Effects of salinity, temperature, and food availability on the ecology of the crustacean copepod

Eurytemora affinis

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Abstract

Climate change has already led to considerable change in the properties of the Baltic Sea. As it progresses, the Baltic Sea will likely experience changes in its temperature and salinity and an increase in eutrophication. Therefore, it is necessary to understand how species are affected by environmental factors, especially those that are projected to change. Zooplankton is the most abundant multicellular organism on earth, and among these, the copepod is the most common. In the Baltic Sea, *Eurytemora affinis* is among the most important copepods, and further knowledge is needed to further our understanding of *E. affinis* in a changing climate. In the present thesis, I conducted a field study to measure the body sizes of two *E. affinis* populations to determine to what extent temperature, salinity, and chlorophyll *a* (Chl. *a*) affected *E. affinis* ecology. Populations were sampled over approximately four months in two areas in the western Gulf of Finland with contrasting characteristics in terms of salinity, temperature, and Chl. *a* concentration. The study showed that *E. affinis* was not significantly affected by salinity but experienced significant variation in body size in relation to temperature and Chl. *a*. Individuals were, additionally, larger in the early summer. Interestingly, most of the variation in body size was a product of the sampling site, and the individuals sampled in the area with more extreme conditions, Pojoviken Bay, were significantly larger (151.4 μm or 21.9%) than those sampled in the other area, Storfjärden. The results strengthened the notion that *E. affinis* is robust against climate change, as the environmental factors had a relatively small effect. Furthermore, the results indicated that there might be unmeasured variables behind the body size variation between populations. The variation could be due to food availability acting as a buffer against the environmental factors, or conceivably due to differences in food quality. This difference could also be because of genetic adaptations, and the Pojoviken Bay population could be a sibling species. Further studies, such as multigenerational experiments and genetic analyses, are needed to determine what lies behind the substantial body size differences between these two areas.

Keywords: • zooplankton • copepods • *Eurytemora affinis* • salinity • temperature • chlorophyll *a* • climate change

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1. Introduction

1.1 Functional traits

Functional traits are biochemical, physiological, structural, morphological, or behavioral characteristics of organisms that influence performance or fitness (Nock et al. 2016). In most cases, functional traits affect fitness through performance measures, for instance, growth rate mating success, or competitive ability. A functional trait can, additionally, affect other functional traits (Geber & Griffen, 2003).

Trait-based approaches have proved useful in determining mechanisms behind community assembly in terrestrial plant communities (Edwards et al. 2012), and over the last decade, several zooplankton traits have been described and used in studies (Hébert et al. 2016). These traits can be useful when examining zooplankton community assemblages and their responses to climate change. In zooplankton, traits might include feeding traits, such as feeding modes, survival traits, meaning traits enhancing longevity by reducing predation risk, and reproductive traits, such as hermaphroditism (Litchman et al. 2013). In this present study, body size was chosen as the measurement for *E. affinis* ecology. This choice was motivated by body size being a significant predictor of several traits, including ones that influence ecosystem functioning and it is, additionally, a typical denominator for most physiological rates in zooplankton (Hébert et al. 2016). Body size is a predictor of clutch size, and by proxy, reproduction, in *E. affinis (*Crawford & Daborn, 1986; Hirche, 1992; Ban, 1994; Lloyd et al. 2013; Souissi et al. 2021), and since adult body size, along with carbon density, has a disproportionate influence on the ecology and physiology of zooplankton (Litchman et al. 2013), body size will likely be highly relevant in understanding *E. affinis* responses to a variety of environmental factors.

1.2. The crustacean copepod, *Eurytemora affinis*

Zooplankton is vital to the functioning of ocean food webs due to their abundance and prominent ecosystem roles. The most common zooplankton are the copepods, which are the most abundant multicellular animals on earth (Richardson, 2008). Copepods are the most important secondary producers in the oceans and they represent the interface between primary producers, microzooplankton, and planktivores. A myriad of fish larvae and other plankton feeders, such as mysid shrimps and the three-spined stickleback, are dependent on food resources produced during the summer in the northern hemisphere, and the copepods are the main prey for these taxa (Engström-Öst et al. 2015). Copepods constitute a hugely important part of global marine food webs, as well as regional ones, and changes in their abundance could lead to hugely impactful bottom-up effects (Richardson, 2008).

Eurytemora affinis is a common euryhaline grazer in many estuaries and other brackish watersheds in the Northern Hemisphere, existing in a variety of different salinities (Kuismanen et al. 2020). In the Baltic Sea, *E. affinis* is one of the most important copepods (Hernroth & Ackefors, 1979; Viitasalo, 1992). Copepod crustaceans are generally considered to be robust against climate change, partly due to their diel vertical migration which forces them to endure large gradients of physio-chemical factors, such as salinity and temperature (Almén et al 2014). *E. affinis* is no exception and can tolerate both high and low salinities, (Devreker et al. 2004). Salinity is still, along with temperature, one of the most important factors that regulate zooplankton diversity (Kuismanen et al. 2020), and biodiversity in general, in the Baltic Sea (Ojaveer et al. 2010).

1.3. The Baltic Sea

The Baltic Sea is a large, strongly stratified, brackish water ecosystem surrounded by nine countries that rely on it economically, The Baltic Sea has a surface area of 420,000 km^2 and a small total water volume of 21,700 km^3 due to its shallow nature (mean depth 54 m) (Jansson et al. 2020). It is also rather isolated from other seas, only having a small connection to the North Sea, which is why water exchange in the Baltic Sea is a process

that takes roughly 30 years (HELCOM, 2018a). The hydrological conditions which account for the brackish water gradient are controlled by the freshwater budget through precipitation, rivers, and water exchange with the North Sea (Neumann, 2010). Due to the hydrological characteristics, the Baltic Sea has a wide range in salinity varying from 15-18 PSU at Øresund to 0-2 PSU in the northeastern parts of the Baltic Sea (HELCOM, 2018a), and overall, it has a salinity of 24.7 g kg^{-1} (Meier et al. 2022a). The temperature of the Baltic Sea is primarily controlled by atmospheric heat fluxes, which warms the upper water column in the summer, while the deeper layers are strongly influenced by surface temperatures in the previous winter. This creates a seasonal thermocline that restricts the vertical mixing of the water layers in summer (HELCOM, 2013).

The projected climate change occurring in the next 100 years will have an appreciable effect on the Baltic Sea as it has several attributes that make it particularly vulnerable to the ongoing changes (Neumann, 2010). Among other issues, it is one of the most polluted seas on the planet (Kabel et al. 2012), it is rather enclosed and therefore has low biodiversity, and the long winter limits its productivity. The Baltic Sea also suffers from the typical anthropogenic factors such as contamination, litter, eutrophication, light and sound pollution, invasive species, fishing, hunting, and habitat loss (HELCOM, 2018a).

1.4. Environmental conditions

The Baltic Sea is characterized by its unique salinity gradient which varies both vertically and horizontally. The species richness, or lack thereof, is generally attributed to the low salinity of the Baltic Sea, and this low salinity is further exacerbated by climate change (HELCOM, 2018a). Climate change affects salinity, and sea surface salinity decreased in the Gulf of Finland between 1979 and 2015 (Almén et al. 2015). Additionally, salinity has been declining due to increased precipitation (Helama et al. 2017), whereas the deep-water salinity has increased due to saltwater inflow (Mohrholz et al. 2015). Earlier projections indicated that the salinity would continue to decrease (BACC Author Team, 2008; BACC II Author Team, 2015), however, the accounting for global mean sea level rise has brought doubt to said projections (Meier et al. 2022b), and it is, therefore, unsure whether the Baltic Sea will freshen (Meier et al. 2022a).

Between 1982 and 2006, the Baltic Sea experienced a 1.35 °C temperature increase, a change seven times larger than the global rate (Belkin, 2009). This increase is considered extreme even for a coastal sea (Kniebusch et al. 2019). Summer sea surface temperatures are projected to rise roughly 2 °C in the southern parts of the Baltic Sea and 4 °C in the northern parts by 2098. Moreover, since the surface water is projected to warm more than the deep layers, vertical stratification is likely to become stronger (HELCOM, 2013) which could be exacerbated by increased saltwater inflows associated with sea-level rise (Meier et al. 2022b).

Chlorophyll *a* (Chl. *a*) concentration is a measurement of phytoplankton biomass, which is generally dominated by dinoflagellates and diatoms in spring and cyanobacteria in summer (Brando et al. 2021), and it is commonly used as an indicator for eutrophication (HELCOM, 2018b; Brando et al. 2021). Chl. *a* concentration is therefore controlled by the availability of the major nutrients, phosphorus, and nitrogen. In the Baltic Sea, the input of nitrogen and phosphorus has increased drastically in the last 150 years due to, among other factors, industrialization, and agriculture (HELCOM, 2013). Consequently, the average Chl. *a* trend for the 1997–2020 period was 0.5% per year, this positive trend was observed in most of the Baltic Sea (von Schuckmann et al. 2021). Furthermore, temperature increases are likely to positively affect Chl. *a* concentration since sufficient temperature levels for cyanobacteria blooms are reached earlier, prolonging and strengthening said blooms (Neumann et al. 2012).

