounded in *E. carolleeae* and more oval in *E. affinis*. At the same time, prosome lengths and clutch sizes in females of both species had a linear relationship (*E. affinis*, $r^2 = 0.59$, $p < 0.05$; *E. carolleeae*, $r^2 = 0.35$, $p < 0.05$).

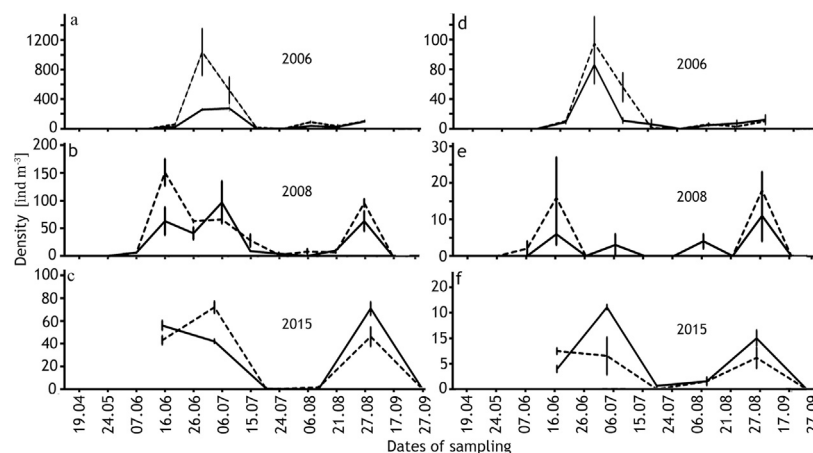


Figure 5 Population density changes in males (dotted lines) and females (solid lines) of *Eurytemora affinis* (a, b, c) and *Eurytemora carolleeae* (d, e, f) during the 2006 (a, d), 2008 (b, e), and 2015 (c, f) summer seasons.

Table 2 Mean values of reproductive parameters in females of *Eurytemora carolleeae* and *Eurytemora affinis* from the Gulf of Finland. Mean \pm standard deviation.

Species/measuring	Egg number	Egg size	Prosoma L (μm)	Prosoma W (μm)	Clutch L (μm)	Clutch W (μm)	Number of studied individuals
<i>E. carolleeae</i>	61.7 \pm 2.4	76.7 \pm 0.8	830.2 \pm 7.0	310.1 \pm 6.4	476.6 \pm 14.7	252.3 \pm 11.5	20
<i>E. affinis</i>	34.0 \pm 1.4	77.6 \pm 0.57	744.0 \pm 15.5	247.9 \pm 5.3	466.8 \pm 11.1	226.9 \pm 5.1	23

3.6. DNA polymorphism data and hybridization between the species

Morphological observation revealed clear differences between the two species, and specimens exhibiting intermediate characters were not typically seen during the study period. Very rare specimens (about 1%) with intermediate features were observed and they were tentatively presumed to be hybrids.

These intermediate phenotypes usually featured intermediate numbers of eggs in the egg sac, intermediate egg sizes, body sizes, or caudal rami shapes. Some had segment-like divisions in setae and genital somite with outgrowth, as in *E. carolleeae*, yet they always differed from the morphology of *E. carolleeae* type specimens by a wing-like outgrowth in the distal part of body, a diagnostic character of *E. affinis*.

Genetic analyses were performed with a complete data set of 86 sequences (75 original and 11 previously published; Sukhikh et al., 2016b). The obtained sequences were compared with existing sequences of *Eurytemora* and deposited in GenBank (accession numbers 18S rRNA KX400968–KX400986; COI KX400987–KX401004, KX401042–KX401328; nITS KX401005–KX401041). The ITS and 18S nuclear genes were analyzed together with the COI gene in order to determine whether hybridization occurs and, if so, whether only F1 individuals are observed or are there subsequent generations of introgression.

Sixteen *E. carolleeae* COI sequences and thirteen *E. affinis* COI sequences were analyzed. Samples sources were: eight *E. carolleeae* and eight *E. affinis* sampled from Neva Bay, four *E. affinis* and four *E. carolleeae* sampled from Luga Bay and 3 *E. carolleeae* from Chesapeake Bay. In both species, a 544 b.p. COI product was amplified. Overall, *E. carolleeae* (COI) sequences contained 38 polymorphic sites and 13 haplotypes; *E. affinis* sequences contained 4 polymorphic sites and 4 haplotypes. The level of pairwise divergence in the COI gene between the two species was 15%, which is indicative of high divergence between these 2 species.

