



Physiological plasticity is key to the presence of the isopod *Idotea baltica* (Pallas) in the Baltic Sea

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ABSTRACT

The low salinity of the Baltic Sea presents a physiological challenge to marine species. The marine isopod *Idotea baltica* is notably dominant among the shallow sublittoral of the Baltic Sea in association with *Fucus vesiculosus*, with permanent populations documented in salinities as low as 3 psu. To investigate the role of physiological plasticity in the successful colonisation of the Baltic by *I. baltica* three populations from the Swedish coast were here studied, one from the Kattegat (Malmö) and two from the Baltic Sea (Kalmar and Öregrund). These three sites cover the geographic range of this species within the Baltic Sea on the Swedish coast, and also the salinity range of this species within the Baltic Sea (10–5 psu). Individuals from these populations were exposed in the laboratory to a fully crossed experiment with the factors salinity and food source, to test for differences in the physiology of these populations under different conditions that may indicate local adaptation, or no differences that indicate physiological plasticity to differing salinity and food source. Metabolic rate, growth and thermal tolerance responses did not differ between the three populations across salinity treatments after a 12 week exposure. The results of this study indicate that the physiology of adult *I. baltica* is highly plastic with regard to salinity; this plasticity is likely to have facilitated their colonisation of the Baltic Sea.

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1. Introduction

Understanding a species' distribution is one of the basic problems of ecology (Krebs, 1978). According to Liebig's Law of the Minimum, 'The distribution of a species will be controlled by that environmental factor for which the organism has the narrowest range of adaptability or control' (Bartholomew, 1958). The practical application of this simple concept can be exceptionally complicated in the marine environment where habitats often have hazy boundaries, abiotic conditions are so variable, and the 'environmental factor' could be anything from an abiotic parameter such as oxygen, pH or temperature, through to competition for food or space. When a species colonises a new area, Liebig's Law is a good starting point in the search for what has changed to enable a particular species to inhabit a new area. Successful colonisation is reliant on the ability to survive and reproduce in the new ecological conditions, and as such these individuals must either be preadapted to the new environment or be flexible enough to respond rapidly and adaptively to novel conditions (West-Eberhard, 2003). In physiological terms then, this success relies on a wide reaction norm that encompasses the new ecological conditions, or physiological plasticity allowing the organism to

acclimatise its physiological ranges in response to the new ecological conditions.

Phenotypic plasticity is the ability of an organism (or genotype) to alter its phenotype in response to the environment. In a variable environment such plasticity may facilitate the survival of an individual/population/species in a range of conditions that are not possible with a fixed phenotype (Bradshaw, 1965; Schlichting and Pigliucci, 1998; Sultan, 2003). Plasticity is not in itself an evolutionary process; habitats are often dynamic and in such changeable habitats selective pressure may be on the trait of plasticity itself rather than a directional outcome of this plasticity (Baythovong and Stanton, 2010; de Jong, 2004). For example the porcelain crab *Petrolisthes cinctipes*, which occurs in the intertidal zone of the North-eastern Pacific is emersed during most low tides and as such exposed to a wide range of temperatures on a daily basis, has a far wider thermal tolerance range than the closely related *Petrolisthes eriomerus* which occurs deeper and is rarely emersed (Stillman and Somero, 1996).

There are circumstances where plasticity may be a mechanism of adaptation, and therefore the evolutionary process. When there is a directional change in the environment that favours individuals capable of certain (in this case) physiological capacity, then the effect of selection upon the population may result in a genotypic shift, and thus create a population adapted to local conditions — where it is no longer plasticity that results in physiological tolerance, but a shift in the reaction norm (Baythovong and Stanton, 2010; West-Eberhard,

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2003), a process termed local adaptation. With the exception of unicellular organisms such adaptation is usually witnessed over geological timescales, due to the generational requirements of adaptation, and the required rates of environmental change that are slow enough to facilitate evolution rather than force extinction. However, spatial gradients of environmental change can create a naturally occurring selection experiment, allowing investigation of phenotypic plasticity and adaptation without the caveat of time (Gilchrist and Lee, 2007).

The Baltic Sea is a low salinity marine system with a pronounced spatial gradient in salinity. Its fauna is characterised by this salinity gradient, as the ability to cope with changes in osmotic pressure is a limiting factor in both marine and freshwater species. As one of the youngest brackish systems on the planet, existing in its current form for approximately 4000 years (Emeis et al., 2003), the colonisation of marine species into the Baltic Sea is a natural experiment (Grinnell, 1919) and may provide working examples of local adaptation (e.g. Nielsen et al., 2009) and physiological plasticity, both in terms of osmoregulatory capacity, and also wider factors arising from interactions within the unique Baltic ecosystem.