1.5. Objective of the thesis

Climate change is likely to cause some variation in the basic ecology of zooplankton and challenge their plasticity in the marine environment. Despite the perceived robustness of zooplankton, populations are still decreasing rapidly regionally or being threatened (Kuosa et al. 2017). Furthermore, *E.affinis* lives on the edge of an optimal salinity and a decline in salinity could directly affect the fitness of local populations (Kuismanen et al. 2020). Therefore, further knowledge is needed to properly qualify the threat to zooplankton.

The objectives of this thesis were to (1) gain a better understanding of two *E. affinis* populations in two areas with contrasting hydrologic characteristics, and (2) to understand to what extent the environmental factors (salinity, temperature, and Chl. *a*) affect the body size of the copepod and, lastly, (3) to identify how time affects body size. The hypotheses were that (1) body size varies by location, and (2) the environmental factors significantly affect body size, and it is likely that temperature negatively affects body size, whereas both Chl. *a* and salinity positively affect body size. Finally, (3) time is expected to influence body size with larger body sizes appearing in early summer when the water temperature is lower (Daufresne et al. 2009).

2. Material and methods

2.1 Study sites

The study sites were in Tvärminne and the Pojoviken Bay by the Hanko peninsula, located in the Gulf of Finland, northern Baltic Sea. The samples were taken at two sites, the Sällvik deep (60°01′10.8″N, 23°28′08.7″E) in Pojoviken Bay and Tvärminne Storfjärden (59°52′56″N, 23°15′14″E) in the archipelago zone (Figure 1).

Storfjärden is located in the archipelago zone as an intermediary between the open sea and estuary (Katajisto, 2006), where most of the water column originates from Baltic proper surface water. It has a maximum depth of 33 m and experiences thermal stratification from June to September (Jäntti et al. 2011). The stratification is, however, easily broken due to upwellings. Niemi (1975, as cited in Katajisto, 2006) states that salinity varies from around 5-7 PSU, with higher salinities near the bottom. The bottom temperature varies from 2 °C in winter and up to 12-14 °C in summer, especially as stratification is disturbed.
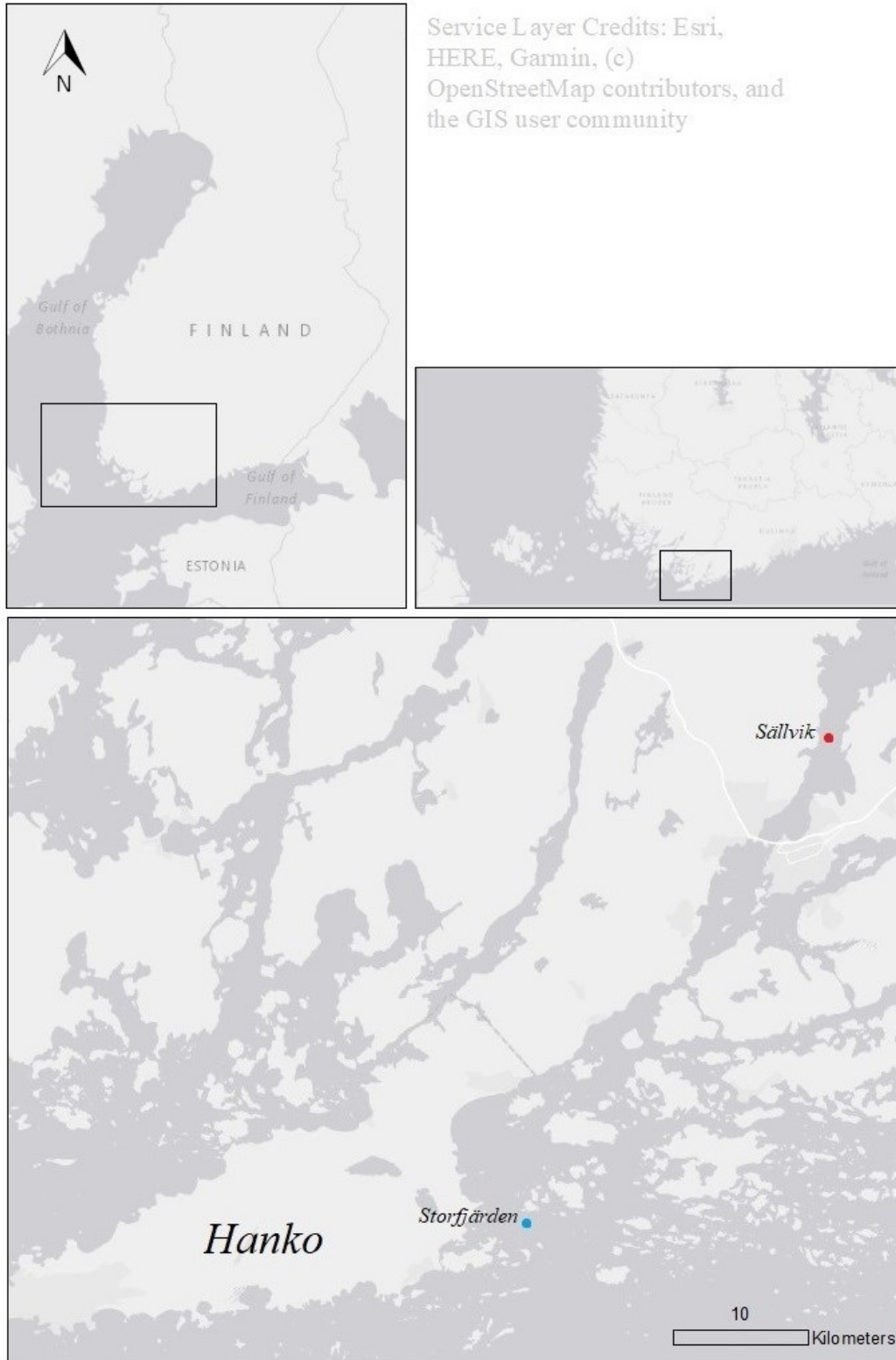
Figure 1. Map of the study area by the Hanko Peninsula, Finland. The blue dot shows the sampling site in Storfjärden close to Tvärminne Zoological Station, and the red dot shows the Sällvik deep in Pojoviken Bay. The map was created using ArcGIS® software by Esri. ArcGIS®.

Pojoviken Bay can be characterized by its permanent pycnocline and its limited inflow due to the 6 m sill which connects it to the Baltic archipelago (Niemi, 1982). It has a vertical salinity gradient of outflowing oligohaline surface water ranging from 0–3 PSU and saline deep water ranging from 4–5.5 PSU. The bottom temperature varies from 1 °C in winter to 4–5 °C in the summer (Katajisto, 2006), while surface temperature frequently rises as high as 23–24 °C in the summer (Örnmark & Holmberg, 2015). Pojoviken Bay has a pycnocline at around 10 m depth, which prevents the mixing of the warm, fresh surface water with the cold saline layer. The deepwater also suffers from low oxygen due to stagnant conditions in the summer (Katajisto, 2006).

2.2 Field sampling

Between May 10 and September 2, 2021, 18 measurements (9 per site) of temperature, salinity, and Chl. a were taken. Water samples were taken at 5 m intervals using a Limnos sampler from the surface down to a depth of 30 m, whereafter temperature and salinity were measured using a VWR salinity meter.

Triplicate Chl. a samples were from a depth of 5 m, using a Limnos sampler. Of the water collected for Chl. a measurement, 100 ml was filtered through 25 mm Whatman GF/C glass fiber filters, which were stored in 20ml scintillation vials in a (-18 °C) freezer. The filters were analyzed later. The filters were submerged in 10 ml of ethanol (96%) and determined the following day by fluorometry (Varian Cary Eclipse Fluorescence Spectrophotometer), using a 96-well microplate reader and Chl. a concentration was expressed as µg /L.

Zooplankton sampling was done twice per month, at the same time as water sampling (Table 1). The samples were collected using a 200 µm net lowered down to 30 m and raised to the surface to acquire a sample of the entire water column. The zooplankton samples were then transferred to a 24 L cooler containing water taken from below the thermocline, ~10-20 m depth. The laboratory work was conducted the same day as sampling, during which zooplankton samples were kept in a large climate room with a temperature resembling the Baltic Sea (12 °C), oxygenized using an aquarium pump. Using

a microscope, roughly 50 (when possible) female *E. affinis* carrying egg sacs were sorted, picked, and transferred to a plastic Eppendorf microtube (2 ml) containing commonly used preservative non-toxic iodide acid Lugol's solution. The tubes were then stored in a (+4 °C) fridge for later analysis. The body size measurements were taken using a Nikon DS-L3 camera mounted on a Leica MZ12 stereomicroscope by measuring the prosome length (hereafter referred to as body size) of each individual *E. affinis* (Figure 2).

