CLIMATE CHANGE DRIVEN HYPOSALINITY AS A SELECTIVE AGENT IN THE LITTORAL MESOHERBIVORE *IDOTEA BALTHICA*

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Abstract

Climate change will include a decrease in seawater salinity in the Baltic Sea. We quantified the effects of the projected future desalination on survival of the early life stage of the littoral herbivore *Idotea balthica*. We collected egg-bearing *Idotea* from three range-margin Baltic Sea populations, we exposed half of each brood to either current (6 ‰) or future salinity (3.5‰). We genotyped a subsample of each brood to analyse patterns of allelic change and to identify genomic regions targeted by selection. The survival was overall reduced by hyposalinity and broods varied in response to hyposalinity implying genetic variation in tolerance, with a stronger decrease in genetic diversity in future salinity. Finally, we identified proteins with crucial roles in basic cellular functions. This study indicates that projected future northern Baltic Sea hyposalinity will not just hamper *I. balthica* survival, but its selective pressure may also affect genetic diversity and cell physiology.

Keywords: climate change, herbivore, genetic diversity, isopods, salinity, RADseq

Introduction

Projections of future climate describe the expected changes in marine abiotic conditions on a global scale (Stocker et al., 2013), and there is increasing evidence on how these changes affect the life and distributions of aquatic organisms and ecosystem functions (Beaugrand and Kirby, 2018; Brierley and Kingsford, 2009; Doney et al., 2012; Knights et al., 2017; Sorte et al., 2010). Most marine climate research has focused on warming and ocean acidification (Hansen et al., 2006; Sunday et al., 2011; Wernberg et al., 2012), but future climate will also modify salinity of many coastal areas through variation in precipitation and evapotranspiration (Boyer et al., 2005; Durack et al., 2012). A shift in water salinity could have important consequences on marine organisms, possibly reshaping their distribution ranges (Kefford et al., 2007; Kinne, 1993; Kirst, 1989).

Climate change modifies the selective environment of marine species and often reduces population sizes thereby hampering allelic variation for specific genomic loci (Charlesworth et al., 1997). As reviewed by Pauls et al. (2013), such reduction in genetic variation is driven by two alternative but not mutually exclusive processes. The first is a shift in the distributional range and a decrease in population size, particularly at the trailing range edge facing the most challenging conditions, and the consequent random reduction of genetic variation because only a portion of the original genetic pool remains. Since the potential for adaptation relies on the overall genetic variation upon which the selection can act, such a genetic drift can be deleterious population persistence (Hoffmann and Sgrò, 2011). The second is the change in local selection regimes, and a micro-evolutionary change in allele frequencies as a response to selection. When the local conditions change, selection may lead to locally adapted, differentiated populations and/or development of phenotypic plasticity (Pauls et al., 2013). As a consequence the most advantageous alleles become fixed, thus reducing the overall genetic variation, also known as a selective sweep (Braga et al., 2019). Thus, genetic variation may diminish due to both random and deterministic processes. Population sensitivity

to environmental stressors and its potential to adapt to changing environment are strongly associated with the overall genetic variation, and its loss can therefore be a threat for species' persistence under future climate changes (Nowak et al., 2009). Furthermore, high amount of genetic variation implies high level of heterozygosity which by counterbalancing the occurrence of deleterious mutations is expected to increase the viability of a species (Chapman et al., 2009).

Despite multiple studies indicated that evolutionary changes due to climate change might happen on a short gap of few generations, there is little evidence from experimental studies that these microevolutionary responses are actually occurring (Gienapp et al., 2007). Modern molecular biology implements the use of restriction site-associated DNA sequencing (RADseq) to produce huge numbers of polymorphic markers to understand how and why species evolve their genetic background (Lowry et al., 2017). For example, in the Atlantic cod *Gadus morhua*, Poćwierz-Kotus et al. (2014) used SNPs to show how salinity could be the major driver of genetic clustering between populations of western and eastern Baltic. In the Atlantic herring (*Clupea harengus*), Guo et al. (2016) highlighted hundreds of loci suggesting that the genetic differentiation between populations form Atlantic and the Baltic Sea is the result of a long term adaptation to low salinity and selection for hyposalinity tolerance. In the isopod *Idotea balthica*, De Wit et al. (2020) showed a strong pattern of genetic structure within the Baltic Sea together with a decrease of heterozygosity among populations, likely driven by the salinity gradient. Laboratory studies attempting to identify molecular markers linked to tolerance to environmental changes are still an uncommon approach. Among these studies, Feng et al. (2019) challenged the swimming crab *Portunus trituberculatus* with short term hyposalinity stress and identified from the survivors SNP loci responsible for adaptation to low salinity resistance.

Climate change may also enforce alterations in littoral communities through changes in biotic interactions and, consequently, impede functioning of the ecosystem (Blois et al., 2013). Among such biotic interactions, top-down regulation of producers through herbivory is particularly important for

littoral ecosystem function as herbivory on both macrophytes and microalgae has the potential to regulate producer abundance, production and community composition (Hillebrand 2009; Poore et al. 2012; Verges et al. 2016). Climate change may affect herbivory function in several ways, both directly by affecting the herbivore performance and indirectly through changes in herbivore community and in food availability and quality (Kotta et al., 2019; Poore et al., 2013; Rothäusler et al., 2017; Rugiu et al., 2018; Vergés et al., 2014). Knowledge on how herbivory function will be affected by the climate change has just started to build up, and such understanding is essential for predicting changes in structure and function of littoral communities.

In the brackish Baltic Sea, the isopod *Idotea balthica* (Pallas, 1772) is abundant in macrophyte stands such as *Fucus* spp. (Haahtela, 1984). *I. balthica* is an euryhaline species, capable of withstanding a broad range of salinity going from 33‰ in the North Sea to 2.7 ‰ in the eastern and northern Baltic Sea where the distributional limit of this species is limited by hyposalinity (Leidenberger et al., 2012). In these regions, *I. balthica* face both annual and seasonal variation in seawater salinity that can range 1-6 ‰ in the Gulf of Finland and 4-7‰ in the Gulf of Bothnia due to seasonal and annual variation in the ratio between precipitation and evaporation (Snoeijs-leijonmalm et al., 2017). Baltic populations of *I. balthica* rely mostly on *F. vesiculosus* and filamentous algae as food source, thus exerting strong top-down control on some of the most important Baltic macrophytes (Jormalainen and Ramsay 2009; Haavisto and Jormalainen 2014). The grazing impact of *I. balthica* may also modify the competition between perennial macrophytes and ephemeral algae, as its grazing pressure might help the perennial ones to fight epibiotism (Goecker and Kåll, 2003; Orav-Kotta and Kotta, 2004). Hence, *I. balthica* is a key littoral herbivore and as such any change in its abundance and/or distribution might have consequences on the community composition, production and energy transfer of littoral food webs.