In terms of the 18S rRNA gene (length of 1690 bp), 15 sequences were successfully obtained for *E. carolleeae* and 9 for *E. affinis*. There were no observed nucleotide differences between the species and no polymorphic sites were observed. This suggests that the 18S rRNA gene is more useful in wide phylogenetic analysis of Copepoda, and less useful in work with closely related species.

ITS gene sequences were obtained and analyzed (*E. carolleeae* $n = 17$; *E. affinis* $n = 12$) from samples collected as follows: 14 *E. carolleeae* and 12 *E. affinis* sampled from Neva Bay; and three *E. carolleeae* from Chesapeake Bay. Due to polymorphism, ITS amplicons were 791 bp from *E. carolleeae* and 783 bp from *E. affinis*. Overall, *E. carolleeae* ITS

sequences (794 bp in length, including sites with alignment gaps) contained one polymorphic site, whereas *E. affinis* ITS sequences (795 bp in length, including sites with alignment gaps) had no polymorphic sites. The level of pairwise divergence, in the ITS1-5.8S rRNA-ITS2 region between the two species, was 4.9%. *E. affinis* sequences from the Loire and Seine Rivers were not available.

4. Discussion

4.1. Distribution of invasive *E. carolleeae* in Europe

The presence of the invasive *E. carolleeae* species in European waters has only been reported in specific locations, namely: the Gulf of Finland, the Gulf of Riga, Amsterdam channels (Sukhikh et al., 2013), Kiel Bight; Mecklenburg Bight, the Arkona Sea, the Bornholm Sea, the Eastern Gotland Sea (Wasmund et al., 2013) and perhaps in British waters (Gurney, 1931) (Fig. 1). The presence of *E. carolleeae* in these areas is a noteworthy result since there are many previous reports, from a wide variety of European fresh and marine waters, showing no evidence of *E. carolleeae*.

Accurate identification of different species is necessary due to the fact that they feature evident differences in physiology, and those differences may cause harmful changes in ecosystem function or productivity. Population shifts may eventually have important consequences for biodiversity, biogeography, conservation, or fisheries management (Gelembiuk et al., 2006; Knowlton, 1993; Lee, 2000). Such invasions might have important implications for disease transmission as well. *Eurytemora* are major hosts of many pathogens, including *Vibrio cholerae*, *V. vulnificus*, and *V. parahaemolyticus* (Colwell, 2004; Lee et al., 2007; Piasecki et al., 2004). They are also probable hosts and vectors for plerocercoids that can infect some fish species (Arnold and Yue, 1997).

E. carolleeae was not found in the Elbe, Schelde, Seine, Loire or Gironde estuaries, nor was it detected in the lake near Paris, the Vistula Lagoon, or the Gulf of Bothnia (the Baltic Sea) in 2006–2011 (Table 1). In addition, we have previously shown that it is not present in samples from White Sea rock pools (Sukhikh et al., 2016a,b), in the White Sea itself (pers. comm. of Polyakova N.V.), or in the Northern Dvina River. In addition, species lists from the Pechora Estuary (Cherevichko, 2017; Fefilova, 2015), the Caspian Sea, and the Volga River drainage basin (Lazareva et al., 2018; Sukhikh et al., 2018) did not include *E. carolleeae*. Finally, previous genetic studies of the *E. affinis* species complex in a number of locations (the Swedish coast – Gorokhova et al., 2013;

Winkler et al., 2011; the Elbe, Schelde, Seine, Loire, and Gironde estuaries – Winkler et al., 2011) have not detected *E. affinis*.

4.2. Population dynamics of *E. carolleae* and *E. affinis* in the Gulf of Finland

Seasonal study of *E. carolleae* and *E. affinis* in Luga Bay revealed no substantial differences in their population dynamics. The highest densities were observed during early summer of 2006 for both species (Fig. 4a). These maximal densities may be the result of dredging activity in the Luga Bay study area that occurred during the summer of 2006 (Spiridonov et al., 2011). This event caused resuspension of nutrients in the water column which, in result, induced an increase of phytoplankton (the main food source for *Eurytemora*) density (Spiridonov et al., 2011).

The lowest population densities (both species) were observed during the summer of 2015 (Fig. 4c). The period was characterized by unusually low temperatures, including a minimum of 12.8°C in June. The conditions likely reduced phytoplankton densities, and the effect is a possible reason for the decreased population densities recorded for both *E. affinis* and *E. carolleae*. Nevertheless, no overall correlation was found between population density and water temperature during the summer.