Figure 2. A female Eurytemora affinis carrying an egg sac. The red line shows the prosome length measurement. This Individual was sampled on 10 June 2021 in Sällvik, Pojoviken Bay.

2.3 Statistical analyses

The graphs and statistical analyses were performed in RStudio, build 351. Linear mixed models were chosen using the *lmerTest* (Kuznetsova, 2017) and *MuMIn* (Bartoń, 2022) packages and created using the *lme4* package (Bates et al. 2013). Sampling event (1–9), which corresponds to a date (Table 1), was classified as a fixed effect, and sampling station (1, 2) as a random effect which was used to examine the relationship between body size and sampling event, temperature, salinity, and Chl. *a.* Additionally, a generalized linear model was used to examine the differences in body size between sampling stations.

Both the linear mixed models and the generalized linear model were tested to determine if they fulfilled the assumptions for said models. The assumption of linearity and homoscedasticity was verified by plotting model residuals in P-P plots, and the assumption of

normally distributed residuals was verified through Q-Q plots. Levene's and Shapiro-Wilk tests were additionally used to confirm the interpretation of the plots. Finally, the assumption of no multicollinearity was met as VIF-values were low.

Table 1. Sampling events and the corresponding dates. For sampling events 1 and 4, the dates differed between sampling sites. Both zooplankton and water samples were taken during these.

| SAMPLING EVENT	Corresponding date	
	Storfjärden	**Pojoviken Bay**
1	10/5/2021	12/5/2021
2	31/05/2021	31/05/2021
3	10/06/2021	10/06/2021
4	21/06/2021	24/06/2021
5	05/07/2021	05/07/2021
6	26/07/2021	26/07/2021
7	02/08/2021	02/08/2021
8	17/08/2021	17/08/2021
9	02/09/2021	02/09/2021

3. Results

3.1 Hydrography

The pycnocline in Pojoviken Bay occurred between 5–10 m during all sampling events, except during mid-summer when the pycnocline rose to 5 m (see Appendix Figure A, Figure B). Temperatures above the pycnocline in Pojoviken Bay were consistently higher than 0–5 m temperatures in Storfjärden, and the mean surface temperature was 3.48 °C higher in Pojoviken Bay. The total mean temperature was 0.52 °C higher in Storfjärden (Figure 3) than in Pojoviken Bay, which was also the case for the mean bottom (30 m) temperature where there was a 1.08 °C difference. Surface salinity in Pojoviken Bay varied between 0.8 and 2.3 PSU, while bottom salinity varied between 4.5 and 4.6 PSU.

Variation in Storfjärden was larger, varying between 6.2 and 4.8 PSU at the surface, and 7.1–5.6 PSU at the bottom. Mean salinity was 2.26 PSU higher in Storfjärden than in Pojoviken Bay.

Chl. *a* concentration was, on average, 6.66 µg/L higher in Pojoviken Bay (9.69 µg/L) than in Storfjärden (3.02 µg/L), and in Pojoviken Bay Chl. *a* peaked June 10th at 26.48 µg /L, in Storfjärden Chl. *a* peaked July 5th at 9.12 µg/L (Figure 3). Finally, the results showed that there was no significant correlation between environmental factors ($r = {<}0.5$).
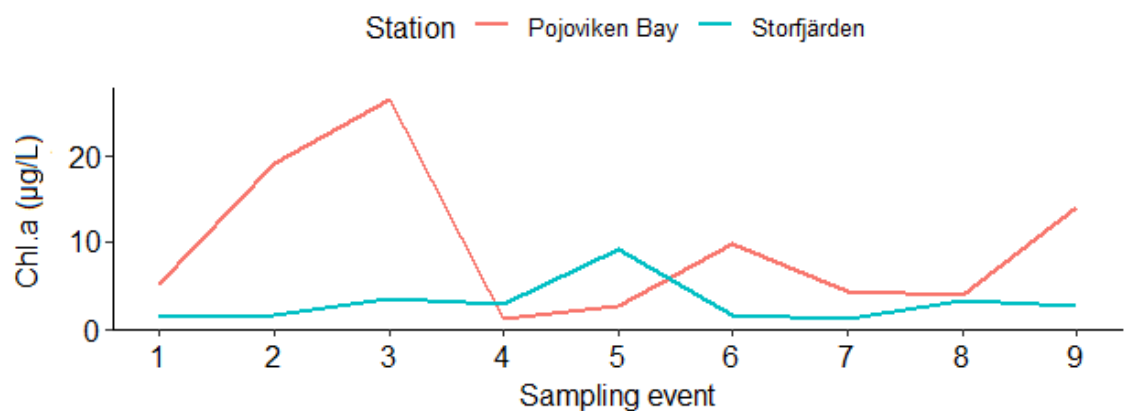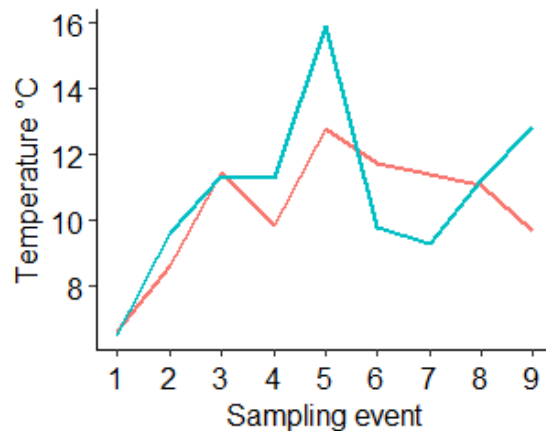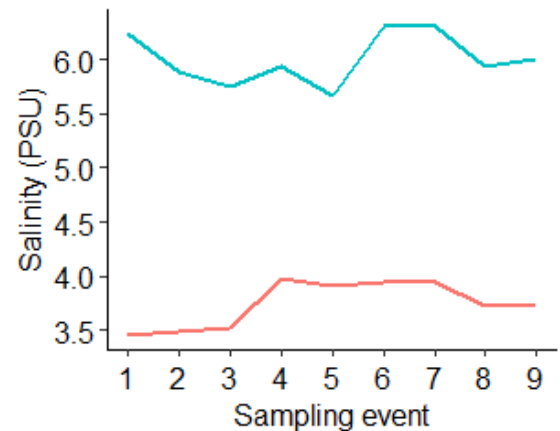

Figure 3. The mean values of the environmental factors, Chl. *a,* temperature and salinity plotted over time. The red line shows Pojoviken Bay values, and the blue line shows Storfjärden values.

3.2 Body size, and the impact of location

In total, 952 individuals were measured, 494 from Pojoviken Bay and 458 from Storfjärden. The average body size in Pojoviken Bay was 21.9%. (151.4 μm on average) larger (840.4 μm, SE = 3.07) than the average body size in Storfjärden (689 μm, SE = 3.32, Table 2), and this pattern was consistent throughout all sampling events. The generalized linear model showed that the relationship between sampling sites and the body size of *E. affinis* was significant (p<0.001, t = 32.8, SE = 4.86, Figure 4).

Table 2. Profile of sampling events and the mean body size, sample size, and standard deviation across sampling sites.