In the present study, we tested how the projected desalination in the northern Baltic Sea affects survival of *I. balthica*, using newly-born juveniles, the putatively most sensitive life-history stage to

111 osmotic stress. We focus specifically on the occurrence of genetic variation in tolerance and the impact
112 of hyposalinity on selection and genetic variation. For this, we exposed laboratory-born broods of
113 juveniles from three populations originating near the distribution range-margin of the species to current
114 (6 ‰) and future projected salinity (3.5 ‰, Meier et al. 2006). This allowed us to estimate the mortality
115 effect of desalination, geographic variation in tolerance among populations and existence of genetic
116 variation in tolerance within populations. Further, we estimated the consequences of hyposalinity
117 treatment to allelic diversity using single nucleotide variation in genome sequences. Finally, we
118 identified some genes in the regions involved in allelic loss, which could be possible targets of natural
119 selection.
120
121 2. **Material and Methods**
122 *2.1. Sample collection*
123 We sampled *I. balthica* from three populations (Närpes = N, Rauma = R and Parainen = P)
124 between the 8th and the 18th of June 2016 in the low salinity northern parts of the Baltic Sea, at the edge
125 of the distributional range for this species (Fig. 1-a,c). From each population, we collected randomly 40
126 (for population P) to 45 (for population N and R) gravid females from a *F. vesiculosus* stand and
127 transported them in cooling boxes to the Archipelago Research Institute (University of Turku) at Seili
128 (60° 14’ N, 21° 58’ E). We stored the gravid isopods individually in glass jars (0.5 L) with 6 ‰ local
129 seawater, aeration and we kept the room temperature at 18° C. We provided *F. vesiculosus* and
130 *Cladophora spp*. as food and substrate and replaced both water and food every 5 days. We checked
131 daily each jar to keep track of the emerging juveniles. When the juveniles emerged from the marsupium
132 (Fig. 1-b,c), we counted them and separated them from the mother keeping each brood in its own jar.
133 The average number of juveniles per brood was 25 ± 9.2 for N, 22 ± 12 for P and 23 ± 6 for R. We

included only the broods with at least 30 juveniles in the present study. We got a total of 42, 21 and 31 such broods from the populations of N, P and R, respectively.

On the 02-Aug-2016 the experiment started. We split each brood into two batches, each one in a 0.25 L jar, filled with water at 6 ‰ salinity gathered locally (Fig. 1-c). This salinity is within the average ambient summer salinity for this region (Meier et al., 2006), and we marked each jar to keep track of the broods. One half of each brood was kept in 6 ‰ while the other one was gradually acclimated to hyposalinity (3.5 ‰) by reducing salinity by 0.5 ‰/day. We chose the level for the hyposalinity treatment based on the 2.5 ‰ decrease in summer surface seawater salinity expected by climate model predictions for the northern marginal region of the Baltic Sea by 2070-2099 (model RCAO-ECHAM-A2-REF, Meier & Eilola 2011).

We monitored the survival (number of alive juveniles present in each jar) daily and replaced water and food (freshly collected *Cladophora spp*.) every five days. We ended the experiment after 22 days, when 70 % of the juveniles exposed to 3.5 ‰ hyposalinity had died (Fig. 1-c). We stored juveniles from each brood in ethanol at both the start and end of the experiment: 10 juveniles at start, and all the remaining alive ones at end of the experiment in both the current and future salinity. Samples were stored at -20°C and then sent to Tjärno Sven Lovén Center (Sweden) for the DNA extraction and Illumina 2b-RAD sequencing (Fig. 1-c).

2.2 Bioinformatic analysis

We prepared 2b-RAD libraries (Wang et al., 2012) from the DNA using a modified version of the laboratory protocol developed by Mikhail Matz, available at: https://github.com/DeWitP/BONUS_BAMBI_IDOTEA. In brief, we extracted DNA from a pool of juveniles, using a DNeasy Blood & Tissue kit (Qiagen) with a standard protocol, including an RNAse treatment at the end of the tissue lysis step. The pool at start of the experiment composed of about 10

158 individuals from randomly selected ten broods, separately for the two populations (N) and (R). The
159 broods from the third population P were not included in this analysis due to their initially low number in
160 the experiment and the high sequencing cost of adding a third pool. At the end of the experiment, we the
161 pooled all remaining survivors, separately for the current and future conditions and the two populations
162 (numbers are given in supplement 1). We chose to use broods from these two populations as the
163 survival analysis did not indicate any among-population variation in tolerance to salinity and we had a
164 higher number of broods from these populations than from the third population. The DNA template
165 (100-200 ng) was fragmented using the type 2b endonuclease enzyme BcgI, after which adapters were
166 ligated to the ends of the excised 36-bp fragments. Fragments were then amplified with barcoded
167 adapters, after which they were pooled equimolarly into three 24-sample pools (20 broods + 4 technical
168 replicates). All pools were sequenced in an Illumina HiSeq 2500 machine, 50bp single-end, at the
169 Swedish National Genomics Infrastructure's SNP & SEQ platform at Uppsala University.
170 All bioinformatic analyses were run on the University of Gothenburg computer cluster 'Albiorix'
171 (http://albiorix.bioenv.gu.se/). All commands used in the analyses can be found here:
172 https://github.com/DeWitP/BONUS_BAMBI_IDOTEA. First, Illumina adapters and poor-quality read
173 ends (Q < 20) were clipped and trimmed using the fastx toolkit
174 (http://hannonlab.cshl.edu/fastx_toolkit/index.html). A draft genome assembly for *I. balthica* (NCBI
175 BioProject: PRJNA599581) was used as a reference for mapping the 2b-RAD data, using bowtie2
176 (Langmead and Salzberg, 2013) (Information for the genome project can be found at
177 https://github.com/The-Bioinformatics-Group/Idotea_genome_project). The resulting alignment files
178 were sorted using samtools (Li et al., 2009). As the numbers of juveniles in many of the broods were too
179 low for accurate estimation of allele frequencies, we merged the data from all broods within the
180 populations (N and R) and within the sampling points (the start of the experiment, after 22 days in
181 current salinity and after 22 in future salinity; Table 1) using samtools merge, after which the pooled

data was analysed using the Popoolation2 pipeline (Kofler et al., 2011). In brief, the samtools mpileup tool was used to count numbers of the four different nucleotides as well as gaps in reads mapping to each position in the reference (using a nucleotide Q > 10 as a cutoff point for counting a nucleotide), after which this information was passed on only for the sites with two alleles present and a minimum sequencing coverage of 20 into a counts file for downstream analyses.

The counts file was further filtered to include only 26597 sites where two alleles were present in both populations at the start point (minor allele read frequency > 0.05%, in order to exclude sequencing errors), in order to examine differences in allele loss between current and future salinity. These loci were tentatively annotated as "genic" or "not genic" using the automated annotation associated with the draft genome assembly used for mapping. The number of alleles lost in each treatment and population, as well as lost alleles shared by both populations, were assessed by counting loci for which no reads with alternative alleles were found. We then examined the probability of presence of the second allele for each count (as a measure of allelic loss) by assigning a "1" to each brood if the second most common allele was present and a "0" if it was not and only one allele was present for each locus (see "statistical analysis" below). We used a G^2 test to check whether the amount of shared and not shared alleles lost in both populations was dependent on the salinity condition.