The marine environment holds a stable osmotic concentration whereas brackish waters (such as the Baltic Sea) fluctuate and put demand on both degree and timing of osmoregulation in the organisms. Aquatic invertebrates vary in osmoregulatory capacity and many may even osmoregulate at low salinity but osmoconform at high salinity (Lockwood, 1962). The marine isopod *Idotea baltica* is a euryhaline species i.e. able to adapt to a wide range of salinities by osmoregulating through active ion transport (Postel et al., 2000). The species is found throughout northern Europe in fully marine conditions, but is also present in the shallow sublittoral macroalgal belts of the Baltic Sea down to salinities as low as 3 psu around Hudiksvall on the Swedish Coast (Leidenberger et al., 2012) where it is key species based both on its top down role as a grazer with a controlling influence on the macroalgal (primarily *Fucus vesiculosus*) community within the shallow sublittoral (Engkvist et al., 2000; Svensson et al., 2004), and as a bottom up food source in the littoral zone for several fish species (Leidenberger et al., 2012), including the commercially important *Gadus morhua* (cod) and *Clupea harengus* (herring) (Salemaa, 1978). *I. baltica* is one of very few marine invertebrates to be found at such permanently low salinities in the Baltic Sea. Within the Baltic Sea the association with *F. vesiculosus* presents an additional challenge as this macroalga uses phlorotannins as a grazer defence (Tuomi et al., 1989) and *F. vesiculosus* within the Baltic Sea has significantly higher levels phlorotannins than its fully marine dwelling counterparts (Nylund et al., 2012). Phlorotannins are documented to reduce gut absorption efficiency within *I. baltica* and act as a grazing deterrent (see review: Jormalainen and Honkanen, 2008). As such the *I. baltica* living in the Baltic Sea face not only a greater (energetically costly) osmoregulatory demand than their counterparts in fully marine habitats, but must also contend with higher levels of grazer defence in their host plant.

Despite the energetic cost of living in low osmotic concentrations and the astringent phlorotannins in their food, *I. baltica* is abundant within the Baltic Sea. A recent review of *Idotea* species within the Baltic (Leidenberger et al., 2012) concluded *I. baltica* distribution within the Baltic Sea was limited by salinity, but a high tolerance to low salinity characterised their distribution. Meanwhile Jormalainen et al., 2001 suggests local adaptation of *I. baltica* to the phlorotannin rich local *F. vesiculosus*. As such, the prevalence of *I. baltica* in the Baltic is as a result of either preadaptation to low salinity and high grazer defence, or because they are flexible enough to respond rapidly and adaptively to these conditions (West-Eberhard, 2003). The species is a brooder, with a low dispersive capacity and long-range dispersal is sporadic and limited, achieved only through adults rafting on floating debris (Gutow et al., 2006), though such rafting has not been documented within the Baltic Sea. With a large geographical distance to cover from the more marine Kattegat seawaters into the Baltic Sea up to Hudiksvall (approx. 1200 km), the brooding

characteristics of *I. baltica* and low dispersal of this species indicate colonisation of the Baltic Sea was achieved relatively slowly, over generations. With a generation time of less than a year, it is feasible that the populations of *I. baltica* along the salinity gradient of the Baltic Coast are locally adapted and their colonisation of low salinity waters was facilitated by adaptation. Alternatively the species may be highly plastic in salinity and grazer defence tolerance.

The focus of this study is to understand the mechanism of successful Baltic colonisation by *I. baltica*. We compare the physiological responses of three geographically distant populations of *I. baltica*, one from the Kattegat (Malmö) and two from within the Baltic Sea (Kalmar and Öregrund), to investigate differences in their physiological tolerances to the salinity gradient along the Baltic Sea, and in addition the variability of food grazer defence (and therefore food quality) between localities in the Baltic Sea. The aim of this study is to determine whether colonisation by this species into the Baltic Sea was opportunistic due to pre-existing high physiological plasticity, or the result of the evolutionary process of local adaptation.

2. Methods

2.1. Field collection

All collections were carried out in October 2009 from three moderately exposed sites on the Swedish coast (see Fig. 1 for map): Malmö (55°57.0299 N, 12°89.6592E) in the Kattegat (salinity; 10 psu), and Kalmar (56°65.1083 N, 16°33902E) (salinity; 7 psu) and Öregrund (60°34.8682 N, 18°453861E) (salinity; 5 psu) in the Baltic Sea, see Fig. 2 for salinity ranges at these sites. These sites were selected to encompass the *I. baltica* range along the Baltic salinity gradient and span approx. 1300 km of the Swedish coastline. Isopods were collected by hand from a depth of 0.5–1 m by gathering *F. vesiculosus* plants at the site. Each algal frond was then searched and *I. baltica* individuals were placed into a separate container on *F. vesiculosus* free from other fauna; the macroalga was used as both refuge and to keep the isopods moist. Containers with animals and macroalgae were transported back to the laboratory without additional water within 7 h of collection where all materials (isopods and macroalgae) were divided between 2 pre-prepared 75 l containers of aerated water of the same salinity as the field site (based on seasonal average data from the Swedish Meteorological and Hydrological Institute). Additional containers of *F. vesiculosus* were cleaned of fauna and epiphytes and placed in –80 °C freezer for artificial food preparation.

2.2. Experimental set-up

Isopods from each population (Malmö, Kalmar and Öregrund) measuring between 11 and 16 mm were weighed and measured before being randomly allocated to one of 3 salinities (5, 7 or 10 psu) and one of three feeds (Malmö, Kalmar and Öregrund). The feeds and salinities relate to the in situ salinities and *Fucus* at each collection site, thus creating a fully crossed experimental design with 9 treatment combinations for each population. Ten replicate individuals were used in each treatment (total 90 isopods per population). Additional animals from each population were maintained in the large containers with fresh *Fucus* until required for thermal tolerance experiments (see methodology below).

