Over one decade of invasion: the non-indigenous cladoceran *Evadne anonyx* G.O. Sars, 1897 in a low-salinity environment

Marilyn Kalaus* and Henn Ojaveer

Estonian Marine Institute, University of Tartu, Lootsi 2a, 80012 Pärnu, Estonia

E-mail: marilyn.kalaus@ut.ee (MK), henn.ojaveer@ut.ee (HO)

*Corresponding author

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**Abstract**

Invasive species are often in focus in the non-indigenous species (NIS) research while low-abundance species receive relatively little attention. However, tracking NIS dynamics since the early stages of an invasion provides valuable information on the ecology of invasions. In the current paper, we investigated the invasion history and population dynamics of the small-bodied cladoceran *Evadne anonyx* G. O. Sars, 1897 in the Gulf of Riga (Baltic Sea) almost since its first detection in 2000. The species already was widespread the Gulf of Riga in 2001 and has been found in nearly every subsequent sample collected during summer months. However, the abundance of the species remained low, seldom 100 individuals m\(^{-3}\). Both, salinity and water temperature affected the spatial distribution and population abundance of *E. anonyx*. The species was found to occur only sporadically at salinities below ca. 6. To obtain reliable presence/absence and density estimates on this small-bodied cladoceran, the entire zooplankton sample needed to be analysed.

**Key words:** distribution, invasion, Ponto-Caspian, population dynamics, sample analysis, Baltic Sea

**Introduction**

Invasive species are a component of non-indigenous species (NIS) that can alter the structure and function of marine ecosystems and may result in appreciable socio-economic impacts to humans (Bax et al. 2003); consequently, they have become a special area of study. However, NIS that are currently rare might subsequently become invasive should environmental conditions become more suited for their expansion, but this might take several decades (e.g. Witte et al. 2010). Unfortunately, our current knowledge of invasive processes in many ecosystems is far from sufficient to predict invasive species expansions and their overall impacts with changing environmental conditions (Richardson 2011).

The study of a NIS population from an early invasion stage allows for a more complete understanding of the dynamics of such populations (O’Connor 2013; Alam et al. 2013). However, due to the scarcity of basic inventory studies in many marine waters (ICES 2012), marine invasions can go unnoticed, perhaps for several decades. For example, the Ponto-Caspian hydromedusa *Blackfordia virginica* Mayer, 1910, remained unrecognised in South American waters for over fifty years (Freire et al. 2014). A second important factor is the lack of personnel with the ability to recognise and distinguish NIS from native organisms (e.g. Geller et al. 2010 and references therein).

To-date, 95 multicellular NIS have been recorded in the Baltic Sea since 1900 (Galil et al. 2014). Several of the most recent invasions (post 1990) originate from the Ponto-Caspian region, with transport on commercial vessels the most vector for introduction (AquaNIS 2013). The small cladoceran *Evadne anonyx* G.O. Sars, 1897, is one of these NIS and is native to the Caspian Sea (Rivier 1966) and estuarine areas of the Black and Azov Seas (Mordukhai-Boltovskoi and Rivier 1987; Rivier 1998). In the Baltic Sea, *E. anonyx* was first recorded in the Gulf of Finland in 1999 and a year later found in the Gulf of Riga (GoR) (Rodionova and Panov 2006; Põllupüü et al. 2008). In 2006, the species was recorded in the Gulf of Gdansk (Bielecka et al. 2014), then two years...
Table 1. Sampling details in the Gulf of Riga (GoR) and the outer station of Pärnu Bay (PB). Sampling depth in PB was 10m.

<table>
<thead>
<tr>
<th>Year</th>
<th>GoR Survey period</th>
<th>PB Station depth range (m)</th>
<th>GoR Number of samples</th>
<th>GoR Number of samples</th>
<th>PB</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>19.07–24.07</td>
<td>-</td>
<td>26–54</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>2002</td>
<td>25.07–31.07</td>
<td>-</td>
<td>20–25</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>2006</td>
<td>26.07–31.07</td>
<td>08.06–02.10</td>
<td>20–45</td>
<td>13</td>
<td>34</td>
</tr>
<tr>
<td>2007</td>
<td>24.07–30.07</td>
<td>03.05–11.10</td>
<td>16–40</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>2008</td>
<td>23.07–29.07</td>
<td>09.05–17.09</td>
<td>20–35</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>2009</td>
<td>22.07–28.07</td>
<td>15.05–10.08</td>
<td>10–30</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>2010</td>
<td>21.07–27.07</td>
<td>25.05–31.08</td>
<td>10–40</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>2011</td>
<td>26.07–01.08</td>
<td>10.05–22.09</td>
<td>22–45</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td>2012</td>
<td>25.07–31.07</td>
<td>09.05–09.10</td>
<td>9–46</td>
<td>22</td>
<td>21</td>
</tr>
</tbody>
</table>

later in several parts of the open Baltic Sea (from the Arkona basin to the Landsort Deep and Askö) and has even extended its range into the Bothnian Sea (ICES 2009).