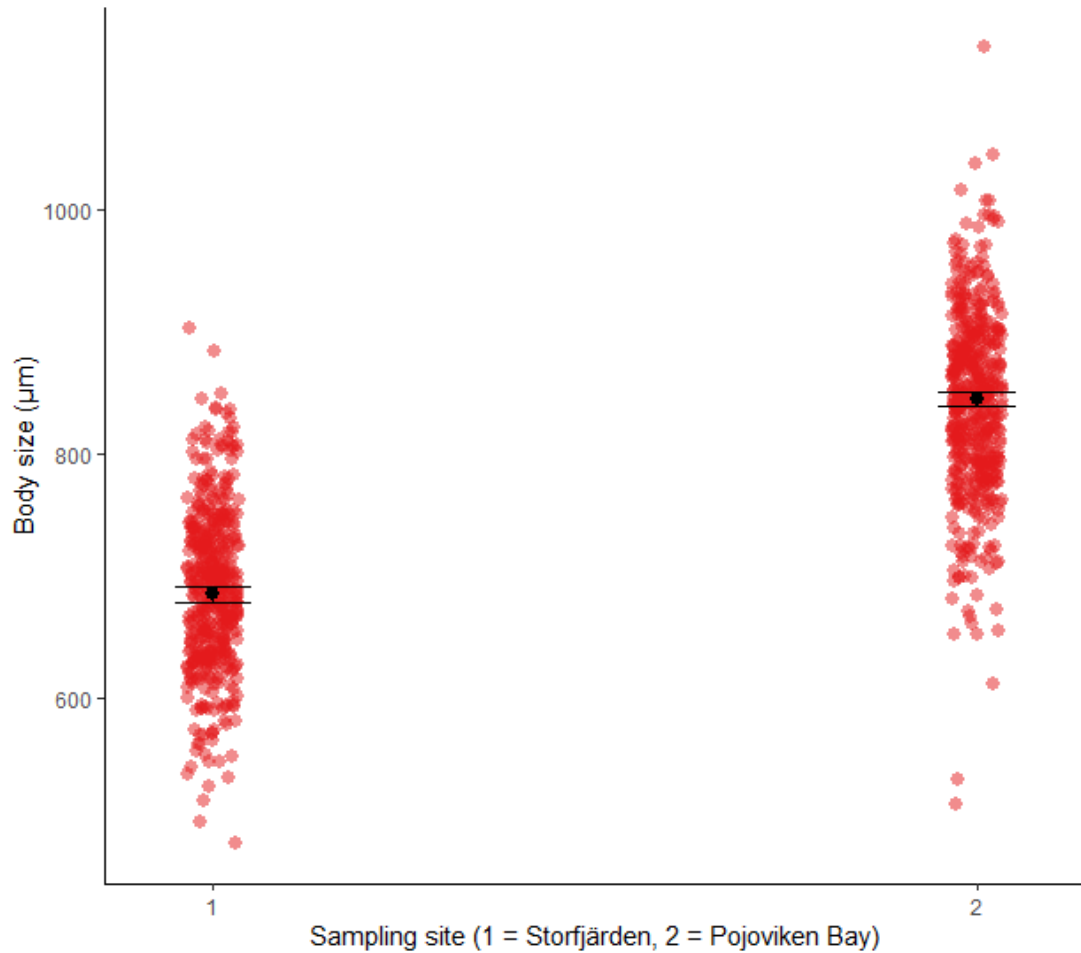| SAM-PLING EVENT	POJOVIKEN BAY			STORFJÄRDEN		
	Mean body size μm	SD	Sample size	Mean body size μm	SD	Sample size
1	895.87	58.87	57	728.97	55.31	40
2	849.03	69.64	50	745.96	63.8	51
3	821.97	69.28	54	682.59	77.1	39
4	893.98	54.33	63	676.67	43.89	51
5	846.48	71.27	53	666.50	53.46	64
6	808.39	77.20	56	698.95	68.67	58
7	815.24	46.40	48	696.02	40.34	49
8	817.96	82.03	57	681.56	72.77	59
9	805.17	57.64	56	632.84	37.41	47
	Mean 840.49		**N** 494	**Mean** 689.06		**N** 458

Figure 4. Plot showing body size as a function of sampling site. The black dots represent the average body size, 840.4 μm in Pojoviken Bay and 689 μm in Storfjärden. The lines show ± SE.

3.3 Effect of salinity, chlorophyll a, temperature, and time on body size

The results of the first linear mixed model showed that the relationship between salinity and body size was nonsignificant (p = 0.9, t = 0.066, SE = 12.77, Figure 5), therefore, salinity was excluded from the model.
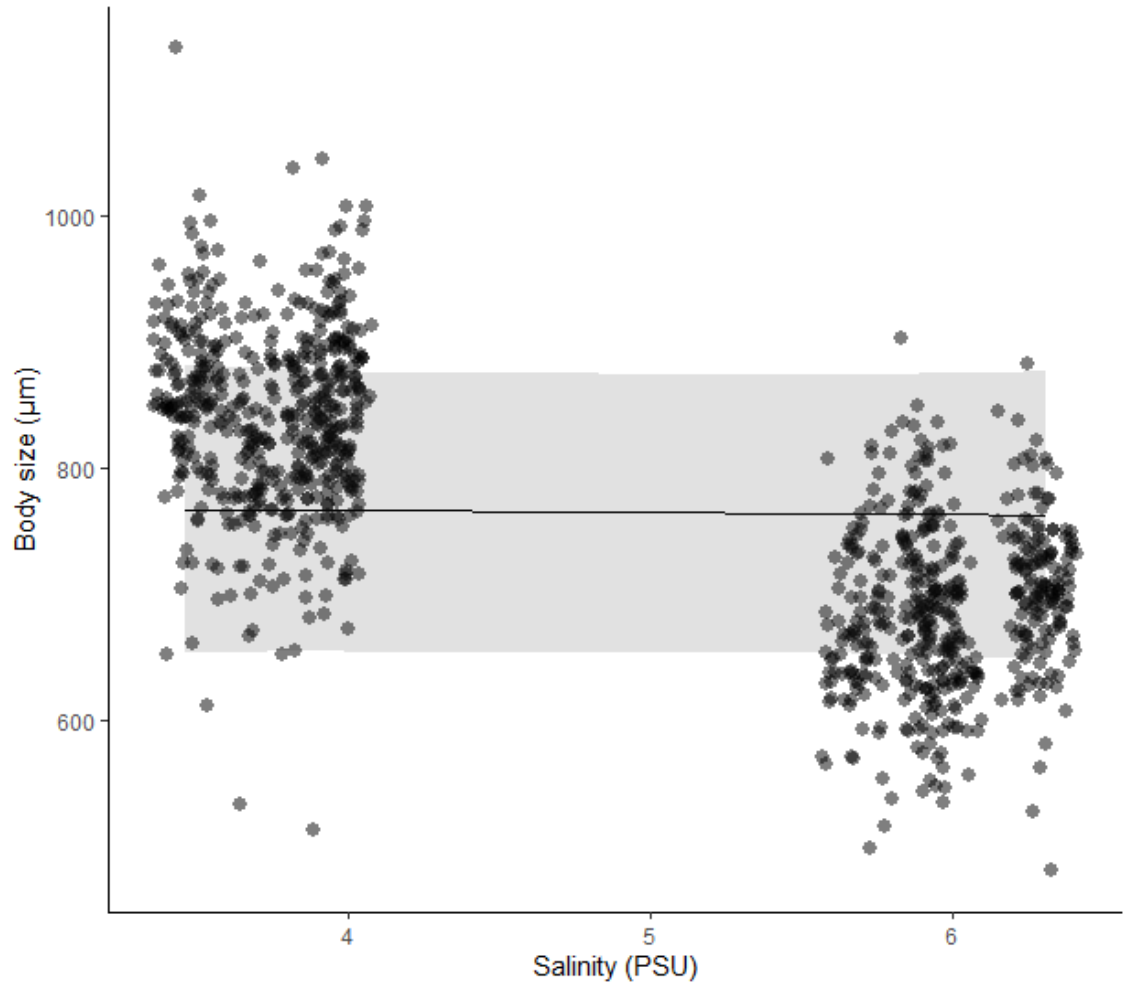
Figure 5. The first linear mixed model illustrated which shows body size as a function of salinity. The grey area shows the 95% confidence interval of the prediction, and the line shows the slope based on the estimate.

Models of interactions between fixed effects showed that the interactions, such as temperature × salinity (p = 0.33, t = 0.96, SE =1.33) and temperature × Chl. *a* (p = 0.19, t = 1.2, SE = 0.02), were not significant. The best model indicated that random effects accounted for a large proportion of the model variance (Station: 12804 ± 113.15, Residual: 4082 ± 63.89, Table 3), indicating considerable variation across sampling stations. Indeed, 72.17% of the variation in body size was explained by station (R^2 = 0.7217) whereas the fixed effects explained 4.82% of the variance (R^2 = 0.0482).

Table 3. Results of the best linear mixed model showing the relationship between *Eurytemora affinis* body size and fixed effects (environmental factors). The random effect is the sampling sites.

	Body size			
Predictors	*Estimates*	*CI*	*t-value*	*p*
Intercept	*867.61*	709.24-1025.98	10.751	**<0.001**
Sampling event	-8.22	-10.05 - -6.40	-8.851	**<0.001**
Temperature	-4.42	-6.59 - -2.25	-4.004	**<0.001**
Chlorophyll *a*	-2.1	-0.28 - -0.14	-5.972	**<0.001**
Random effects				
$\sigma2$	4082.43			
$\tau00$ Sampling site	12803.75			
ICC	0.76			
^{N} Sampling site	2			
Observations	952			
Marginal R^2 / Conditional R^2	0.048 / 0.770			

σ2: Population variance, within-subject. **τ00:** Random intercept variance, between subject-variance. **ICC:** Intraclass-correlation coefficient, used for R^2 calculation. **N:** Number of effects, sampling sites in this case. **Marginal R^2/Conditional R^2:** Marginal R^2 describes the variance explained by the fixed effects, whereas conditional R^2 describes the variance explained by both the fixed and random effects. **CI:** 95% confidence interval of the predictors, shows lower and upper bound. **t-value:** Measures the number of standard errors the coefficient deviates from 0. Higher values indicate significance. **p:** the p-value used for significance testing.