Each isopod was secured in a 250 ml pot with two large mesh panels to allow water exchange and then placed in one of two 5 l aquaria/treatment (5 pots per aquaria). In this size range the sex ratio was approximately 50:50. No brooding females were used. Approximately 1 g wet weight (WW) of artificial *Fucus* food (~1 cm × 2 cm × 1/2 cm 'stick', see below) was placed in each pot with the isopod, which allowed ad libitum feeding. All isopods were within the experimental set-up for a period of one week prior to the start of the exposure. Salinity was adjusted over this 7 day period

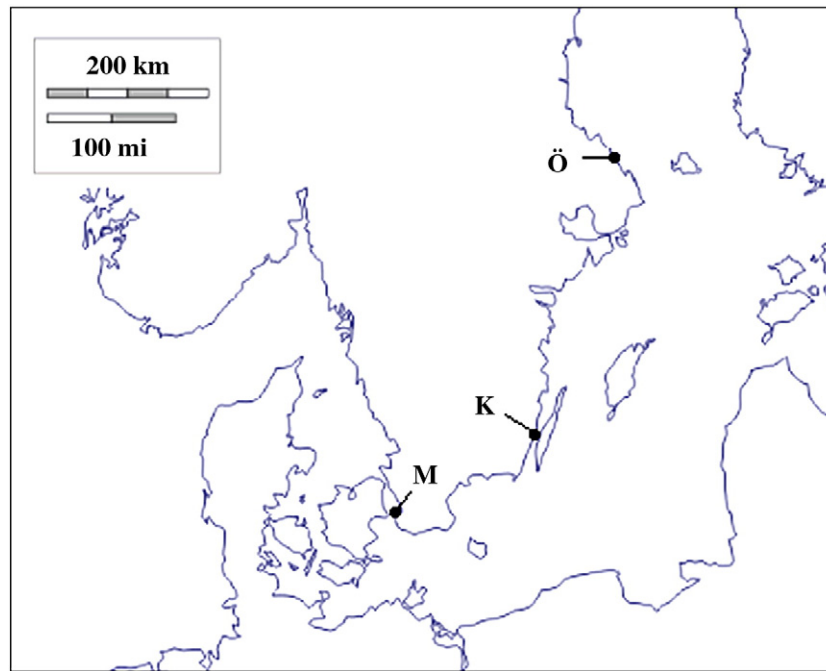


Fig. 1. Map showing the geographical locations of the three population Malmö, Kalmar and Öregrund collection sites. M = Malmö, K = Kalmar and Ö = Öregrund.

to achieve the experimental salinities required for all the different treatments.

All aquaria were maintained at 12 °C with continuous aeration. Food and water were changed weekly for the course of the 12 week experimental exposure.

Experimental salinities were achieved by mixing deep sea water (~32 psu) with tap water. Tap water was selected as it was the closest mineralogical composition to natural limnic water available; local water is from a nearby lake and treatment is minimal (filtration, CO₂ acidification to kill bacteria and addition of chlorine. Both CO₂ and chlorine should be off-gassed before supply). Adjusted salinity water mixtures were bubbled with air for a minimum of 6 h as a precaution to remove any traces of chlorine before it was used for the animal containers.

2.3. Artificial food and food consumption

To consider local differences in food characteristics *F. vesiculosus* from each site was used. Due to the potential for the macroalgae to react differently to the salinity treatments, the plants (apical parts) were freeze dried and made into a fine powder. One g algal powder was rehydrated in 4 ml Milli-Q water (MQ) and mixed with an agar

mixture (360 mg agar with 5 ml MQ) that was heated to boiling in a microwave. The resulting mixture was poured into a Petri dish mould and left to cool to create a *Fucus* food mix in an agar matrix.

A fresh batch of artificial food was made each week (from the same stock of algal powder, stored in an airtight container at room temperature). Start and end (blotted) weights were recorded for each food 'stick' at weeks 6 and 12. In addition a food stick was placed in a pot with no isopod to measure incidental weight change.

The consumption for each individual was calculated as percentage weight change of the food and standardised per gramme of animal. There was a small (<5%) increase in weight of the control food samples due to water uptake, and food consumption measurements were corrected for this. The food sticks maintained their form throughout immersion.

2.4. Metabolic rate

Oxygen consumption was used as a proxy for metabolic rate, and measured using closed bottle respirometry. After the 12 weeks exposure individuals were placed in a 120 ml glass titration bottle with oxygen saturated water of the same salinity as experienced in the experimental exposure. Bottles were sealed with a glass stopper and placed in a waterbath (12 °C) in darkness for 60 min. The bottles were gently turned every 15 min to mix the water and avoid oxygen stratification within the bottle. Start and end oxygen values for each bottle were measured with a Unisense OXY-meter with a Clark-type oxygen probe (Unisense OX 100 micro sensor). Blank chambers were run with each set of incubations to measure background respiration. Oxygen consumption was calculated as the difference between the start and end oxygen values, corrected for background respiration, salinity and atmospheric pressure, and standardised per gramme of animal WW. Oxygen did not drop below 90% saturation in any chamber.