Further, we subsequently applied a stringent filter, keeping only loci where both alleles remained in both populations in the current salinity (to control for laboratory selection), but where one had been lost in both populations in the future treatment. These were considered to be the prime candidate targets of natural selection due to low salinity. Nucleotide sequences ("contigs") from genomic regions surrounding these SNP loci were extracted from the draft genome assembly, after which filtered contigs were searched for evidence of possible open reading frames (ORFs) by using the web search within NCBI database (https://www.ncbi.nlm.nih.gov/orffinder/) with the default options within

205 "refseq_protein" and "Swissprot" databases for the taxon Arthropoda (taxid: 6656). We considered
206 significant only the protein matches with e-value < 0.05.
207 The raw data have been submitted to NCBI database (BioProject PRJNA668696).
208
209 *2.3 Statistical analysis*
210 Statistical analysis were performed using R version 3.5.2 (R Development Core Team, 2018).
211 Binary responses in terms of survival and probability of presence of the second allele (hereafter allelic
212 loss) for each contig were investigated using generalized linear mixed models (GLMERs). We fit each
213 GLMER by maximum likelihood with Laplace Approximation implemented within the R package
214 "lmer4" (Bates et al., 2015). For the survival response, we included in the model Salinity (two levels:
215 current and future salinity) as a fixed factor and Population and brood, nested under Population, as
216 random factors, and the interactions of the fixed and random factors. For the analysis of allelic loss, we
217 included Salinity (fixed factor) and Population, and their interaction. Individuals within brood were used
218 as replicates in the survival analysis, individuals pooled within populations were the replicates in the
219 allelic loss analysis. In both cases, after constructing the full models, we removed the non-significant
220 factors sequentially by using the Akaike information criterion (AIC) to select the best model (Carey &
221 Wang 2001). The significance of fixed effects on both the survival and allelic loss was tested using a
222 Wald test. The significance of the random effects and the random-by-fixed effect interactions was tested
223 by X^2-tests of the differences in the 2 × log likelihood value of that factor included versus excluded
224 from the model (likelihood ratio test Bolker et al. 2008). In all models, we estimated the marginal (R^2_m)
225 and conditional (R^2_c) coefficients of determination that describe the variance explained by the fixed
226 factors alone and both the fixed and random factors together, respectively, following the procedure
227 given by Nakagawa and Schielzeth (2013).
228

3. Results

3.1 *Survival of Idotea*

After 22 days, we found a negative effect of hyposalinity on survival, as 76.9% of juveniles survived in the current while only 33.5% survived in the future hyposalinity (Table 2). The three populations did not differ in survival and all the three populations were equally hampered by hyposalinity (Fig. 2, Table 2). However, broods within populations differed in their survival (Table 2) and, most interestingly, also their responses to hyposalinity varied (Fig. 3, Table 2). The statistical model explained 43% of the variation in survival ($R^2_c = 0.43$), of which 23% was explained by Salinity ($R^2_m = 0.23$).

3.2 SNP analysis

The 2b-RAD libraries generated on average 7.57 ± 5.72 (μ ± SD) millions of reads per brood. The mapping rate was 59.7 ±12.3%, and the sequencing depth was 41.7 ± 31.2 % (Table S1).

The analysis of allelic loss included a total of 26597 alleles, and the Salinity explained 14.9% of the genetic variation in the probability of presence of the second allele ($R^2_m = 14.9$, $R^2_c = 0.16$). Future salinity reduced the probability of presence of the second allele across both populations, and, overall, the second allele was lost in 0.7 % of broods exposed to the current salinity and 3% in the ones exposed to the future salinity (Table 2). However, the model indicated that allelic loss did not differ among the two populations included into this analysis (Table 2). Further, the amount of shared alleles lost in both populations was dependent on the salinity (contingency table test: $G^2 = 10.9$, DF=1, $p < 0.001$) and the number of lost shared alleles was higher for the survivors to the future conditions than for current ones, being respectively 8.4 % and 3.4 % (Fig. 4a-b). Out of the 26597 loci, 760 (2.85 %) were associated with genic regions according to the preliminary genome annotation. Within the alleles lost in future conditions (shared by both populations) the corresponding proportion was 2.5 % (3 / 120), and thus does

not indicate any overrepresentation of coding regions in lost alleles. It is worth noting here though, that the genome sequence is highly fragmented and not well annotated.

The stringent filtering for loci putatively affected by selection identified 91 SNPs where the second most common allele was lost in both populations in the future climate treatment but remaining in both populations in the current salinity treatment. These SNPs were located in 84 different genomic contigs, for which we found a significant match with the ORFs for 51 proteins, with an ORF's length of 582 ± 589 nucleotides and 193 ± 196 amino acids (mean ± SD, Table S2). We divided the proteins manually into four functional categories: cellular responses, regulation of the cell function, protein synthesis, and intra- or extra-cellular signalling. The highest number of proteins were attributed to cellular responses, while the number of proteins attributed to cellular signalling was the lowest (Fig. 4c, Table S2).

4. Discussion

Our study shows that *I. balthica* juveniles from all three populations poorly acclimate to 3.5 ‰ hyposalinity, thus indicating that the predicted future desalination enforced by the climate change could be beyond their tolerance range. Previous studies, based on performance of adult specimen, describe broad salinity tolerance of the species. Indeed, laboratory experiments indicate that adults can withstand quick salinity variation from 25 to 7 ‰ (Horlyck, 1973) as well as long-term ones from 10 to 5 ‰ (Wood et al., 2014). Poor survival of adult individuals in the simulated projected future salinities, in combination with warming, has been shown for populations round the Baltic Sea (Rugiu et al., 2018). However, osmotic tolerance may differ throughout the development of invertebrates, and juvenile life stages are likely to be more sensitive than adults. This is supported by previous studies showing lower survival in hypersaline water in young than adult stages of freshwater gastropods and mites (Kefford et al., 2007). Also, the efficiency of osmotic regulation, both to hypo- and hypersalinity, might develop

277 throughout the life stages as shown for the isopods *Cyathura polita* (Kelley and Burbanck, 2006) and
278 *Sphaeroma serratum* (Charmantier and Charmantier Daures, 1994). Further, the acclimation to
279 variations in water salinity includes ionic regulation (mainly of Ca^{+} and Na^{-}) in specialised cells on the
280 gills and through their active transport involving several enzymes (Charmantier, 1998). For some
281 species, the activity of enzymes such as ATPases starts or improves in post larval stages resulting in
282 ontogenic changes in salinity tolerance (Bouaricha et al., 1991). Thus, lower tolerance of juvenile stages
283 provides a rationale for the low survival in 3.5‰ salinity found here. Understanding such differences in
284 acclimation among life stages of the species is crucial because the tolerance of the most sensitive life-
285 history stage will define their persistence and future distributional range when facing climate change.
286 We found variation in survival among broods within but not among populations, and this
287 variation was also present when comparing their responses to future salinity. Indeed, some broods better
288 tolerated the exposure to 3.5 ‰ than others, most likely implying occurrence of within-population
289 genetic variation in hyposalinity tolerance. Population genetic breeding designs to detect genetic
290 variation are not too commonly conducted in marine organisms but few examples exist (Edwards,
291 2013). Among these studies, Galletly et al. (2007) used full-sib approach to describe how the effect of
292 copper pollution on the hatching of the tunicate *Stylea plicata* varies among broods, thus indicating the
293 presence of genetic basis of such resistance. Hemmi and Jormalainen (2004), using a half-sib design,
294 showed both heritability of and maternal effects on growth of *I. balthica*, together with environmental
295 influence of eutrophication. Sunday et al. (2011) studied larval growth of the urchin *Strongylocentrotus*
296 *franciscanus* and the mussel *Mytilus trossolus* and found that the urchin hosted higher phenotypic and
297 genetic variation than the mussel in larval size when exposed to future CO_2 conditions. As the present
298 study involved a full-sibling design, such variation could be due to additive genetic variation,
299 dominance effects, or even due to maternal or epistatic influences (Bernardo, 1996), and our
300 experimental design does not allow to demarcate among these possibilities. The brood-by-salinity -

interaction in a quantitative trait such as survival suggest existence genetic variation in phenotypic plasticity of traits behind salinity tolerance. As climate change will enforce environmental selection, this plasticity in responses to salinity might be important to cope with future climate change by providing a substrate for natural selection.