The absence of *Eurytemora* species in the 2017 samples was possibly due to a shift of the resident marine zooplankton community to a riverine one, since summer 2017 was rather rainy and river flow had increased. During the same sampling period, *Eurytemora* species were observed in more or less usual densities in the central part of Neva Bay of the Gulf of Finland (pers. comm. of Litvinchuk L.), an area unaffected by river outflow-associated salinity decreases. In the summer 2018, *E. carolleae* in Luga Bay was also observed in usual density.

Throughout the study period, the population density of *E. affinis* was several times higher than that of *E. carolleae* in Luga Bay (Fig. 4). However, in September 2010 and in August 2015, Neva Bay samples contained only *E. carolleae*; this suggests a major shift in zooplankton populations, featuring a replacement of *E. affinis* by invasive *E. carolleae* (Fig. 3). However, the shift in zooplankton was temporary since samples devoid of *E. affinis* were recorded only those two times. Interestingly, both summers 2010 and 2015 featured unusual temperatures: hot 2010 and cold 2015. Record heat levels were observed in summer 2010, resulting in the warmest summer of the last 100 years in the region (<https://en.wikipedia.org>; https://en.wikipedia.org/wiki/2010_Northern_Hemisphere_summer_heat_waves). Consequently, during that summer, the warmest water temperatures were also recorded. Water temperatures above 15–20°C are known to be unfavorable for *E. affinis* (Devreker et al., 2008, 2010; Dur et al., 2009; Hirche, 1992; Knatz, 1978).

These uncommon temperature conditions probably negatively affected native *E. affinis* populations, yet without reducing population densities of invasive *E. carolleae*. The temperature tolerance of the invasive copepod species is possibly wider as water temperatures in its native Chesapeake Bay range between 5 and 25°C (Kimmel et al., 2006). *E. carolleae* is also characterized by high egg

productivity (Pierson et al., 2016), which could favor its rapid spread in the area. In the eastern part of the Gulf of Finland, yearly mean water temperature varied between 0 (winter) and 18–20°C (summer) (<http://weatherarchive.ru/Sea/Ust-luga/July>). In such an environment, invasive species may be more successful than native ones in fast changing environmental and temperature conditions. Furthermore, *E. carolleae* densities were not observed to depend on summer temperatures in different years.

In 2010 and 2015, analysis of Luga Bay samples did not reveal replacement of *E. affinis* by *E. carolleae*. This indicates that site-specific factors likely play a significant role in the population dynamics of the species. In fact, the population density trends are similar to the other years studied (Fig. 3) even though the proportions of *E. carolleae* were slightly higher during these two years (30% in 2010 and 14% in 2015). The relatively lower 2010 densities of *E. carolleae* in Luga Bay, in comparison to Neva Bay, could be due to the sample collection timing. Plankton samples were not collected during August, as in other years, but later, at the end of September, when water temperature was 18°C. However, during September of 2008 and 2015, water temperatures were not higher than 15°C, and neither *Eurytemora* species was found there. These observations reinforce the possibility that temperature fluctuations may affect the development of both species in the Gulf of Finland.

Luga Bay is known to be one of the most important regions in the Gulf of Finland for fish feeding, breeding, and spawning (Golubkov, 2009). Therefore, it is possible that fish predation on copepods was higher in Luga Bay. Prosome size (length and width) was larger in *E. carolleae* than in *E. affinis* (Table 2); this makes them more susceptible to visual predators. It has been demonstrated that fish eat larger zooplankton first and small ones afterwards (Brooks and Dodson, 1965). In addition, this invasive species has a larger egg sac (Table 2), and it was shown by Mahjoub et al. that fish prefer to feed on ovigerous females. Therefore, with their bigger prosomes and egg sacs, *E. carolleae* may be more visible to fish predators and more susceptible to subsequent predation. Therefore, in addition to temperature, fish predation pressure may be one of the limiting factors in population growth of *E. carolleae* in Luga Bay. Ideally, laboratory experiments would test these hypotheses.