Additionally, the model showed that the relationship between the fixed effects – temperature ($p<0.001$, t = -4, SE = 1.1), Chl. *a* ($p<0.001$, t = -5.9, SE = 0.03), sampling event ($p<0.001$, t = -8.8, SE = 0.92) – and body size were significant (Figure 6).
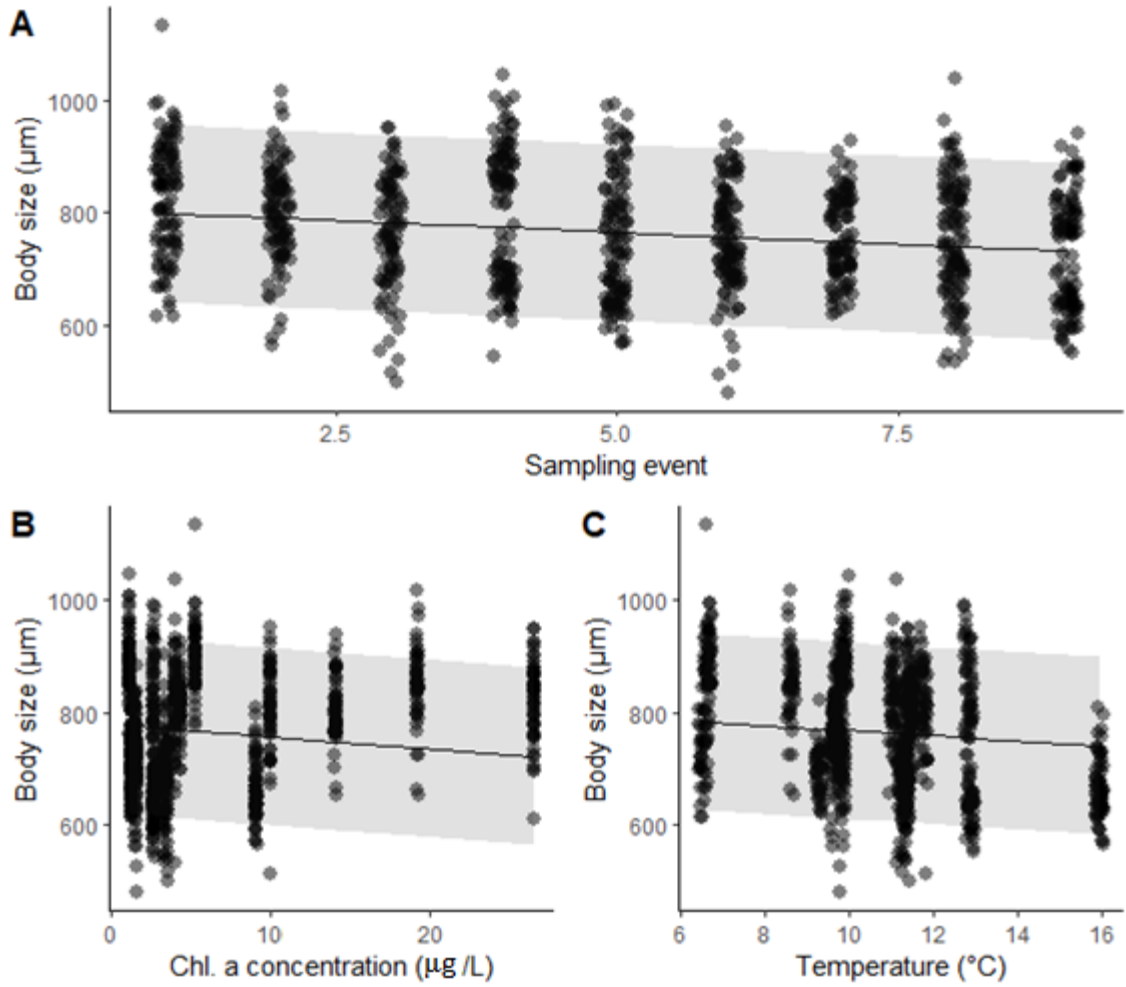
Figure 6. The best linear mixed model illustrated. **A)** Body size as a function of time- **B)** Chl. *a* concentration **C)** temperature. The grey area shows the 95% confidence interval of the prediction, and the line shows the slope based on the estimates (see Table 3).

4. Discussion

The results showed that *E. affinis* individuals sampled in Pojoviken Bay were on average 151.4 μm (21.9%) larger than the ones sampled in Storfjärden. This variance in body size was shown to be predominantly due to location rather than salinity, temperature, Chl. *a,* or time. Contrary to what was hypothesized, the relationship between body size and salinity was nonsignificant, whereas the relationship between the other predictor variables and body size was significant. However, the predictor variables only accounted for a small amount of the variance (4.82%).

4.1 Environmental factors

4.1.1 Salinity

The lack of relationship between salinity and body size is in contrast to the stated hypotheses in this study, but nevertheless, in accordance with other studies (Katona, 1970; Souissi et al. 2016; Kuismanen et al. 2020). Since it does not appear that salinity significantly affects body size, it has been shown to have a significant effect on the hatching success (Kuismanen et al. 2020) and development (Karlsson et al. 2018) of *E. affinis*. *A. tonsa* hatching success has also been shown to be affected by salinity *(*Holste & Peck, 2006). Furthermore, deviation from optimal salinity ranges can be stressful, and the increased energy spent on osmoregulation could affect enzyme activities of *E. affinis* (Cailleuad et al. 2007). Experiments have demonstrated that only the most extreme salinities, 0 and 35 PSU, affect *E. affinis* nauplii survival (Devreker et al. 2004). Similarly, experiments comparing reproduction at different salinities showed that only the highest salinity, 25 PSU, impacted reproductive parameters (Devreker et al. 2009). This suggests that *E. affinis* has a tolerance to a wide range of salinities. Furthermore, *E. affinis* seems to prefer low salinities (Michalec et al. 2010; Devreker et al. 2010), this preference could alter the spatial distribution of *E. affinis* in the Baltic Sea if salinity continues to decrease.

4.1.2 Temperature

In accordance with previous studies (Ban, 1994; Lloyd et al. 2013) a small negative relationship between temperature and body size of *E. affinis* was observed in this study, meaning that as temperature decreased, body size increased. Notably, previous studies suggest body size only seems to be significantly affected by extreme temperatures (7 °C, 24 °C) in experimental conditions (Souissi et al. 2016; Souissi et al. 2021). Similar negative relationships have also been observed in the copepods *Temora longicornis* and *Pseudocalanus elongates* (Breteler & Gonzalez, 1988). Body size has, as previously described, been shown to be a predictor of clutch size (Crawford & Daborn, 1986; Hirche, 1992; Ban, 1994; Lloyd et al. 2013; Souissi et al. 2021), indicating higher fecundity for larger females, which suggests that rising temperatures will affect fecundity. Additionally, Souissi et al. (2021) found that *E. affinis* appears to switch between a K-strategy in colder conditions, and an r-strategy in warmer conditions, suggesting trade-offs in relation to temperature changes.

Temperature is likely to affect *E. affinis* abundance, Mialet et al. 2010 found that, in nature, maximal abundances were seen in temperatures varying from 10 to 15 °C. Moreover, Jansson et al. (2020) observed that in the Gulf of Riga, as temperature increased, the amount of lower complexity organisms like rotifers and cladocerans increased, making them dominate over larger organisms such as *E. affinis*. Bradley (1991, as cited in Kimmel & Bradley, 2001) noted that seasonal succession between *Acartia tonsa* and *E. affinis* was dependent on critical salinities and temperatures, with *E. affinis* dominant at low temperatures and mid-range salinities (1 °C and 11ppt). Since temperatures are rising (IPCC 2021), these are possible future problems *E. affinis* might face.

4.1.3 Chlorophyll *a*

Chl. *a* concentration (µg/L), a proxy for food availability for the copepods, was on average higher in Pojoviken Bay, and the largest concentrations were also found here. As hypothesized, the results showed that there was a significant relationship between body size and food availability. The observed relationship was, however, negative, and a decrease in

food availability increased body size. This effect was small, and a decrease of 10 μg/L would only account for a 21 μm change in body size. These results contrast previous results where *E. affinis* body size was positively correlated to food availability (Ban, 1994), and additionally, Breteler & Gonzalez (1988) demonstrated that body size was positively correlated with food availability in *T. longicornis* and *P. elongates*. Moreover, it has been shown that calanoid copepods respond to increases in food availability with rapid reproduction (Viitasalo et al. 1995) and that food shortage has a larger effect on *E. affinis* egg production than body size (Ban, 1994).

Copepods are better equipped to tolerate environmental stress, such as low salinity, when food availability is high, for which tolerance is substantially increased when food concentrations are elevated (Lee et al. 2013). This might be beneficial for *E. affinis* in Pojoviken Bay since the extremely low salinities (<1 PSU) and high temperatures (>24 °C) could potentially be offset by high food availability, especially since Chl. *a* values were high enough to categorize Pojoviken Bay as eutrophic (Håkanson, 2008). It is not only quantity that matters, but the quality of the food is also relevant, and even if Chl. *a* concentration is high, they could be dominated by cyanobacteria like *Aphanizomenom* and *Nodularia,* which are not the preferred foods for copepods (Bouvy et al. 2001; Hu et al. 2006). Furthermore, it has been shown that cyanobacteria can negatively affect copepods: Koski et al. (1999) found that *E. affinis* fed with toxic and non-toxic *Nodularia* produced deformed egg-sacs and experienced lowered hatching success, and mortality rose with *Nodularia* ingestion. Similarly, egg production was negatively affected by ingestion of *Nodularia* in *Acartia sp,* and *Nodularia* was quite common in Storfjärden (Engström-Öst et al. 2015). Thus, food quality could be a potential explanation for the observed negative relationship between Chl. *a* and body size and potentially a reason for the difference between sites.