Our results indicate that the lower survival in the future salinity will coincide with allelic loss, as the probability of finding the second most common allele among survivors was much lower there than in the current salinity. Further, part of the allelic loss will target functionally relevant regions of the genome. Indeed, we show that the effects of future desalination may not only hamper the abundance of *I. balthica*, but also end up in reducing its genetic variation in traits that, at least to some extent, can be targeted by natural selection. In addition, the fact that more of the alleles shared than not shared by both the populations were lost in future salinity is an indication of a deterministic allele loss, i.e. selective pressure of low salinity on the genetic diversity of *I. balthica*. Further, the selection for the salinity tolerant individuals affects the allele frequency of other functionally relevant genes, thus affecting the genetic variation also in genomic regions not directly involved in salinity tolerance but still crucial for the isopods. Such reduction in genetic variation has been reported for changes in abiotic factors such as temperature and pH due to climate change (reviewed in Kelly, 2019; Nadeau et al., 2017). However, environmental selection for the most tolerant trait does not necessarily translate into reduced fitness for a species. Temporal environmental variation among generations might still contribute to build up genetic variation by promoting different traits at different times, so that none of the genotypes will be overrepresented (Kelly et al., 2003). For instance, it has been showed for *Drosophila subobscura* that the variation in seasonal temperature helped the development of the response to heat shock (Bergland et al., 2014). Unless this phenomenon takes place and buffers the effect of selective mortality, the decreased genetic variation might effects might hamper the population's capability to tolerate environmental changes (Allendorf, 1986) and lead to genetic erosion (Hoffmann and Willi, 2008). Such

erosion of genetic diversity could decrease the amount of non-adaptive as well as adaptive variants and affect the population's potential for evolutionary adaptation (Jump et al., 2009).

Our study highlighted potential selection by hyposalinity on loci of critical importance for the cell biology and possibly involved into the potential for invertebrate's populations to persist under changing salinity. Among them, the Grainyhead-like proteins are transcriptional factors important for the maintenance of epidermal barriers but also for immunocompetence in Drosophila (Paré et al., 2012). The keratin-associated proteins are responsible for the keratin structure, which is the main component of the cuticle that covers the whole body of crustaceans and ensure them both protection and water permeability (Willmer, 2006). The actin-like proteins, which are involved into osmotic adjustments in decapods, and their gene expression in the gills is responsive to hyposalinity as a structural components of the gills, where the osmoregulation takes place in crustaceans (Havird et al., 2016).

Hyposalinity selected for lower variation in several loci responsible for the regulation of growth and associated functions. These included the homeodomain finger proteins, which regulate the epidermal growth factor pathway and are important for the development of eyes, limbs and gonads in arthropods (Sharabi et al., 2013). Further, the Calcium ion binding protein (AGAP006686) affect cuticular mineralization, thus, influencing moulting cycle, and their regulation controls the intracellular Ca^{2+} concentration (Gao, 2004). The O-linked N-acetyl glucosammine transferase acts in synthesis and mobilization of vitellogenin and nutrients to the developing ovary in decapods (Wong et al. 2008). Rhodopsin proteins are among the endocrine factors responsible for the food intake (Cardoso et al., 2012) but act also in the formation of visual pigments in photoreceptors (Koyanagi and Terakita, 2008). Finally, many proteins were responsible for fundamental biological cell processes. Such proteins include the reverse transcriptase, acting in the nucleotidyl transferase activity and part of the repair pathway for single nucleotide base excision repair and RNA-directed DNA polymerase activity, which is essential for DNA duplication and Elongation factor-2. This list included the G-protein coupled receptor,

a neuropeptide with myotropic and diuretic activity found in invertebrates such snails and ticks (Holmes et al., 2003). The alpha protein-kinase plays an important role in a multiple cellular processes such as protein translation, Mg^{2+} homeostasis and intracellular transport in the amoeba *Dictyostelium discoideum* (Middelbeek et al., 2010). Alkaline ceramidases are involved into the production of sphingosine, which has a major role in cell growth, trafficking and apoptosis as described in Mao and Obeid (2008). The present study highlights several functionally important loci, variation of which is eroded in hyposaline conditions and which are potential targets for selection due to hyposalinity.

To conclude, our study indicates that the future decline in salinity of the northern Baltic Sea will have a negative effect on the survival of *I. balthica*, as the juvenile stages will cope poorly with such environmental change. We also documented that hyposaline conditions decreased the genetic diversity through environmental selection in several fundamentally important loci. The potential disappearance of this species due to future desalination might affect multiple trophic levels both in terms of reduced top-down regulation of producers through herbivory and of bottom-up energy flow through reduced prey availability for fish.

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Literature cited

Allendorf, F.W., 1986. Genetic drift and the loss of alleles versus heterozygosity. Zoo Biol. 5, 181–190.

Bates, D., Mächler, M., Bolker, B.M., Walker, S.C., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–45. doi:10.18637/jss.v067.i01

Beaugrand, G., Kirby, R.R., 2018. How Do Marine Pelagic Species Respond to Climate Change? Theories and Observations. Ann. Rev. Mar. Sci. 10, 169–197. doi:10.1146/annurev-marine-121916-063304

Bergland, A.O., Behrman, E.L., O'Brien, K.R., Schmidt, P.S., Petrov, D.A., 2014. Genomic Evidence of Rapid and Stable Adaptive Oscillations over Seasonal Time Scales in *Drosophila*. PLoS Genet. 10. doi:10.1371/journal.pgen.1004775

Bernardo, J., 1996. Maternal Effects in Animal Ecology Maternal Effects in Animal Ecology. Am. Zool. 36, 83–105.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.S.S., 2008. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24, 127–135. doi:10.1016/j.tree.2008.10.008

Bouaricha, N., Thuet, P., Charmantier, G., Charmantier-Daures, M., Trilles, J.P., 1991. Na+-K+ ATPase and carbonic anhydrase activities in larvae, postlarvae and adults of the shrimp *Penaeus japonicus* (Decapoda, Penaeidea). Comp. Biochem. Physiol. -- Part A Physiol. 100A, 433–437. doi:10.1016/0300-9629(91)90495-X

Boyer, T.P., Levitus, S., Antonov, J.I., Locarnini, R.A., Garcia, H.E., 2005. Linear trends in salinity for the World Ocean, 1955-1998. Geophys. Res. Lett. 32, 1–4. doi:10.1029/2004GL021791

Braga, R.T., Rodrigues, J.F.M., Diniz-Filho, J.A.F., Rangel, T.F., 2019. Genetic Population Structure and Allele Surfing During Range Expansion in Dynamic Habitats. An. Acad. Bras. Cienc. 91, e20180179. doi:10.1590/0001-3765201920180179

Brierley, A.S., Kingsford, M.J., 2009. Impacts of Climate Change on Marine Organisms and Ecosystems. Curr. Biol. 19, R602–R614. doi:10.1016/j.cub.2009.05.046

Cardoso, J.C.R., Félix, R.C., Fonseca, V.G., Power, D.M., 2012. Feeding and the rhodopsin family G-protein coupled receptors in nematodes and arthropods. Front. Endocrinol. (Lausanne). 3, 1–22. doi:10.3389/fendo.2012.00157

Carey, V.J., Wang, Y.-G., 2001. Mixed-Effects Models in S and S-Plus. J. Am. Stat. Assoc. 96, 1135–1136. doi:10.1198/jasa.2001.s411