2.5. Growth

Animal length and weight were measured at the start and end of the experiment. Length was measured from the tip of the head to the tip of the tail, determined using callipers accurate to 0.1 mm.

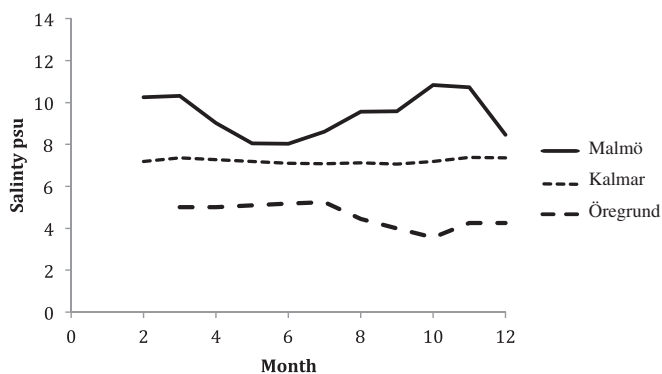


Fig. 2. Box and whisker plot showing salinity range for each site, data from SMHI SHARK database.

Blotted WW was measured on a Mettler Toledo AT200 balance accurate to 0.1 mg. Most individuals moulted during the experiment. The cast exuviae were removed from the containers during routine cleaning.

2.6. Acute thermal tolerance

Upper and lower thermal lethal limits (UTL and LTL respectively) were determined for all three populations of *I. baltica*. Individuals from each population were randomly assigned to two groups, one of which was acclimated to 12 °C, the other to 18 °C for two weeks prior to the thermal tolerance experiments. These temperatures were chosen to represent high summer and spring/autumn temperatures (Fig. 3). Winter temperatures were avoided as it is not known how deep *I. baltica* migrate during the winter, and therefore what temperatures they experience. The thermal tolerance experiments commenced at either 12 °C or 18 °C, corresponding to the acclimated temperature of the group tested. Individual animals were placed in a well of a 6 well culturing plate with aerated water of the original salinity for each population. Each well also contained a strip of fake algae made from inert plastic, which the isopod clung to when exhibiting normal behaviour. The well plate was placed into a Heto DT-2 electronic thermostat controlled water bath and heated or cooled at a rate of 1 °C/3 min. Temperature within wells was measured using a digital thermometer. At each temperature survival was recorded for each individual. Six replicate individuals were used for each population. Preliminary trials highlighted two determinants of lethal limit, a behavioural indicator and death. The behavioural indicator consisted of spasms accompanied by lying on side or back and death was determined as the cessation of pleopod movement for longer than 1 min. Lower thermal lethal limits could not be determined as the animals survived at –1.8 °C for 4 h, where the water was frozen solid.

2.7. Phlorotannin content

Phlorotannin content of 25 *F. vesiculosus* plants was quantified colorimetrically using the Folin–Ciocalteu method (van Alstyne, 1995). Apical parts of algae were freeze-dried, ground to a fine powder and 12 to 15 mg powder was extracted in 1.5 ml aqueous acetone (60% by volume) on a vortex in the dark for 18 h. The extracts were centrifuged and 100 µl of the supernatant was diluted with MQ water to a final volume of 8 ml. Thereafter, 0.5 ml Folin–Ciocalteu's reagent (Merck, Art. 109001) and subsequently 1.5 ml sodium carbonate were added to the samples. After 2 h incubation in the dark the absorbance at 740 nm was measured using a spectrophotometer. Phloroglucinol (1,3,5-trihydroxybenzene, Sigma, Art. 6099-90-7) was used as standard.

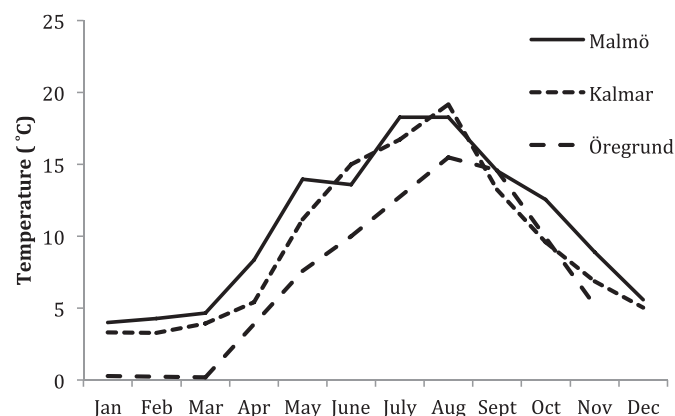


Fig. 3. Monthly sea surface temperature 2008, based on data from SMHI SHARK database.

Plant samples for phlorotannin analysis were collected at the same time and locations as the *F. vesiculosus* collected for use as food during the course of the experiment. In addition processed artificial food used in the experiments was tested – both fresh, and after 3 days of residing in seawater.

2.8. Statistical analyses

PERMANOVA analyses were performed using the statistical programme PRIMER 6 with the PERMANOVA + add-on. PERMANOVA analysis provides an ANOVA analysis without the limitations of a normally distributed dataset, thus no transformation of data was required. Where there were significant factors or interactions, PERMANOVA pair-wise tests were conducted to detect which levels were responsible for significant differences. ANCOVA was carried out using MINITAB 16, with Kolmogorov–Smirnov test for normality of data performed prior to analysis.