From the study of the long-term dynamics of eleven NIS in the NE Baltic Sea, it was clear that temperature significantly affected abundance/biomass of most of these species (Ojaveer et al. 2011). *E. anonyx* typically appears in the mesozooplankton community in May, reaches peak abundance during July, and disappears in October (Rodionova and Panov 2006; Põllupüü et al. 2008). The species rarely exceeds 100 individuals m⁻³ (Põllupüü et al. 2008) but is consistently present when water temperatures exceed 12–15°C and salinity is >5, with a maximum abundance at 17–19°C (Rodionova and Panov 2006; Põllupüü et al. 2008). Within its native environment (Caspian Sea), *E. anonyx* abundance peaks at temperatures of 16–20°C and salinity of 12–13 (Mordukhai-Boltovskoi and Rivier 1987; Rivier 1998). Thus, the salinity and temperature conditions are important controlling the seasonal population dynamics of *E. anonyx*.

The aim of this study was to investigate: i) the seasonal and long-term abundance of *E. anonyx* in one of the distinct sub-basins of the Baltic Sea (GoR) with sampling having commenced from about the date of its first detection; and ii) how its distribution and abundance might be influenced by variation salinity and water temperature.

Materials and methods

Study area

The GoR has an area of 16 330 km² and a volume 424 km³. It is a relatively shallow (mean depth 26 m and maximum depth >60 m) and semi-enclosed sub-basin in the NE part of the Baltic Sea. The basin receives freshwater from a large drainage area (134,000 km²), which mostly enters at the southern part of the basin. Salinity varies from 0.5 to 7 and there is no permanent halocline. Oxygen concentrations above 5 mL L⁻¹ in most areas. In summer, the surface temperature may attain 20°C (Kotta et al. 2008).

Pärnu Bay (PB) has an area of 700 km² and a volume 2 km³. This is a shallow area with an average depth of 5 m in the NE part of the GoR. PB is ice-covered during most winters. During the ice-free season, the water column is well mixed. During July and August, surface temperatures may rise to 24°C, and salinity varies from almost fresh water in the Pärnu River mouth to 7 in the open bay. Hydrological conditions are determined by meteorological processes, river discharge (Pärnu River, freshwater inflow 2 km³ annually), and water exchange with the open part of the GoR (Kotta et al. 2008).

Sampling and sample analysis

Zooplankton samples were collected during daytime with a Juday net (mouth area 0.1 m², 90 µm bar-mesh) hauled vertically from the bottom to the surface. Samples were preserved in a 4% formalin and seawater solution. In the open GoR, zooplankton samples were obtained during hydroacoustic surveys for pelagic commercial fish performed mainly in July during 2001–2012 (see Table 1). There was no sampling during 2003 to 2005. Sampling in PB was at two stations, located in the inner and outer part (depths 5 and 10m, respectively), during May to October of 2006–2012 (Figure 1). Results of zooplankton sampling prior to 2006 are reported by Põllupüü et al. (2008) and these data were included in the present study. Samples from station 2 in PB were obtained bimonthly during June-August, whereas
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sampling in May and September-October took place monthly. At PB station 1, samples were collected monthly. In all, 132 samples were collected from the open part of the GoR and 143 samples from PB (Table I and Supplementary material Tables S1–S2). To obtain the abundance of E. anonyx, each sample was examined in its entirety.

In PB, temperature and salinity were measured throughout the water column during zooplankton surveys using CTD (model SAIV SD204; SAIV A/S, Environmental Sensors & Systems, Laksevag, Norway). For the GoR, we used the mean sea-surface temperature (SST) and sea-surface salinity (SSS) values for each survey period, determined from the daily modelled data obtained from Omstedt (2011, updated).