Chapman, J.R., Nakagawa, S., Coltman, D.W., Slate, J., Sheldon, B.C., 2009. A quantitative review of heterozygosity-fitness correlations in animal populations. Mol. Ecol. 18, 2746–2765. doi:10.1111/j.1365-294X.2009.04247.x

Charlesworth, B., Nordborg, M., Charlesworth, D., 1997. The effects of background and interference selection on patterns of genetic variation in subdivided populations. Genet. Res. (Camb). 70, 155–174. doi:10.1534/genetics.115.178558

Charmantier, G., 1998. Ontogeny of osmoregulation in crustaceans: A review. Invertebr. Reprod. Dev. 33, 177–190. doi:10.1080/07924259.1998.9652630

Charmantier, G., Charmantier Daures, M., 1994. Ontogeny of osmoregulation and salinity tolerance in the isopod crustacean *Sphaeroma serratum*. Mar. Ecol. Prog. Ser. 114, 93–102. doi:10.3354/meps114093

De Wit, P., Jonsson, P.R., Pereyra, R.T., Panova, M., André, C., Johannesson, K., 2020. Spatial genetic structure in a crustacean herbivore highlights the need for local considerations in Baltic Sea biodiversity management. Evol. Appl. 13, 974–990. doi:10.1111/eva.12914

Doney, S.C., Ruckelshaus, M., Emmett Duffy, J., Barry, J.P., Chan, F., English, C.A., Galindo, H.M., Grebmeier, J.M., Hollowed, A.B., Knowlton, N., Polovina, J., Rabalais, N.N., Sydeman, W.J., Talley, L.D., 2012. Climate Change Impacts on Marine Ecosystems. Ann. Rev. Mar. Sci. 4, 11–37. doi:10.1146/annurev-marine-041911-111611

Durack, P.J., Wijffels, S.E., Matear, R.J., 2012. Ocean Salinities Reveal Strong Global Water Cycle Intensification During 1950 to 2000. Science (80-.). 336, 455–458. doi:10.1126/science.1212222

Edwards, S. V., 2013. Next-generation QTL mapping: Crowdsourcing SNPs, without pedigrees. Mol. Ecol. 22, 3885–3887. doi:10.1111/mec.12401

Feng, Y., Zhang, D., Lv, J., Gao, B., Li, J., Liu, P., 2019. Identification of SNP markers correlated with the tolerance of low-salinity challenge in swimming crab (*Portunus trituberculatus*). Acta Oceanol. Sin. 38, 41–47. doi:10.1007/s13131-019-1428-0

Galletly, B.C., Blows, M.W., Marshall, D.J., 2007. Genetic mechanisms of pollution resistance in a marine invertebrate. Ecol. Appl. 17, 2290–2297. doi:10.1890/06-2079.1

Gao, Y., 2004. Characterization and expression of plasma membrane Ca2+ ATPase (PMCA3) in the crayfish *Procambarus clarkii* antennal gland during molting. J. Exp. Biol. 207, 2991–3002. doi:10.1242/jeb.01101

Gienapp, P., Teplitsky, C., Alho, J.S., Mills, J.A., Merilä, J., 2007. Climate change and evolution: Disentangling environmental and genetic responses. Mol. Ecol. 17, 167–178. doi:10.1111/j.1365-

294X.2007.03413.x

Goecker, M.E., Kåll, S.E., 2003. Grazing preferences of marine isopods and amphipods on three prominent algal species of the Baltic Sea. J. Sea Res. 50, 309–314. doi:10.1016/j.seares.2003.04.003

Guo, B., Li, Z., Merilä, J., 2016. Population genomic evidence for adaptive differentiation in the Baltic Sea herring. Mol. Ecol. 25, 2833–2852. doi:10.1111/mec.13657

Haahtela, I., 1984. A hypothesis of the decline of the bladder wrack Fucus vesiculosus L. in SW Finland in 1975-1981. Limnologica.

Haavisto, F., Jormalainen, V., 2014. Seasonality elicits herbivores' escape from trophic control and favors induced resistance in a temperate macroalga. Ecology 95, 3035–3045. doi:10.1890/13-2387.1

Hansen, J., Sato, M., Ruedy, R., Lo, K., Lea, D.W., Medina-Elizade, M., 2006. Global temperature change. Proc. Natl. Acad. Sci. U. S. A. 103, 14288–14293. doi:10.1073/pnas.0606291103

Havird, J.C., Mitchell, R.T., Henry, R.P., Santos, S.R., 2016. Salinity-induced changes in gene expression from anterior and posterior gills of *Callinectes sapidus* (Crustacea: Portunidae) with implications for crustacean ecological genomics. Comp. Biochem. Physiol. - Part D Genomics Proteomics 19, 34–44. doi:10.1016/j.cbd.2016.06.002

Hemmi, V., Jormalainen, A., 2004. Genetic and environmental variation in performance of a marine isopod: effects of eutrophication. Oecologia 140, 302–311. doi:10.1007/s00442-004-1574-7

Hoffmann, A.A., Sgrò, C.M., 2011. Climate change and evolutionary adaptation. Nature 470, 479–485. doi:10.1038/nature09670

Hoffmann, A.A., Willi, Y., 2008. Detecting genetic responses to environmental change. Nat. Rev. Genet. 9, 421–32. doi:10.1038/nrg2339

Holmes, S.P., Barhoumi, R., Nachman, R.J., Pietrantonio, P. V., 2003. Functional analysis of a G

protein-coupled receptor from the Southern cattle tick *Boophilus microplus* (Acari-Ixodidae) identifies it as the first arthropod myokinin receptor. Insect Mol. Biol. 12, 27–38. doi:10.1046/j.1365-2583.2003.00384.x

Horlyck, V., 1973. The osmoregulatory ability in three species of the genus *Idotea* (Isopoda, Crustacea). Ophelia 12, 129–140.

Jormalainen, V., Ramsay, T., 2009. Resistance of the brown alga *Fucus vesiculosus* to herbivory. Oikos 118, 713–722. doi:10.1111/j.1600-0706.2008.17178.x

Jump, A.S., Marchant, R., Peñuelas, J., 2009. Environmental change and the option value of genetic diversity. Trends Plant Sci. 14, 51–58. doi:10.1016/j.tplants.2008.10.002

Kefford, B.J., Nugegoda, D., Zalizniak, L., Fields, E.J., Hassell, K.L., 2007. The salinity tolerance of freshwater macroinvertebrate eggs and hatchlings in comparison to their older life-stages: A diversity of responses - The salinity tolerance of freshwater macroinvertebrate eggs and hatchlings. Aquat. Ecol. 41, 335–348. doi:10.1007/s10452-006-9066-y

Kelley, B.J., Burbanck, W.D., 2006. Osmoregulation in Juvenile and Adult *Cyathura polita* (Stimpson) subjected to salinity changes and ionizing gamma irradiation (Isopoda, Anthuridea). Chesap. Sci. 13, 201. doi:10.2307/1351064

Kelly, C.K., Chase, M.W., de Brujin, A., Fay, M.F., Woodward, F.I., 2003. Temperature-based population segregation in birch. Ecol. Lett. 6, 87–89.