3. Results

3.1. Metabolic rate

Metabolic rate was not significantly different between the three populations of *I. baltica*, nor affected by food source or salinity (Fig. 4).

3.2. Phlorotannin content

Phlorotannin contents of the *F. vesiculosus* from the three sites were significantly different (Tables 1a & 1b) with phlorotannin levels significantly higher at the Kalmar site compared to Öregrund and Malmö (Fig. 5a). There was no clear pattern associated with progression into the Baltic or salinity of collection site. The effect of phlorotannins on the individuals can be measured by the proxies of growth and survival, as in Kubanek et al. (2004). However measurement of phlorotannin levels within the artificial food sticks revealed a significantly lower phlorotannin content (Fig. 1b) than fresh food, moreover after 3 days in seawater there were no differences in phlorotannin content between the different food sources. As a result of these measurements no conclusions can be drawn on the food source as a factor.

3.3. Food consumption

None of the populations of *I. baltica* showed increased or decreased food consumption as a result of altered salinity (Fig. 6). Thus, food consumption was not different for populations fed under local salinity conditions compared with the non-local conditions.

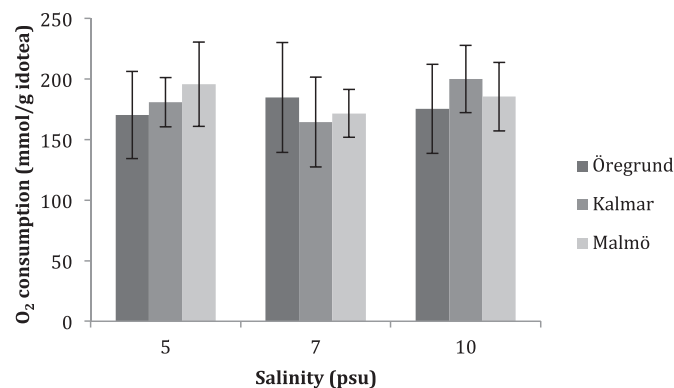


Fig. 4. Oxygen consumption (mean ± 95% confidence intervals) of *Idotea baltica* populations from three locations off the Swedish coast (Öregrund 5 psu, Kalmar 7 psu, and Malmö 10 psu). Each measured in their own and altered salinities after 12 weeks of exposure. Data for the different food sources were also non-significant and were pooled.

Table 1a

PERMANOVA results comparing phlorotannin levels in the *Fucus vesiculosus* from the three source sites which were used as the three alternatives within the treatment factor 'food quality'.

Source	d.f.	SS	MS	Pseudo-F	P(perm)	Unique perms
Re	2	1787	894	80.2	≤0.0001	9949
Res	72	802	11.1			
Total	74	2590				

Significant results shown in bold.

3.4. Growth and survival

Survival was 79%, with no difference between treatments. All individuals gained weight and length over the course of the 12 week experiment (Fig. 7). However, weight (mg) increase was not significantly different between the three populations or salinity and interactions thereof (ANCOVA, Table 2).

3.5. Thermal tolerance

The upper thermal lethal limit (UTL) of the three populations was on average 35 °C at 12 °C acclimation with no significant difference between the three populations (Tables 3a, 3b and 3c, Fig. 8a). All *I. baltica* exhibited plasticity in their thermal tolerance, with significantly higher UTL (Table 3a) in the individuals acclimated to 18 °C. The critical thermal maximum (CTM, Behavioural limit) was also significantly different between the acclimation temperatures for the Öregrund and the Kalmar population, but not for the Malmö population (post hoc test, Table 3c, Fig. 8b). Here the CTM was not significantly different based on acclimation temperature, whereas there was a significant difference in the CTM between the two acclimation temperature treatments for both Kalmar and Öregrund (Table 3b). The lower thermal lethal limit (LTL) could not be determined as the animals from all populations survived at −1.8 °C and below this point the water began to freeze solid. The test individuals taken below this temperature survived freezing and resumed normal activity when the water was thawed. Further testing would have become a function of both time kept in frozen water as well as temperature, so the test for LTL was abandoned.

4. Discussion

This investigation supports the hypothesis that *I. baltica* within the Baltic Sea are physiologically plastic to salinity. The change from marine to brackish and freshwater habitats is physiologically challenging, and successfully accomplished by relatively few (Hutchinson, 1957). The major forcing factor in the Baltic is osmotic stress (Remane and Schlieper, 1971). Thus the means by which the species that live in the Baltic Sea have obtained the physiological capacity to do so holds a potential wealth of information regarding processes of adaptive evolution and physiological plasticity. Baltic populations in general, have a lower genetic diversity compared to Atlantic populations and the Baltic acts as refuge for unique evolutionary lineages (Johannesson and André, 2006). In the case of the isopod *I. baltica* the results of this investigation indicate no local adaptation of populations along over 1300 km of the Swedish coast from Malmö to

Table 1b

PERMANOVA post hoc pair-wise tests of phlorotannin levels in *Fucus vesiculosus* between sites.