The native Evadne nordmanni Lovén, 1836, and the alien E. anonyx are routinely examined in PB as part of the Estonian marine monitoring program using HELCOM COMBINE recommendations. This methodology is based on sub-sample analysis of zooplankton samples (HELCOM 2013). To obtain reliable estimates of infrequent small-bodied NIS, such as E. anonyx, we performed a comparative analysis and compared the efficacy of the total sampling method and the sub-sampling method. We scored all E. anonyx and E. nordmanni separately in sub-samples and then calculated the percentage of E. anonyx to those of the genus Evadne in each sub-sample. We then applied this ratio to the genus Evadne to obtain an abundance estimate obtained for the total sample and then compared these with the total-sample count and both expressed as the number of individuals m⁻³.

Morphologically, E. anonyx and E. nordmanni are very similar (Figure 2). The distinction was made using two characteristics outlined by Rivier (1998): (i) formula of the setae – number of setae in the exopodites of thoracic limbs I–IV – for E. anonyx 2.2.2.1 and for E. nordmanni 2.2.1.1; and (ii) shape of cauda – for E. anonyx the cauda is formed of rounded tubercles and the cauda of E. nordmanni has two conical pointed outgrowths.

Statistical analysis

The analyses were undertaken using the open-source language R version 3.0.2 (R Core Team 2013). Log-transformed abundances of E. anonyx were modelled as a response to water temperature and salinity. Temperature and abundance was expected to show a seasonal and interannual variability, which we separated by fitting the generalized additive model (GAM) smoother (R library mgcv; Wood 2006) through abundance and the temperature, using the week of year as a predictor variable. A linear model was fitted using either the GAM predicted seasonal values of abundance and the temperature, or residuals from the seasonal GAMs. Correlation in the first (predicted seasonal values of abundance and temperature) indicated the effect of seasonality. The effect of interannual temperature variability on abundance is present should a correlation
between the residuals of seasonal abundance and the temperature exist. Due to sample/data availability, the seasonal relations between the abundance of *E. anonyx* and abiotic environment were investigated for the PB station 2 for June-August period only. Comparisons between the two different methods for analysing samples involved the Student’s *t*-test. Non-zero log-transformed abundances were included, resulting in normal distribution of two samples compared.

**Results**

**Spatio-temporal distribution of *Evadne anonyx***

*E. anonyx* was already widely distributed in the GoR during 2001 – it was present in 126 of 132 samples. The highest abundances occurred in the southern and western parts of the basin (Figure 3; Supplementary material Table S1). During the period 2002 to 2009, mean abundance was somewhat lower than in 2001 (Figure 4). The mean abundance ranged between 3 and 28 ind. m⁻³ with the highest density (173 ind. m⁻³) observed in 2012. Only parthenogenetic females were captured in the GoR samples in summer. When present, *E. anonyx* comprised 0.4–100% of the total abundance of the genus *Evadne*, with a mean 42.7% and standard deviation of 36.6%.

Few *E. anonyx* were obtained from PB samples before 2006 (M. Põllupüü, University of Tartu, Tallinn, Estonia, unpubl. data). Within the inner part of this bay (station 1), a similar situation continued during our study period, we only detected the species in two years (2006 and 2011). In 2006, *E. anonyx* occurred mainly during August with
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abundances of 2 to 50 ind. m\(^{-3}\). In July and August 2011, we observed densities of 2 to 44 ind. m\(^{-3}\). At PB station 2, the abundance of *E. anonyx* was relatively low, although higher than at station 1. *E. anonyx* was found during May to October in all years investigated at densities of 1 to 22 ind. m\(^{-3}\) and was more abundant during 2006–2008. Population numbers declined to a low level in 2009–2011 (Figure 5; Table S2). The highest abundance in a single sample (153 ind. m\(^{-3}\)) was recorded in August, 2006. Unlike the summer samples in the GoR, both partenogenetic and gamogenetic individuals occurred (see also Pöllupüü et al. 2008).