4.1.4 Oxygen and pH

Despite their exclusion from this study, oxygen and pH are increasingly relevant due to climate change. Ocean pH is decreasing due to acidification and future modeling suggests that the Baltic Sea may experience more than a tripling of acidity, indicating a 0.2–0.4 reduction in pH by 2100 (Havenhand, 2012).

Copepods have traditionally been considered quite robust against ocean acidification (Kurihara et al. 2004; Mayor et al. 2007; Vehmaa et al. 2013), but adverse effects such as reduction in body size and slower nauplii development have been observed (Vehmaa et al. 2016). Furthermore, the responses to ocean acidification vary by species (Engström-Öst et al. 2020), and some species like *Acartia bifilosa* and *A. tonsa* have been shown to be negatively affected by lower pH (Cripps et al 2014; Vehmaa et al. 2016). Conversely, *E. affinis* is unlikely to be sensitive to near-future levels of ocean acidification on a physiological level, as demonstrated by Almén et al. (2016).

Oxygen depletion is a common effect of eutrophication, and oxygen deficiency is already a problem in the Baltic Sea, as the extent of oxygen-deficient waters is more than ten times larger now than it was 115 years ago, and this deficiency is still rising (Carstensen et al. 2014; HELCOM, 2018b). Generally, the most apparent consequences of hypoxia occur in benthic ecosystems and organisms, whereas less attention is paid to copepods since they may avoid unfavorable conditions by migrating upward (Elliot et al. 2013; Almén et al. 2014). Copepod responses vary by species, and some species die even when exposed to short-term (9 days) anoxia, while all species from the *Cletodidae* family are seemingly tolerant to anoxia (Grego et al. 2014). Similarly, it has been shown that copepod abundance is adversely affected by low oxygen, possibly due to avoidance (Kimmel et al. 2009; Elliot, 2013). Conversely, a study by Stalder & Marcus (1997) suggests that some copepods do not effectively avoid severe or moderate hypoxia indicating that the decrease in abundance might be due to direct mortality, rather than avoidance. Even if copepods can avoid oxygen-deficient waters, this vertical shift could lead to higher mortality rates by predation (Decker et al. 2004) and some copepods may even take refuge in hypoxic waters to avoid this predation (Kimmel et al. 2009). It is possible that as stratification intensifies in the Baltic Sea, the vertical distribution of *E. affinis* will become significantly more limited and that there will be higher physiological costs associated with living higher up in the water column.

4.2 Adaptation

The results of this study showed that although the environmental factors certainly affected *E. affinis* body size, the effect was rather small. One potential for these results could be genetic adaptations.

Marine to freshwater colonization is no easy feat, but it is one that *E. affinis* has managed multiple times independently (Lee et al. 2011). Since *E. affinis* is quite tolerant of broad salinity gradients (Devreker et al. 2004,) the ability to colonize freshwater habitats has been attributed to said tolerance (Wolff, 2000), however, it has been demonstrated that they might not be so broadly tolerant but rather that they are experiencing evolutionary adaptations. These genomic adaptations have trade-offs, and the populations experience a reduction in high-salinity tolerance in conjunction with an increase in their low-salinity tolerance (Lee et al. 2003). Therefore, there is not simply a broad tolerance, but a shift in the optimal salinity of *E. affinis*.

Shifts in ion-motive enzyme activity (V-type H^+ ATPase, Na^+/K^+-ATPase), which is thought to be critical for freshwater adaptations, have been observed to occur rapidly in *E. affinis* (Lee et al. 2011) and is a prime example of *E. affinis* adaptations. Dominance reversal is when an allele is completely or partially dominant in selective circumstances in which it is favored and recessive in contexts in which it is harmful (Connallon & Chenoweth, 2019). Beneficial dominance reversal has been observed in *E. affinis* and this could be a pivotal mechanism in maintaining genetic variance, aiding it in salinity tolerance and further enhancing *E. affinis's* ability to rapidly adapt (Posavi et al. 2013).

Moreover, it has been shown that multiple invasive populations have responded to selection through parallel use of the same single-nucleotide polymorphisms and genomic loci (Stern & Lee, 2020). This indicates that this process is imitable and repeatable and that *E. affinis* will likely continue its freshwater colonization.

4.3 Taxonomy

As previously discussed, *E. affinis* shifts from marine to freshwater habitats has microevolutionary implications, but it can also have macroevolutionary implications. Rapid evolution has been demonstrated in *E. affinis* and it could, potentially, lead to rapid speciation. This has been observed in populations on the Pacific coast of the USA which are reproductively isolated from their ancestral populations on the Atlantic coast (Lee, 2000; Lee, 2002).

In the early stages of speciation, sibling species have been known to occur. Sibling species are often morphologically indistinguishable but different ecologically (Sukhikh et al. 2013), and in some cases, these genetic differences are only clear in sympatry (Knowlton 1993). Molecular genetic diagnosis is needed to identify sibling species, and analysis of *E. affinis* from the Baltic Sea has revealed the presence of two *E. affinis* clades, one introduced from North America and one native to Europe (Aleksev et al. 2009). These two clades have been defined as two different species, *E. carolleeae* and *E. affinis (*Alekseev & Souissi, 2011) between which the nucleotide difference is as high as 15%. Furthermore, this new invasive *E. affinis* form has recently increased in abundance in the Gulf of Finland (Sukhikh et al. 2013), but if it occurs off the Hanko peninsula is yet unknown.

A comparative study conducted on *E. affinis* populations in North America and Europe revealed that fitness, meaning egg production and reproductive lifespan, was far higher in the North American populations than those in Europe (Beyrend-Dur et al. 2009). The difference in fitness could be a potential explanation for this increase in abundance, and *E. carrolleeae* will likely be more widely distributed in the future (Sukhikh et al. 2013), particularly since *E. carrolleeae* population densities seem unaffected by heatwaves in contrast to *E. affinis* populations (Sukhikh, 2019). However, both coexistence and segregation have been observed in North American *E. affinis* clades, which is probably due to clade-specific physiological tolerances and food source conditions (Favier & Winkler, 2014).

The introduction of sibling species and the potential forming of intermediate forms of said species, as observed in the Caspian Sea (Sukhikh & Alekseev, 2013), can have major

implications for the future of *E. affinis* and could be an explanation for the large variation between study sites observed in this study.

4.4 Error sources and future considerations

In general, there were few error sources, but those that were encountered will be addressed in this section. One of the potential error sources was the variation in sample size (see table 2). The aim was to acquire 50 individuals per sampling event per study site, but this was not always possible. For instance, during sampling event 1 (see table 1), 57 individuals were collected in Pojoviken Bay which was due to the sheer number of individuals in the sample, all visible individuals were selected to avoid simply choosing 50. Conversely, only 40 individuals were sampled from Storfjärden, due to the lack of individuals available in the sample, and similar issues were encountered during sampling event 3. However, there was only a total sample size difference of 36 individuals and most of this difference occurred during the aforementioned sampling events and is unlikely to have skewed the results significantly.

Linear mixed models using sampling site as a random effect were used in the present study, the motivation for this was to account for pseudoreplication. However, if the random effect were to be excluded, and sampling sites were simply viewed as a grouping variable, salinity would explain most of the observed variation. Due to the absence of overlap in salinities, the sampling site and corresponding salinities become synonymous and the within-group variation in response to salinities was insufficient in explaining *E. affinis* body size. This choice will naturally lead to salinity showing a smaller effect than what might be expected, and the study site becomes the main predictor. This choice could have resulted in an underrepresented effect of salinity, this choice was, however, motivated by *E. affinis* being euryhaline (Walter & Boxshall, 2021) and by the fact that previous studies suggest that *E. affinis* body size is relatively unaffected by salinity (Katona, 1970; Souissi et al. 2016; Kuismanen et al. 2020), further suggesting that salinity is unlikely to be a driver of large variations. A potential solution for this issue would have been to include one or more additional sampling sites which would have added more random effects and a potential overlap in mean salinities.

The variation in body size between study sites was large, and it is unlikely that a difference this large could be caused by the environmental factors alone, which was supported by the statistical analyses. Therefore, genetic comparisons, such as nucleotide sequencing, should be conducted to identify what is behind this stark difference in body size. Experimental and multigenerational studies on these contrasting populations could, furthermore, provide useful information regarding their climate responses. Lastly, factors such as abundance, egg production, and nauplii development should be examined to further our understanding of *E. affinis* climate responses.