Groups	t	P(perm)	Unique perms
Kalmar vs. Malmö	6.22	0.001	998
Kalmar vs. Öregrund	16.2	0.001	997
Malmö vs. Öregrund	5.32	0.001	999

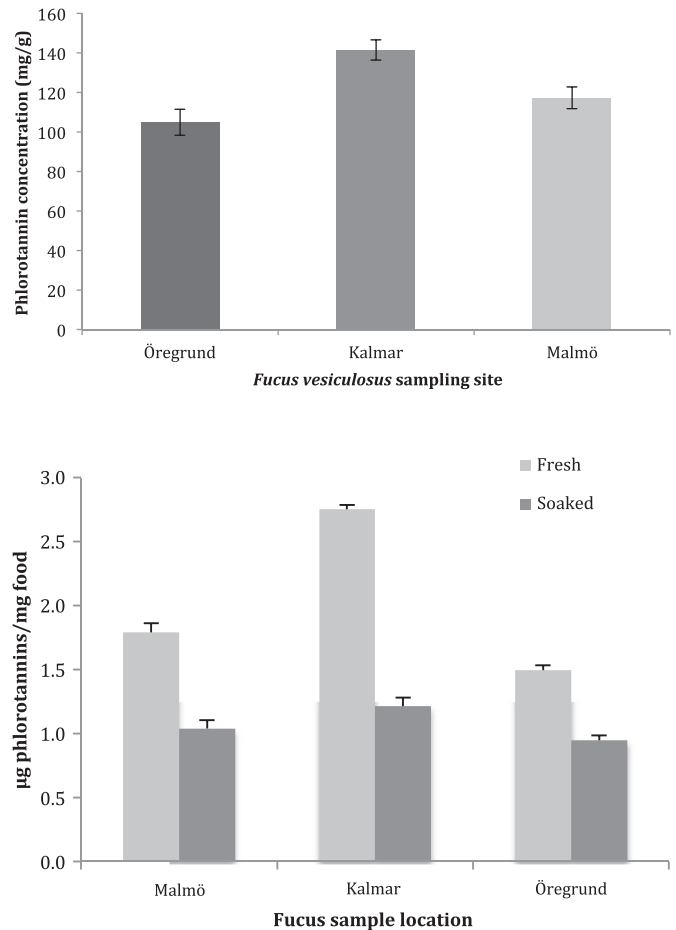


Fig. 5. a: Phlorotannin concentration of the *Fucus vesiculosus* (mg/g) from three geographic locations along the Swedish coast used as the food sources in the experiment. Values shown are means \pm 95% confidence intervals. b: Phlorotannin concentration of the artificial food made from *Fucus vesiculosus* (µg/g) from three geographic locations along the Swedish coast used in the experiment. Values shown are means \pm 95% confidence intervals.

Öregrund in the northern Baltic, which incorporates the transition from the Kattegat into the Baltic Sea itself, and a decrease in salinity from 10 to 4 psu. The fully crossed design revealed no difference between populations or treatment in survival or the measured physiological responses when populations were exposed to reciprocal abiotic (salinity) conditions within the Baltic. Their survival may

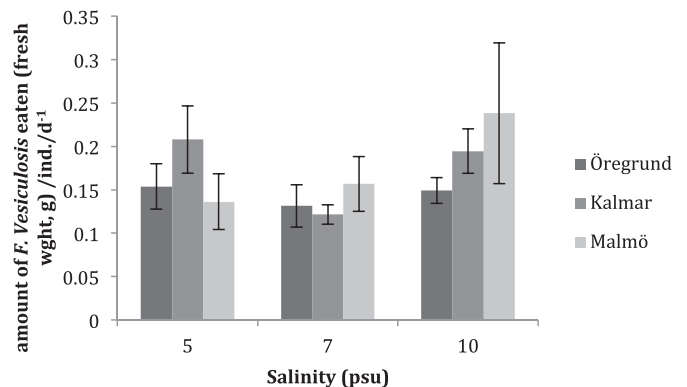


Fig. 6. Food consumption (mean \pm 95% confidence intervals, n = 10) of *Fucus vesiculosus* by three populations of *Idotea baltica* from three locations off the Swedish coast (Öregrund 5 psu, Kalmar 7 psu, and Malmö 10 psu). Consumption measured under three salinity conditions (shown).