Relation of *E. anonyx* abundance to environmental conditions

There was a highly significant positive correlation between the abundance of *E. anonyx* and SSS for the GoR ($R^2=0.223$, $p<0.001$, GAM; SSS range 6.1–6.7). The relationship with SST was also statistically significant ($R^2=0.11$, $p=0.02$, GAM; SST range 16.1–20.8 °C). In the outer part of PB at station 2, we found a positive relationship between the abundance of *E. anonyx* and water temperature ($R^2=0.241$, $p<0.001$, GAM; temperature range 4.0–25.6°C). There was no correlation between the residuals of the seasonal GAM curve of abundance and temperature, i.e. the positive linear correlation between the *E. anonyx* abundance and temperature was due to the similar seasonal course of both variables. The response on species abundance was slightly lagged and followed seasonal changes in temperature, both with increasing temperature in spring/summer followed by decreasing temperature in late summer to early autumn (Figure 6). We found no relation between the abundance of *E. anonyx* and salinity in the outer PB ($R^2=0.095$, $p=0.219$; salinity range 3.1–5.6).

Methods comparison

The two methods to estimate *E. anonyx* abundance were not equivalent. Subsampling only detected *E. anonyx* in 55 (46 %) of zooplankton samples where the species was actually present as determined by total count. As well, subsampling overestimated the *E. anonyx* abundance in samples when detected ($t=-4.009$, $df=35$, $p<0.01$, $n=36$, t-test; Figure 7). As a result the sub-sample method was judged unreliable for estimating presence and/or densities of *E. anonyx*.

Figure 4. Abundance (individuals m\(^{-3}\)) of *Evadne anonyx* in the Gulf of Riga in summer during 2001–2012. On each box, the central mark is the median, boxes indicate first and third quartiles, whiskers are 95% confidence bounds, and dots represent outliers.

Figure 5. Abundance (individuals m\(^{-3}\)) of *Evadne anonyx* in the outer station of Pärnu Bay in years 2006–2012 (* data from M. Põllupüü, University of Tartu, Tallinn, Estonia, unpublished data.). On each box, the central mark is the median, boxes indicate first and third quartiles, whiskers are 95% confidence bounds, and dots represent outliers.

Figure 6. GAM predicted values of *Evadne anonyx* abundance vs. GAM predicted seasonal curve of water temperature in the outer Pärnu Bay. Both species abundance and water temperature had weekly resolution.
Discussion

The Baltic NIS has been colonized by several pelagic invertebrates during the past few decades. In addition to *E. anonyx*, *Cercopagis pengoi* Ostroumov, 1891, *Mnemiopsis leidyi* A. Agassiz, 1865, and *Cornigerius maeoticus* Pengo, 1879, have arrived (AquaNIS 2013). The current study provides evidence of a seasonal and spatio-temporal dynamics of *E. anonyx* since the early invasion stage. *E. anonyx* has formed self-sustaining population across the Gulf of Riga since 2001, or perhaps before, and can now be considered to be a common component of the mesozooplankton community. However, densities of *E. anonyx* have remained relatively low throughout the study period.

Põllupüü et al. (2008) stated that *E. anonyx* was not considered to be a high risk species for low-salinity environments in the Baltic Sea because it was suggested that the species is unable to survive at salinities below 9 (Panov et al. 1999; Rivier 1998). Based on the current study, the species can be considered to be a permanent component of the mesozooplankton community above salinities ~6. However, *E. anonyx* clearly suffers under substantial salinity stress across the whole GoR. We also suggest that the somewhat sporadic occurrence of *E. anonyx* in the inner part of PB, where salinity is generally below 6, may be due wind-induced water currents that transport *E. anonyx* from the open GoR. Salinity stress is a feature challenging other NIS in the Baltic Sea, as in case of the comb jelly *Mnemiopsis leidyi* (Jaspers et al. 2011) and Chinese mitten crab *Eriocheir sinensis* H. Milne-Edwards, 1853 (Otto and Brandis 2011).

A recent study in the Gulf of Gdansk in the southern Baltic Sea failed to show a relationship between *E. anonyx* abundance (variability 0.11–6 ind. m⁻³) and salinity (range 4.6–7.5). When compared with our study, the abundance of *E. anonyx* in the Gulf of Gdansk was substantially lower although the salinity range was greater (Bielecka et al. 2014). This might be explained due to the low density of the *E. anonyx* and insufficient spatio-temporal coverage. However, the species may have only recently arrived to this region (Bielecka et al. 2014). In terms of water temperature, the highest densities of *E. anonyx* in the Gulf of Gdansk (Põllupüü et al. 2008; Bielecka et al. 2014) occurred at the same 19°C temperature as observed in the current study.