Kelly, M., 2019. Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. Philos. Trans. R. Soc. B Biol. Sci. 374. doi:10.1098/rstb.2018.0176

Kinne, R.K.H., 1993. The role of organic osmolytes in osmoregulation: from bacteria to mammals. J. Exp. Zool. 265, 346–355. doi:10.1002/jez.1402650403

Kirst, G.O., 1989. Salinity Tolerance of Eukaryotic Marine Algae. Annu. Rev. Plant Physiol. Plant Mol. Biol. 41, 21–53. doi:10.1146/annurev.pp.41.060190.000321

493 Knights, A.M., Firth, L.B., Russell, B.D., 2017. Ecological responses to environmental change in
494 marine systems. J. Exp. Mar. Bio. Ecol. 2015–2018. doi:10.1016/j.jembe.2017.01.016
495 Kofler, R., Pandey, R.V., Schlötterer, C., 2011. PoPoolation2: Identifying differentiation between
496 populations using sequencing of pooled DNA samples (Pool-Seq). Bioinformatics 27, 3435–3436.
497 doi:10.1093/bioinformatics/btr589
498 Koyanagi, M., Terakita, A., 2008. Gq-coupled rhodopsin subfamily composed of invertebrate visual
499 pigment and melanopsin. Photochem. Photobiol. 84, 1024–1030. doi:10.1111/j.1751-
500 1097.2008.00369.x
501 Langmead, B., Salzberg, S.L., 2013. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357–
502 359. doi:10.1038/nmeth.1923.Fast
503 Leidenberger, S., Harding, K., Jonsson, P.R., 2012. Ecology and distribution of the isopod genus *Idotea*
504 in the Baltic Sea: key species in a changing environment. J. Crustac. Biol. 32, 359–381.
505 doi:10.1163/193724012X626485
506 Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin,
507 R., 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25, 2078–2079.
508 doi:10.1093/bioinformatics/btp352
509 Lowry, D.B., Hoban, S., Kelley, J.L., Lotterhos, K.E., Reed, L.K., Antolin, M.F., Storfer, A., 2017.
510 Breaking RAD: an evaluation of the utility of restriction site-associated DNA sequencing for
511 genome scans of adaptation. Mol. Ecol. Resour. 17, 142–152. doi:10.1111/1755-0998.12635
512 Mao, C., Obeid, L.M., 2008. Ceramidases: regulators of cellular responses mediated by ceramide,
513 sphingosine, and sphingosine-1-phosphate. Biochim. Biophys. Acta - Mol. Cell Biol. Lipids 1781,
514 424–434. doi:10.1016/j.bbalip.2008.06.002
515 Meier, H.E.M., Eilola, K., 2011. Future projections of ecological patterns in the Baltic Sea, Oceanografi
516 SMHI Reports, Oceanografi 107.

517 Meier, H.E.M., Kjellström, E., Graham, L.P., 2006. Estimating uncertainties of projected Baltic Sea
518 salinity in the late 21st century. Geophys. Res. Lett. 33, 1–4. doi:10.1029/2006GL026488
519 Middelbeek, J., Clark, K., Venselaar, H., Huynen, M.A., Van Leeuwen, F.N., 2010. The alpha-kinase
520 family: An exceptional branch on the protein kinase tree. Cell. Mol. Life Sci. 67, 875–890.
521 doi:10.1007/s00018-009-0215-z
522 Nadeau, C.P., Urban, M.C., Bridle, J.R., 2017. Climates Past, Present, and Yet-to-Come Shape Climate
523 Change Vulnerabilities. Trends Ecol. Evol. 32, 786–800. doi:10.1016/j.tree.2017.07.012
524 Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R2 from generalized
525 linear mixed-effects models. Methods Ecol. Evol. 4, 133–142. doi:10.1111/j.2041-
526 210x.2012.00261.x
527 Nowak, C., Vogt, C., Pfenninger, M., Schwenk, K., Oehlmann, J., Streit, B., Oetken, M., 2009. Rapid
528 genetic erosion in pollutant-exposed experimental chironomid populations. Environ. Pollut. 157,
529 881–886. doi:10.1016/j.envpol.2008.11.005
530 Orav-Kotta, H., Kotta, J., 2004. Food and habitat choice of the isopod *Idotea baltica* in the northeastern
531 Baltic Sea. Hydrobiologia 514, 79–85. doi:10.1023/B:hydr.0000018208.72394.09
532 Paré, A., Kim, M., Juarez, M.T., Brody, S., McGinnis, W., 2012. The functions of Grainy head-like
533 proteins in animals and fungi and the evolution of apical extracellular barriers. PLoS One 7,
534 e36254. doi:10.1371/journal.pone.0036254
535 Pauls, S.U., Nowak, C., Bálint, M., Pfenninger, M., 2013. The impact of global climate change on
536 genetic diversity within populations and species. Mol. Ecol. 22, 925–946. doi:10.1111/mec.12152
537 Poćwierz-Kotus, A., Kijewska, A., Petereit, C., Bernaś, R., Więcaszek, B., Arnyasi, M., Lien, S., Kent,
538 M.P., Wenne, R., 2014. Genetic differentiation of brackish water populations of cod *Gadus morhua*
539 in the southern Baltic, inferred from genotyping using SNP-arrays. Mar. Genomics 19, 17–22.
540 doi:10.1016/j.margen.2014.05.010

Rugiu, L., Manninen, I., Sjöroos, J., Jormalainen, V., 2018. Variations in tolerance to climate change in a key littoral herbivore. Mar. Biol. 165, 1–11. doi:10.1007/s00227-017-3275-x

Sharabi, O., Ventura, T., Manor, R., Aflalo, E.D., Sagi, A., 2013. Epidermal growth factor receptor in the prawn *Macrobrachium rosenbergii*: function and putative signaling cascade. Endocrinology 154, 3188–3196. doi:10.1210/en.2013-1259

Snoeijs-leijonmalm, P., Shubert, H., Radziejewska, T., 2017. Biological Oceanography of the Baltic Sea. doi:10.1007/978-94-007-0668-2

Sorte, C.J.B., Fuller, A., Bracken, M.E.S., 2010. Impacts of a simulated heat wave on composition of a marine community. Oikos 119, 1909–1918. doi:10.1111/j.1600-0706.2010.18663.x

Stocker, T.F., Quin, D., Plattner, G.K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, B. Midgley, B.M., 2013. Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, in: Researchgate.Net. doi:10.1017/CBO9781107415324.Summary

Sunday, J.M., Crim, R.N., Harley, C.D.G., Hart, M.W., 2011. Quantifying rates of evolutionary adaptation in response to ocean acidification. PLoS One 6, 1–8. doi:10.1371/journal.pone.0022881

Wang, S., Meyer, E., Mckay, J.K., Matz, M. V., 2012. 2b-RAD: A simple and flexible method for genome-wide genotyping. Nat. Methods 9, 808–810. doi:10.1038/nmeth.2023

Wernberg, T., Smale, D.A., Thomsen, M.S., 2012. A decade of climate change experiments on marine organisms: Procedures, patterns and problems. Glob. Chang. Biol. 18, 1491–1498. doi:10.1111/j.1365-2486.2012.02656.x

Willmer, P., 2006. Osmoregulation in Invertebrates. Encycl. Life Sci. 1–11. doi:10.1038/npg.els.0003646

Wong, Q.W.L., Mak, W.Y., Chu, K.H., 2008. Differential gene expression in hepatopancreas of the shrimp *Metapenaeus ensis* during ovarian maturation. Mar. Biotechnol. 10, 91–98.

doi:10.1007/s10126-007-9042-0

Wood, H.L., Nylund, G., Eriksson, S.P., 2014. Physiological plasticity is key to the presence of the isopod *Idotea baltica* (Pallas) in the Baltic Sea. J. Sea Res. 85, 255–262. doi:10.1016/j.seares.2013.05.009

Electronic Supplemental Material

Table S1 showing the information regarding the 2b-RAD libraries displayed by brood.