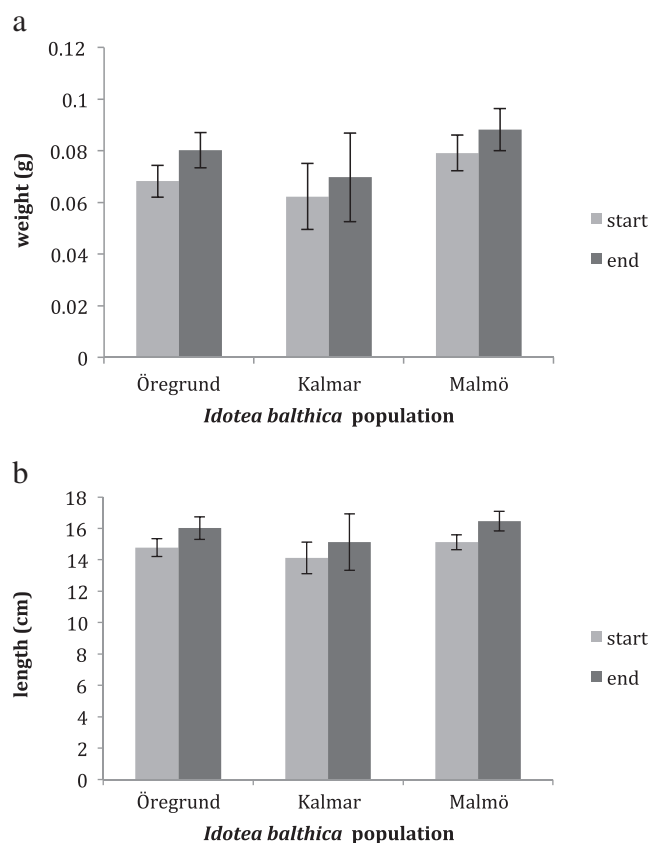


Fig. 7. a. Weight (g WW) and b. length (cm) of *I. baltica* at the start and end of the experimental period (mean \pm 95% confidence intervals). Data for the food source & salinity treatments were non-significant and were pooled.

indicate an ability to change phenotype in response to altered salinity. Critically however the physiological parameters of metabolism, growth and thermal tolerance, were no different across salinity for any of the three populations. An increase in metabolic rate may indicate increased energetic demand that could be linked to altered osmoregulatory, and a decrease in growth or thermal tolerance could be linked to an energetic trade off with one or both of these factors. The absence of such data indicates no energetic or functional compromises in basic physiological function as a result of altered salinity. Osmoregulation in *I. baltica* occurs primarily in the pleopods (Postel et al., 2000) via NaKATPase pumps; thus the efficiency or number of pumps is plastic in adults to facilitate the osmoregulatory demands of altered salinity imposed in this experiment. Additional pumps can be manufactured in a timescale of hours. However osmoregulation is an active process and the ability to survive in low salinity water requires the pumps but also the energy to osmoregulate to an increased level. This plasticity and ability to osmoregulate in such low salinity waters are rare; within the *Idotea* genus only five of the 27 species (*I. baltica*, *I. granulosa*, *I. chelipes*, *I. metallica* and *I. phosphorea*) are found in low salinity waters (Poore and

Table 3a

PERMANOVA results of thermal tolerance testing the upper thermal lethal limit between three populations (pop) of *Idotea baltica* (Malmö, Kalmar & Öregrund) at two acclimations temperatures (temp) (12 & 18 °C).

Source	d.f.	SS	MS	Pseudo-F	P(perm)	Unique perms
Pop	2	2.19	1.09	3.21	n/s	9952
Temp	1	6.79	6.79	19.9	<0.001	9745
Pop \times temp	2	0.735	0.37	1.08	n/s	9945
Res	27	9.2	0.34			
Total	32	19				

Schotte, 2013). The osmoregulatory stress imposed by the Baltic Sea means far fewer marine invertebrates are found there, with entire phyla (e.g. echinoderms) unable to reproduce, and therefore maintain permanent populations below 18 psu (HELCOM, 2009) due to this constraint. Even 'tolerant' euryhaline species such as the green shore crab *Carcinus maenas* are limited to the southern, less brackish Baltic waters. Thus the abundance and prevalence of *I. baltica* in the Baltic Sea may be enabled by their osmoregulatory capacity, but it is also due to the low competition for space and resources that results in few species being able to survive in the ecosystem.

Regarding grazer defence resistance, the *F. vesiculosus* food sources used in this study have higher levels of phlorotannins when compared to *F. vesiculosus* from the west coast of Sweden (Nylund et al., 2012) and even between the experimental sites used in this experiment there were significant differences in phlorotannin levels which showed no pattern related to salinity or distance into the Baltic Sea. However phlorotannins are highly soluble, and retaining representative levels in the processed food samples proved difficult, with much lower levels present on processing, and the full loss of differences between localities after three days in seawater. Given that phlorotannins deter feeding (Hay and Fenical, 1988; Targett and Arnold, 1998) in herbivores including *I. baltica*; and there is pre-existing evidence of food choice being influenced by the host plant's chemical properties (Honkanen et al., 2002) there is a strong cause to test for local adaptation to food source. However the means of doing this is not clear; using fresh algae is difficult where salinity is an additional variable as this will place a stress and may also alter the *Fucus* in itself. As the results of our food tests here showed, creating an artificial matrix from dried *Fucus* also is not sufficient to capture the chemical properties of the food source. Despite the redundancy of the artificial food as a variable (reflecting phlorotannin differences between localities) it did provide a stable and useable food source that was not liable to changes due to salinity. It has previously been surmised that *I. baltica* feed sub-optimally in situ by feeding on the more phlorotannins rich basal parts of *F. vesiculosus*, trading off growth with predator avoidance (Jormalainen et al., 2001), from a nutritional aspect, the food presented in this study was made from primarily the apical parts of *F. vesiculosus* which typically contain a higher proportion of easily digestible soluble carbohydrates (Westemeier and Gomez, 1996) than the basal parts, thus the food used in this study was a relatively high quality food compared to the food chosen in situ. As such it is probable that the isopods were not nutritionally limited during the experiment, even when the salinity treatments increased their energetic demand.