Cladoceran populations have high seasonal variability, partly due to the alternation of partheno- genetic and gamogenetic reproduction modes, and are characterised by a rapid increase in densities when environmental conditions became favourable (Egloff et al. 1997; Rivier 1998). High population abundances are generally sustained only for a relatively short time. Earlier investigations have confirmed that parthenogenetic females dominated in the *E. anonyx* community in the GoR for most of the season (Põllupüü et al. 2008). The decrease in abundance in autumn coincided with the increasing abundance of males and gamogenetic females (Põllupüü et al. 2008). It has been suggested that shift to a gamogenetic reproduction mode is triggered by unfavourable environmental conditions (Egloff et al. 1997; Rivier 1998). As we have found only parthenogenetic *E. anonyx* individuals in the GoR samples in summer, the population was therefore still in the developing phase in all years investigated and likely had not reached the annual abundance peak.

In contrast to *E. anonyx*, the non-indigenous predatory cladoceran *Cercopagis pengoi* may attain greater abundances in the GoR, principally within sheltered coastal areas. The seasonal timing of peak abundance *E. anonyx* broadly matches that of *C. pengoi* (Põllupüü et al. 2008; Ojaveer et al. 2004). As small-bodied, low-motility, species are preferred prey for *C. pengoi* (Simm and Ojaveer 2006), *E. anonyx* may be a valuable prey item for *C. pengoi* during the warmest summer months - a time when the preferred prey *Eubosmina* spp. is less abundant (Ojaveer et al. 2004). *E. anonyx* might therefore facilitate other pelagic alien species in the area as a prey item. *Eudane* spp. are seldom consumed by pelagic fishes in the GoR during summer (Lankov et al. 2010), possibly due to their low densities; however, they have been found in the Baltic herring (*Clupea harengus membras* Linnaeus, 1761) stomachs (M. Kalaus, H. Ojaveer).
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unpubl. data). However, C. pengoi may play an important role in herring and three-spine stickleback (Gasterosteus aculeatus Linnaeus, 1758) diets in the GoR (Lankov et al. 2010). We suggest the ecological role of E. anonyx in the GoR, due to its low abundance, is most likely limited to enriching the species diversity of pelagic communities during the warm season.

As ecosystem conditions may change, the status of a NIS population might change over time. For example, a complex of changes in the environment resulted in a sudden outburst of the comb jelly Mnemiopsis leidyi in the Black Sea and led to significant decline in abundance of pelagic commercial fishes (e.g. Oguz et al. 2008). Therefore, systematic monitoring of NIS population dynamics, and investigating their ecology over time, is essential to track and explain potential changes in ecosystem structure and functioning. The current study provides a baseline of the seasonality and abundance levels for E. anonyx in the GoR under conditions characterised by a relatively warm climate and low salinity. While the densities of E. anonyx remained, in some instances, several orders of magnitude lower than those of many native zooplankton species (e.g., Ikauniece 2001; Lankov et al. 2010) this situation might change if environmental conditions change.

Successful reproduction and establishment of a self-sustaining NIS population under the lower levels of salinity might indicate an osmoregulatory adaption to local conditions. Physiological plasticity has indeed shown to be an important characteristic of several NIS (e.g. Blumenshine et al. 2011 and references therein). To our knowledge, there is no indication that this has happened for E. anonyx. However, unexpected low-salinity preference was recently observed for an invasive gammard Gammarus tigrinus Sexton, 1939 in the Baltic Sea, potentially due to high genetic diversity of invading individuals (Kotta et al. 2013). Thus, further laboratory experiments and investigations in physiology and genetics of E. anonyx is needed to shed some light on the causes and potential future /consequences of this colonization.

We also found that the entire mesozooplankton sample should be examined when scoring the numbers of infrequent small-bodied specimens as sub-sampling may either not capture or poorly estimated numbers of individuals. This is a practical finding that relates to meeting international requirements like EU MSFD Descriptor 2 (European Commission 2010) and when performing basin-scale impact assessments of NIS (Olenin et al. 2007).

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References


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The following supplementary material is available for this article:

**Table S1.** Abundance of *Eudawe anonyx* in the Gulf of Riga (by sampling dates and stations) in summer, 2001–2012.

**Table S2.** Abundance of *Eudawe anonyx* in the outer part of Pärnu Bay (station 2, see Figure 1) by sampling dates in 2006–2012.

This material is available as part of online article from: http://www.aquaticinvasions.net/2014/Supplements/AI_2014_Kalaus_Ojaveer_Supplement.xls