4.3. Reproductive characteristics of the studied species

Study of the reproductive parameters of the two *Eurytemora* species living in sympatry revealed a significant difference in clutch size, but not in egg size. *E. carolleae*, from a summer 2015 sample, was characterized by higher reproductive potential. The invasive *E. carolleae* produced almost double the clutch size (62 eggs female⁻¹) than that of the native *E. affinis* (34 eggs female⁻¹). In Chesapeake Bay (the native habitat of *E. carolleae*), the species is characterized by salinity tolerance, temperature tolerance, and high fecundity (Pierson et al., 2016). Beyrend-Dur et al. (2009) compared two formerly transatlantic *Eurytemora* populations collected from the Seine estuary (France) and from the Saint Lawrence salt marshes (Canada) and showed that American *Eurytemora* had higher fecundity, higher salinity tolerance, shorter

development time, and a longer life span (Beyrend-Dur et al., 2009). These reproductive and physiological differences may enhance the ability of *E. carolleeae* to invade and spread into new areas. This ability may further be enhanced in regions where conditions have become more favorable, over time, due to climate change. A general trend of decreasing salinity in the Baltic Sea is one such example (<https://www.st.nmfs.noaa>).

4.4. Comparison between invasive and native *E. carolleeae* populations

In comparisons between the invasive *E. carolleeae* found in the Gulf of Finland (this study) and the native *E. carolleeae* from Chesapeake Bay (Lloyd et al., 2013), native *E. carolleeae* had a lower clutch size (around 50 eggs female⁻¹) and a smaller prosome length (about 780 µm) at the same water temperatures. Chesapeake Bay is a possible source of invasive copepods (Sukhikh et al., 2013), and it is likely that invasive *E. carolleeae* encountered more favorable environmental conditions in the Gulf of Finland than in its native area. This interpretation is supported by Lajus et al. (2015), who compared levels of fluctuating asymmetry (FA) for populations of *E. carolleeae* from Chesapeake Bay and from the Gulf of Finland. Fluctuating asymmetry represents random deviations from perfect symmetry, and is a proxy for developmental instability (Zakharov, 1989). FA is often used to monitor stress of different origins (Beasley et al., 2013; Graham et al., 2010).

FA was larger for native *E. carolleeae* (Chesapeake Bay), compared to invasive *E. carolleeae* (Gulf of Finland). Interestingly, *E. affinis* from the Gulf of Finland has almost the same FA as the invasive *E. carolleeae* species. This may be the result of generally less stressful environmental conditions in the Gulf of Finland in comparison to Chesapeake Bay. The Gulf features different temperature conditions and fewer salinity changes due to the absence of tides. In fact, the *E. affinis* population from the Seine estuary, with its high tides, had the highest FA of all of the studied populations (Lajus et al., 2015). Those findings fits with our data showing higher FA for native *E. carolleeae* (from Chesapeake Bay) than for invasive *E. carolleeae* (from the Baltic).

4.5. Interaction between sympatric species

Long-term monitoring of the population densities of the two *Eurytemora* species living in sympatry, as well as analysis of their morphological and reproductive parameters, revealed that invasive *E. carolleeae* and native *E. affinis* have remained reproductively isolated from one another. However, rare individuals with intermediate morphological features were observed. Similar cases are known, and hybrids within zooplankton species/lineages are not unheard of in studies of planktonic dispersers, and in particular within Copepoda (Makino and Tanabe, 2009; Parent et al., 2012; Petrusek et al., 2008; Pritchard et al., 2012; Taylor and Hebert, 1993).

Analysis of nuclear ITS genes confirmed that the gene pools of the two studied species have remained largely genetically isolated. More variable (and thus more powerful) molecular markers should be developed to test for the pre-

sence of subtle introgression between these two closely related and sympatric species.

5. Conclusion

We have demonstrated that two *Eurytemora* species (native *E. affinis* and invasive *E. carolleeae*) co-exist in the same area in the Gulf of Finland. Although previously published work has established the presence of these species in the Gulf of Riga and in Amsterdam channels, Wasmund et al. (2013) have demonstrated their expanded co-distribution in Kiel Bight, Mecklenburg Bight, Arkona Sea, Bornholm Sea, and in Eastern Gotland Sea.

The population dynamics of both species are largely parallel. Invasive *E. carolleeae* is usually second to *E. affinis* in terms of density. In addition, the larger body size and different reproductive traits of *E. carolleeae* confer a potential for it to displace native *E. affinis* species. Future work which aims to assess the prospects for further geographic expansion of *E. carolleeae* should take into consideration not only interspecific competition between these two closely related *Eurytemora* species, but also species present at higher and lower trophic levels that interact with *Eurytemora* copepods.

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