Table S2: Full list of the genomic contigs matching open reading frames and the main information for each of them.

575 Table 1 showing the number of individuals used for DNA extractions at the starting point (initial
576 day of the experiment) and juveniles survived at the end of the experiment in current salinity (after the
577 exposure to 6 ‰) and future salinity (after the exposure to 3.5‰).

Population	# broods used	Start	Current salinity	Future salinity
N	10	93	116	36
R	10	107	101	34

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580 Table 2. Generalised mixed model statistics for the probability of survival and allelic loss at the
581 end of the experiment.

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Source of variation	Survival probability			Allelic loss		
	df	Wald χ^2	P	df	Wald χ^2	P
Fixed Factors						
Salinity	1	178.8	<0.001	1	650	0.001
		χ^2	P		X^2	P
Random Factors						
Population		0	1		0	1
Population × Salinity					6.6	0.09
Brood		44	<0.001			
Brood × Salinity		237.6	<0.001			

584 Figure legends

585 Figure 1. a) A map showing the locations of the three sampling sites - Närpes (N), Rauma (R) and
586 Parainen (P) - within the Baltic Sea. b) A photo showing an *I. balthica* juvenile as one week old. C)
587 Schematic illustration of the experimental design with the collection of gravid isopods from the wild,
588 gathering the broods from each individual separately, exposing one half of each brood to current and the
589 other half to future salinity and extracting the DNA from the survivors for downstream applications.
590
591 Figure 2. The mean survival probability (± 95% confidence interval) of juveniles from the
592 populations N (a), P (b), and R (c) under current and future salinity conditions
593
594 Figure 3. Mean survival probability at the end of the experiment for broods from the populations
595 N (a), P (b), and R (c) exposed to the current and future salinity conditions. Each line represent a single
596 brood and combines the brood averages in the two salinities.
597
598 Figure 4. Venn diagrams showing the amount of alleles lost in the populations R and N in current
599 (a) and future (b). The size of circles is proportional to the amount of alleles lost. c) The pie chart shows
600 the proportion of ORFs found a match in NCBI database for the functional categories: regulation of the
601 cellular function (Reg. cell. function), cellular response (Cell. response), cellular signalling (Cell.
602 signaling) and protein synthesis .
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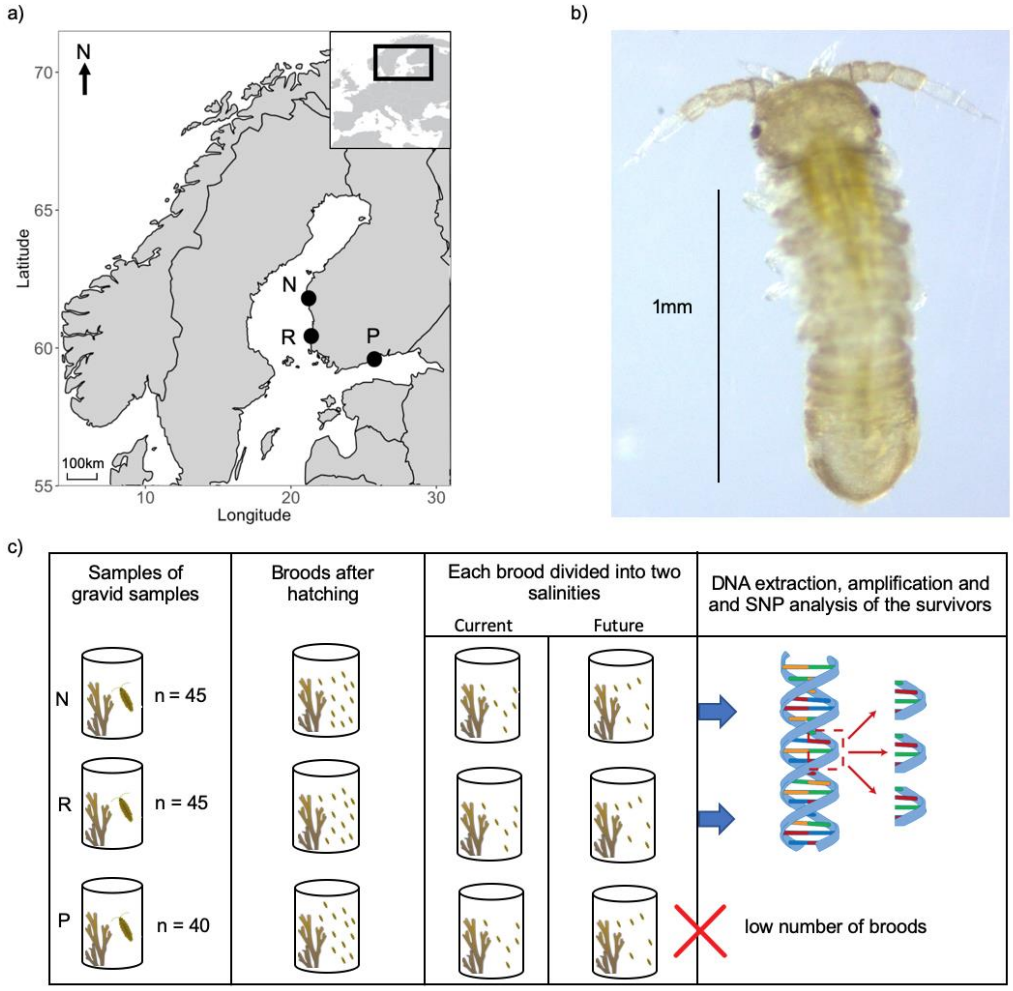
608 Figure 1



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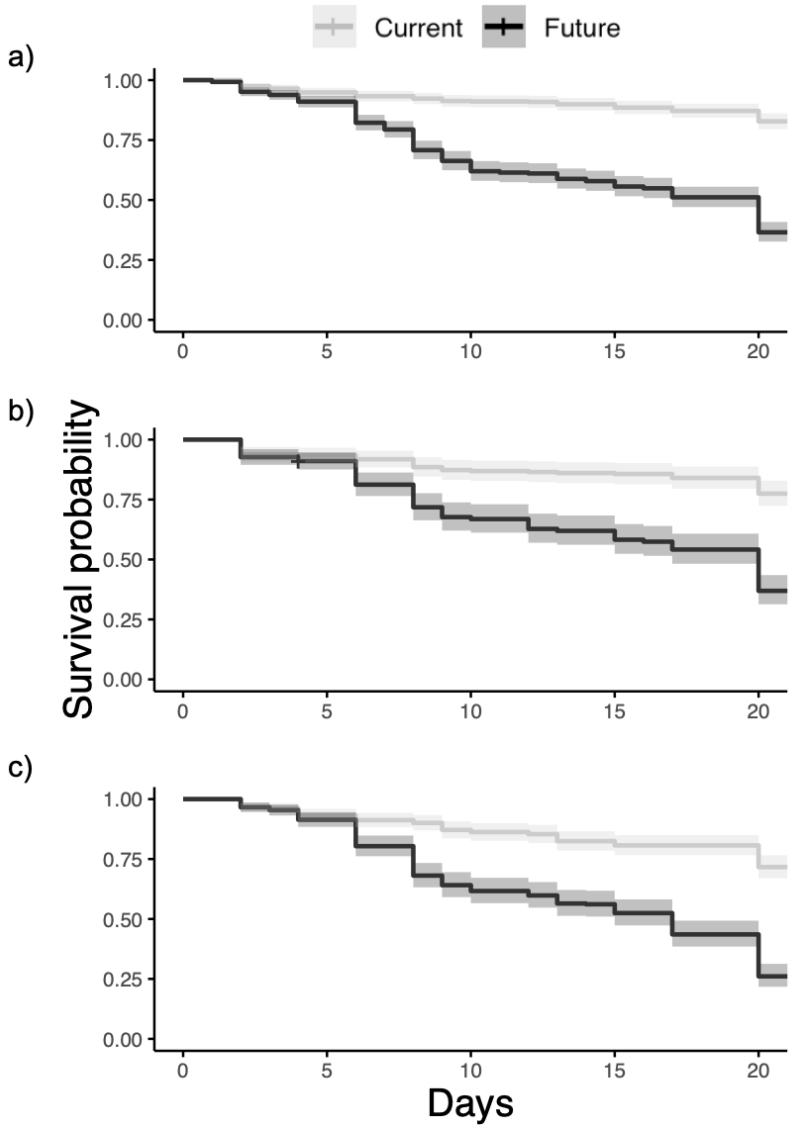
618 Figure 2