5. Conclusions

This study revealed that temperature, time, and Chl. *a* had a significant effect on the ecology of *E. affinis,* while salinity did not have a significant effect. However, the effects of these variables were small, suggesting that *E. affinis* is indeed robust against climate change as has been demonstrated in previous studies (Devreker et al. 2004; Almén et al. 2014), especially regarding salinity. Furthermore, *E. affinis* sampled in Pojoviken Bay, the area representing potential future conditions, were significantly larger, which reinforced this perception of *E. affinis* robustness. Still, body size is not the only thing potentially affected by the environmental factors, and the Pojoviken Bay population might suffer in terms of hatching success (Kuismanen et al. 2020), development (Karlsson et al. 2018), and overall abundance (Mialet et al. 2010). Conversely, since body size is positively correlated with clutch size (Crawford & Daborn, 1986; Hirche, 1992; Ban, 1994; Lloyd et al. 2013; Souissi et al. 2021), the Pojoviken Bay population is unlikely to be adversely affected in terms of fecundity.

Comparisons between study sites revealed that the variation in body size was primarily due to location, not environmental factors, indicating that there could be some unmeasured factors at play. This variation in body size could, for example, be explained by genetic adaptations, such as shifts in ion-motive enzyme activity that are important for freshwater species (Lee et al. 2011). Moreover, the stark contrast in body size opens up the possibility of these two populations being similar but separate, sibling species. *E. affinis*

sibling species have recently been observed in both the Baltic Sea (Sukhikh et al. 2013) and the Caspian Sea (Sukhikh & Alekseev 2013), and sibling species are relatively common in marine environments (Knowlton 1993). Therefore, it is not unreasonable to suggest that the *E. affinis* population in Pojoviken Bay might be a sibling species better suited for freshwater life.

This study suggests that *E. affinis* is unlikely to be a major loser of climate change. However, as sibling species, such as *E. carolleeae,* or other sibling species, increase in abundance, native *E. affinis* populations could be threatened by the interspecific competition presented by these. Thus, future research is needed to determine whether the population in Pojoviken Bay is a sibling species, or if this change could be driving native *E. affinis* populations to produce evolutionary adaptations. However, since temperature and Chl. *a* still had a significant effect on body size, they should not be discounted. Chl. *a* might prove especially important, as it could allow *E. affinis* to better tolerate extreme conditions (Lee et al. 2013). Therefore, experimental studies, such as long-term studies comparing the two populations should be conducted, as it might elucidate the strength of the effects. Nevertheless, genetic research could prove vital when comparing these populations.

Acknowledgments

Summary in Swedish – Svensk sammanfattning

Effekten av salinitet, temperatur och födotillgång på hoppkräftan *Eurytemora affinis* ekologi

I samband med klimatförändringen förväntas stora förändringar i Östersjöns abiotiska och biotiska egenskaper. Salinitet förväntas minska både på havsytan och havsbotten (Helama et al. 2018; Mohrholz et al. 2015) men nyare studier indikerar att det finns osäkerhet angående salthalt (Meier et al. 2022). Temperatur och övergödning (och således Chl. *a*) förväntas samtidigt öka (HELCOM, 2018a; HELCOM, 2018b; HELCOM, 2013). Dessa förändringar förväntas negativt påverka biodiversiteten Östersjön (Ojaveer et al. 2010) samt djurplankton diversiteten (Kuismanen et al. 2020).

Djurplankton är en väsentlig del av marina födovävar på grund av deras abundans och viktiga ekosystem roller. Av alla djurplankton är hoppkräftor vanligast. Hoppkräftor är de vanligaste multicellulära organismerna på jorden (Richardson 2008), och är viktig föda för bl.a. fiskyngel (Engström-Öst et al. 2015), och därmed kan mängdförändringar leda till stora "bottom-up" effekter (Richardson 2008).

Eurytemora affinis är en vanlig hoppkräfta i många estuarier och andra brackvattenområden på norra halvklotet (Kuismanen et al. 2020) och i Östersjön är *E. affinis* en av de viktigaste hoppkräftorna (Hernroth & Ackefors, 1979; Viitasalo 1992).

Hoppkräftor anses vara relativt tåliga mot klimatförändringen eftersom de migrerar vertikalt inom vattenpelaren (eng. water column), vilket tvingar dem att uppleva stora fysikaliska och kemiska gradienter som salthalt- och temperaturgradienter (Almén et al. 2014). *E. affinis* är inget undantag, och upplever samt tolererar stora salthalts-, temperatur, och pH gradienter (Kurihara et al. 2004; Mayor et al. 2007; Vehmaa et al. 2013; Almén et al. 2016).

Syftet med denna avhandling är att undersöka hoppkräftan *E. affinis* reaktion på miljöfaktorer i Östersjön. Detta gjordes genom att studera sambandet mellan funktionella egenskapen (eng. functional trait) kroppsstorlek och salthalt,

temperatur samt Chl. *a* på två områden, ett område som liknar Östersjöns framtidsscenarier och ett område som är mer likt nuläget.

Hypoteserna i min avhandling är:

H1: Kroppsstorlek varierar mellan undersökningsområdena.

H2: Temperatur förväntas ha en negativ effekt på kroppsstorlek, medan Chl. *a* och salinitet förväntas ha en positiv effekt.

H3: Kroppsstorlek varierar över tid, och förväntas vara större under tidig sommar (Daufresne et al. 2009)

Undersökningsområdena för avhandlingen var Pojoviken och Tvärminne vid Hangö halvön. För att besvara på hypoteserna utfördes totalt 18 (9 per undersökningsområde) djurplankton- och vattenprovtagningar, två gånger per månad mellan 10 maj 2021 och 2 september 2021.

Djurplanktonproverna samlades med ett 200 μm nät som sänktes till 30 m djup och sedan lyftes till ytan. Djurplanktonproverna undersöktes därefter i mikroskop, där 50 (om möjligt) *E. affinis* honor med äggsäckar sorterades och plockades, individerna bevarades och deras kroppsstorlek mättes senare med en mikroskop kamera. Vattenproverna togs m.h.a. en Limnos vattenprovtagare, dessa prover togs i 5 m intervaller från ytan till 30 m djup varefter temperatur och salinitet mättes med en VWR salinitetsmätare. Chl. *a* vattenprov togs vid 5 m djup med samma Limnos vattenprovtagare, 100 ml av vattenprovet filtrerades genom glasfiberfilter, och Chl. *a* bestämdes senare genom fluorometri och rapporterades som μg/L. Data från provtagningarna analyserades i R med linjära modeller, linjära blandade modeller (LMM) användes för att undersöka förhållandet mellan kroppsstorlek och tid, temperatur, salinitet samt Chl. *a*. Ytterligare användes en generaliserad linejär modell (GLM) för att undersöka sambandet mellan kroppsstorlek och undersökningsområde. För analyserna inkluderades tid (datum), salthalt, temperatur och Chl. *a* som fasta effekter och undersökningsområdena som slumpmässig effekt.

Totalt mättes 952 *E. affinis* honor, 494 från Pojoviken och 458 från Storfjärden. Analyserna visade att individerna i genomsnitt var 21,9 % större i Pojoviken, och att de

var större i Pojoviken under alla provtagningar. Dessutom var individer samlade under våren i genomsnitt större. Den generaliserade linjära modellen visade att sambandet mellan undersökningsområde och kroppsstorlek var starkt signifikant. De linejära blandade modellerna visade att sambandet mellan kroppsstorlek och temperatur, tid samt Chl. *a* var signifikant, men att det inte fanns något signifikant samband mellan kroppsstorlek och salthalt. För övrigt visade modellerna att undersökningsområdet förklarade 72,17 % av variationen i kroppsstorlek, medan temperatur, tid och Chl. *a* endast förklarade 4,82 % av variationen.

Resultaten förstärkte hypoteserna och kroppsstorlek varierade mellan område (H1) och över tid (H3), och denna storleksskillnad var signifikant. Däremot förväntades ett större samband mellan kroppsstorlek och salthalt, eftersom *E. affinis* verkar föredra låga salthalter (Devreker et al. 2010), men liknande mönster har observerats i tidigare studier (Katona, 1970; Souissi et al. 2016; Kuismanen et al. 2020). I enighet med hypoteserna var sambandet mellan kroppstorlek samt chl. *a* signfikant. Sambandet mellan kroppsstorlek och temperatur liknade tidigare studier (Ban, 1994; Lloyd et al. 2013) och en minskning i temperatur ledde till en liten ökning i kroppsstorlek. Till skillnad från tidigare studier (Breteler & Gonzalez ,1988; Ban, 1994) hade Chl. *a* en negativ effekt. Chl. *a* koncentrationer var dock betydligt högre i Pojoviken, där kroppsstorlek var större. Därmed är det sannolikt. att Chl. *a* har en positiv påverkan, men endast till en viss punkt, eller att det är frågan om mat av dålig kvalitet, exempelvis cyanobakterier som inte föredras av hoppkräftor (Bouvy et al. 2001; Hu et al. 2006). Att individerna från Pojoviken var betydligt större, trots de extrema förhållandena, kunde bl.a. bero på att individerna har ökad tolerans för extrema förhållanden (Lee et al. 2013) när Chl. *a* koncentrationer är höga. Det är även möjligt att *E. affinis* populationen i Pojoviken genetiskt anpassat sig till rådande miljön (Lee. 2003; Lee et al. 2011; Posavi et al. 2013) eller att det är frågan om en större syskonart (Alekseev & Souissi, 2011; Sukhikh et al. 2013).

Resultaten stöder idéen att *E. affinis* är tålig mot klimatförändringen, men ifall syskonarter eller dylika ökar i mängd, kan detta hota *E. affinis* urpsrungsarter. Men fortsatta undersökningar, som genetiska analyser, långtidsanalyser och experiment, krävs för att få en fullständig bild av *E. affinis* i en förändrande Östersjö.

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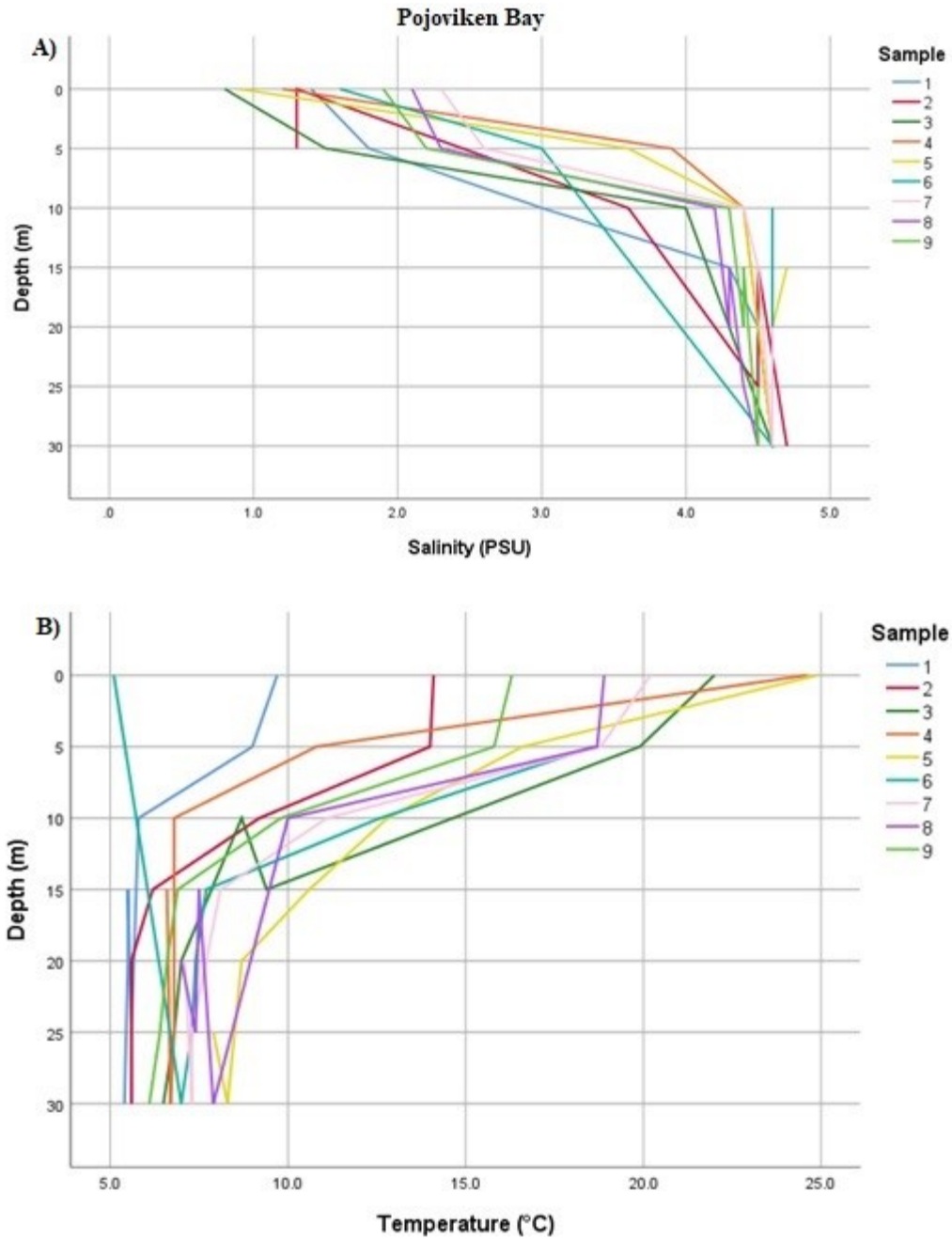
Appendix

Figure A. Environmental profiles of A) salinity and B) temperature during all sampling events in Sällvik, Pojoviken Bay. Measurements were taken at 5 m intervals (0-30 m). Sampling events correspond to a date (see Table 1).
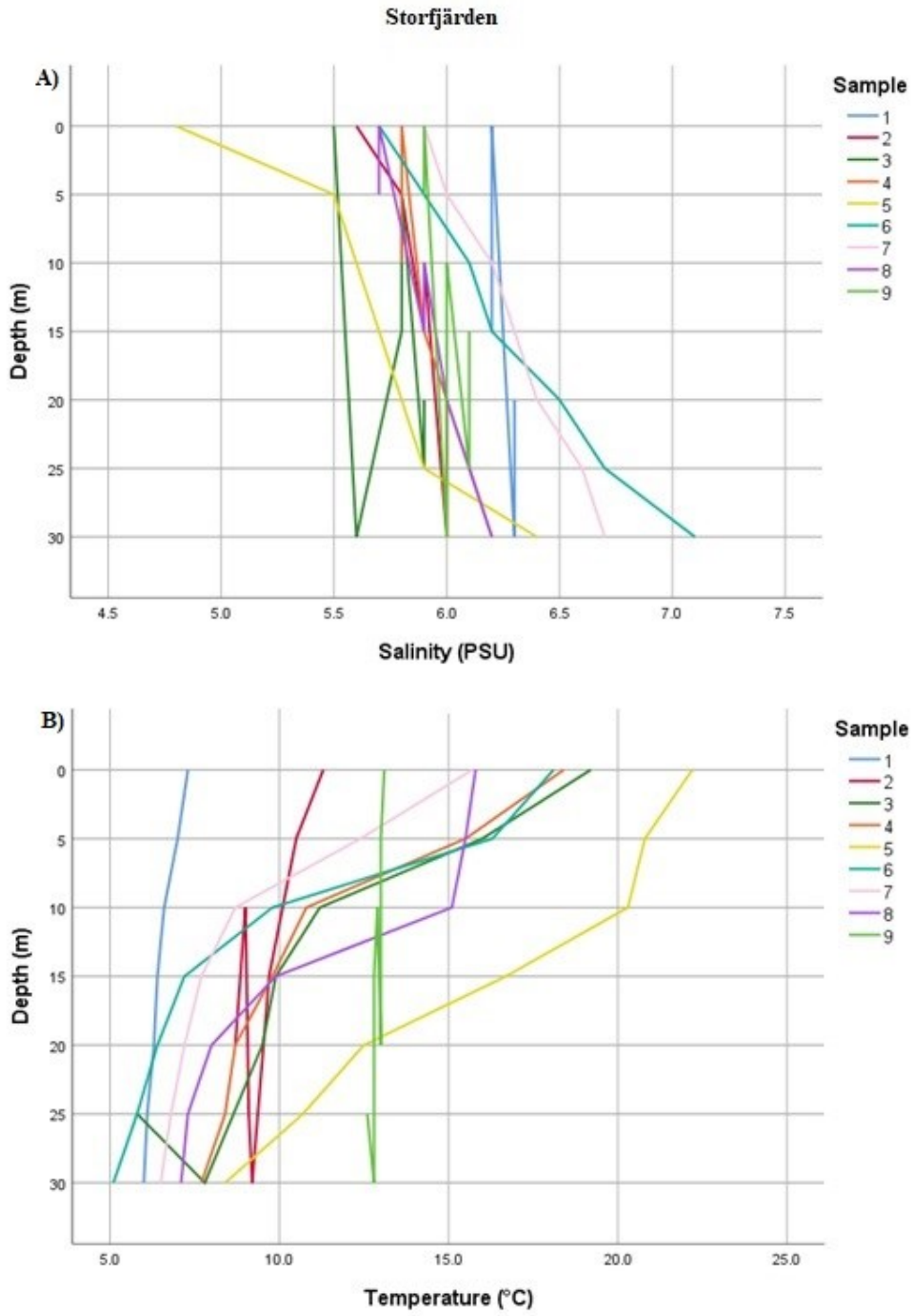
Figure B. Environmental profiles of A) salinity and B) temperature during all sampling events in Storfjärden, Tvärminne. Measurements were taken at 5 m intervals (0-30 m). Sampling events correspond to a date (see Table 1).