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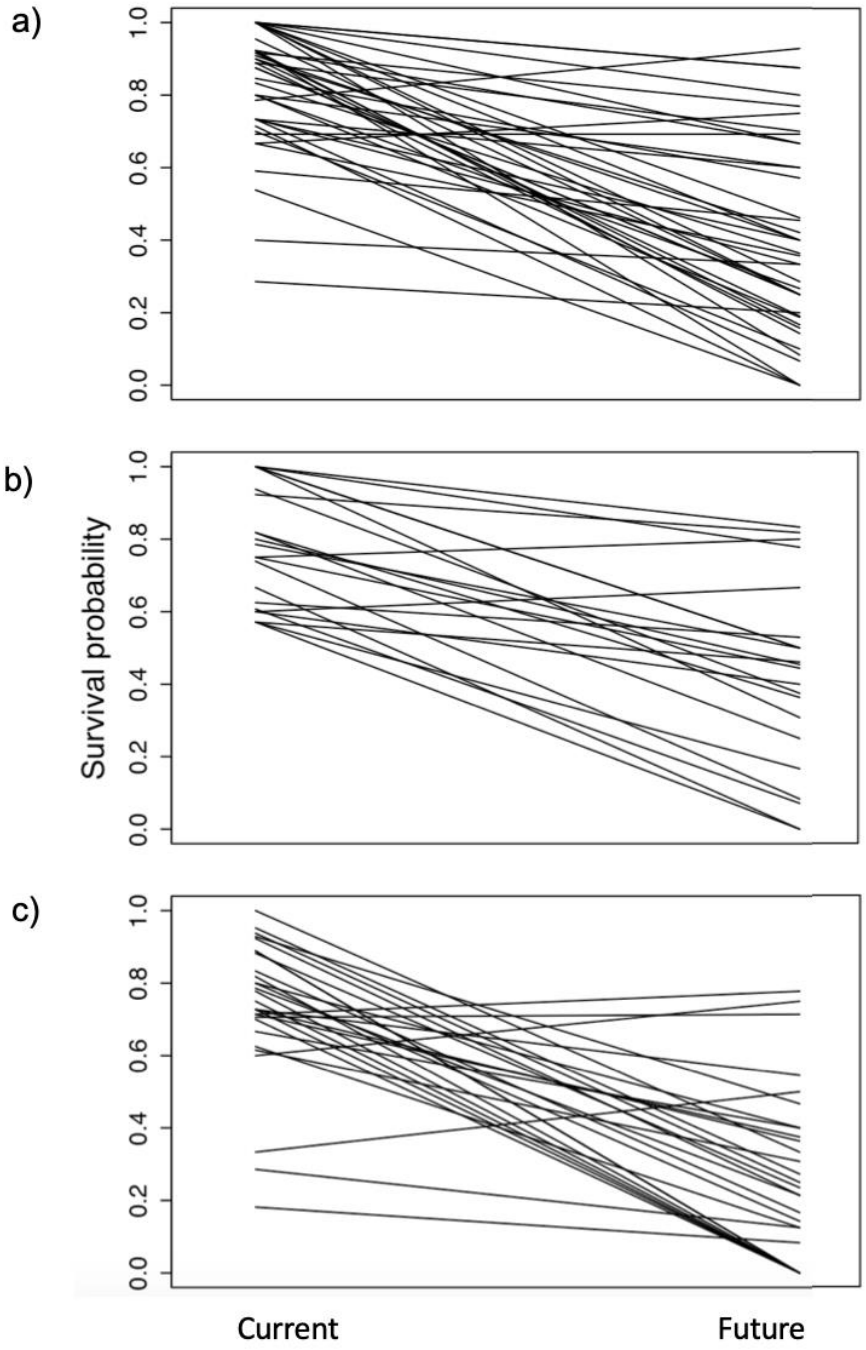
627 Figure 3



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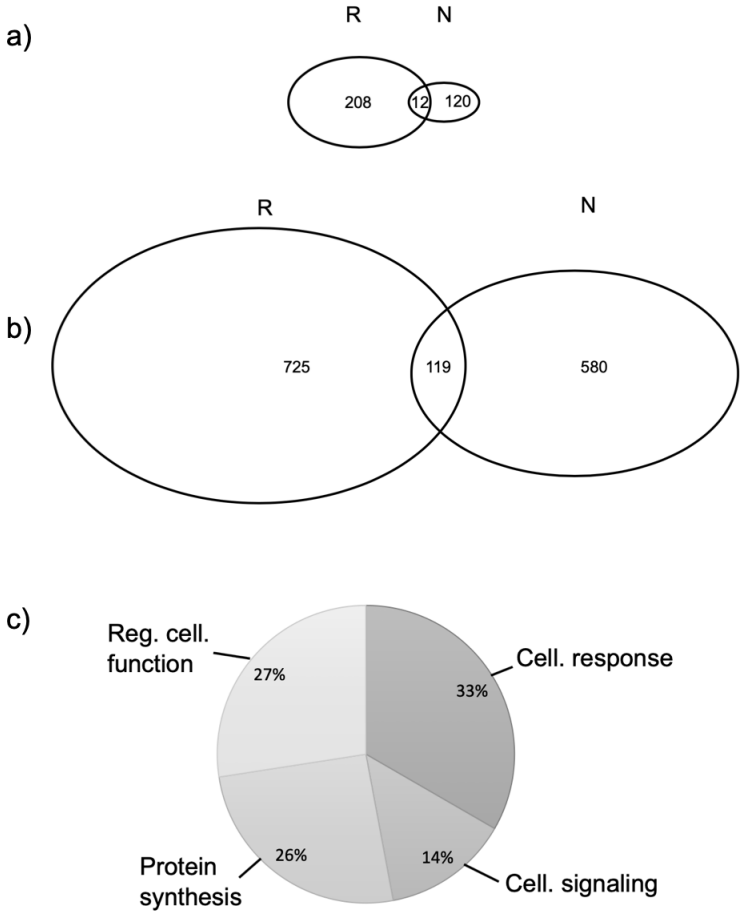
633 Figure 4



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