Table 2

ANCOVA of growth (end weight) with start weight as a covariable across populations (pop) and salinity treatments. Data were square root transformed prior to analysis.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Start weight	1	0.0479	0.0397	0.0397	58.4	<0.001
Pop	2	0.00156	0.000879	0.000439	0.65	0.528
Salinity	2	0.000237	0.000588	0.000294	0.43	0.651
Pop*salinity	4	0.00134	0.00134	0.000336	0.49	0.74
Error	51	0.0346	0.03461	0.000679		
Total	60	0.0858				

Table 3b

PERMANOVA results of thermal tolerance testing the critical thermal maximum between three populations (pop) of *Idotea baltica* (Malmö, Kalmar & Öregrund) at two acclimations temperatures (temp) (12 & 18 °C).

Source	d.f.	SS	MS	Pseudo-F	P(perm)	Unique perms
Pop	2	18.2	9.10	1.65	n/s	9944
Temp	1	200	200	36.2	<0.001	9835
Pop \times temp	2	60.9	30.4	5.51	<0.01	9959
Res	27	149	5.52			
Total	32	435				

Table 3c

PERMANOVA post hoc pair-wise tests of the critical thermal maximum, of the population x temperature interaction at the temperature level.

Population	Groups	t	P(perm)	Unique perms
Öregrund	12 vs. 18	19.1	0.0001	23
Malmö	12 vs. 18	0.724	0.467	69
Kalmar	12 vs. 18	3.41	0.0001	15

The results of the thermal tolerance investigation, in addition to underlining the lack of physiological differences between the three *I. baltica* populations, further emphasise the physiological plasticity of this species. The significant increase in upper thermal lethal limit as a result of higher acclimation temperature demonstrates the adaptability of *I. baltica* to changing environmental factors. Outside of the Baltic this species is exposed to a far greater range of temperatures as a result of exposure at low tide, and such populations would be expected to have a far higher ULT. Without the ecological need for this large thermal tolerance range in the tide-free Baltic Sea this high ULT is not required, but the plasticity of this ULT (demonstrated by the increase with higher temperature acclimation) suggests the Baltic populations have not lost the capability to withstand higher temperatures at least on a short temporal scale. For the Malmö population, which is in contrast to the other two populations located in Kattegat outside the Baltic Sea, acclimation had less effect on the critical thermal maximum. While being exposed to a similar annual temperature range, the Kattegat has current and wind induced influences that affect water flow and can rapidly change the temperature in contrast to the Baltic proper water temperature which is known to be more

stable. Thus, the lack of behavioural change between the 12 and 18 °C for the Malmö population is most likely a result of these animals being regularly rather than seasonally exposed to temperature changes.

The physiological plasticity of *I. baltica* as demonstrated by this study was important for the successful colonisation of the Baltic Sea. It is likely that this physiological plasticity has also been instrumental in determining the wider distribution of this isopod species, in particular within the low saline Black Sea. For species such as *I. baltica* with a generally low dispersion potential, plasticity may be a key survival mechanism in times of environmental change (Ernande and Dieckmann, 2004). Whereas species whose offspring are planktonic are not reliant on local environmental stability to ensure the survival of the next generation, a change to the abiotic environment has the potential to be catastrophic for brooding species with lower dispersal potential. For a species with low dispersal then, plasticity may allow a greater flexibility to environmental change (Ernande and Dieckmann, 2004) and knowledge of such plasticity can allow for the resilience of different ecosystems (in terms of function) to be predicted in light of current and future environmental changes e.g. increasing temperature, changing salinity and even ocean acidification. In range edge populations' plasticity may play an important role in a species' success and ability to cope with environmental variability (Gilchrist and Lee, 2007). However, maintaining a large tolerance range can be expensive and thus in order to live at the extreme range end the individuals there lose scope at the other end of the scale. For example, a trade off is seen in the osmoregulatory capacity of juvenile *Salvelinus alpinus* (Arctic Char) where those reared in freshwater have a lower salinity tolerance to those reared in marine conditions (Staurnes et al., 1992). This study demonstrates that all the *I. baltica* along the coast of the Baltic have the plasticity necessary to live throughout its salinity range, however to further investigate the invasion into the Baltic Sea, potential tradeoffs should be considered. Such tradeoffs may include, do *I. baltica* in the Baltic retain their ability to tolerate full salinity seawater, as in the Arctic Char example above? Increased temperature has been shown to shorten the lifespan of *I. baltica* (Tuomi et al., 1988), while it is already documented that individuals in low salinity are smaller than their fully marine counterparts which may affect brood size and therefore fecundity, in females.

4.1. Conclusions

The plasticity to 'regain' a wider thermal or salinity range is valuable to the persistence of the population if the environmental variability is unusually high. Fields et al. (1993) suggest stages in a species adaptability to increasing temperature as a result of climate change; at moderate environmental change physiological acclimatization (plasticity) is the key, then at a larger scale of environmental change phenotypes with more extreme tolerances are selected for; and finally if the environmental perturbation exceeds the tolerance range of the species then evolutionary adaptation at the genetic level is required for the species to survive. These stages can also be applied to salinity. Despite the large salinity gradient in the Baltic Sea our study indicates that this gradient is merely a moderate environmental change with respect to the tolerance range of *I. baltica* and as such Fields et al.'s (1993) first stage of coping, physiological plasticity is utilised to cope by these populations in the Baltic Sea.

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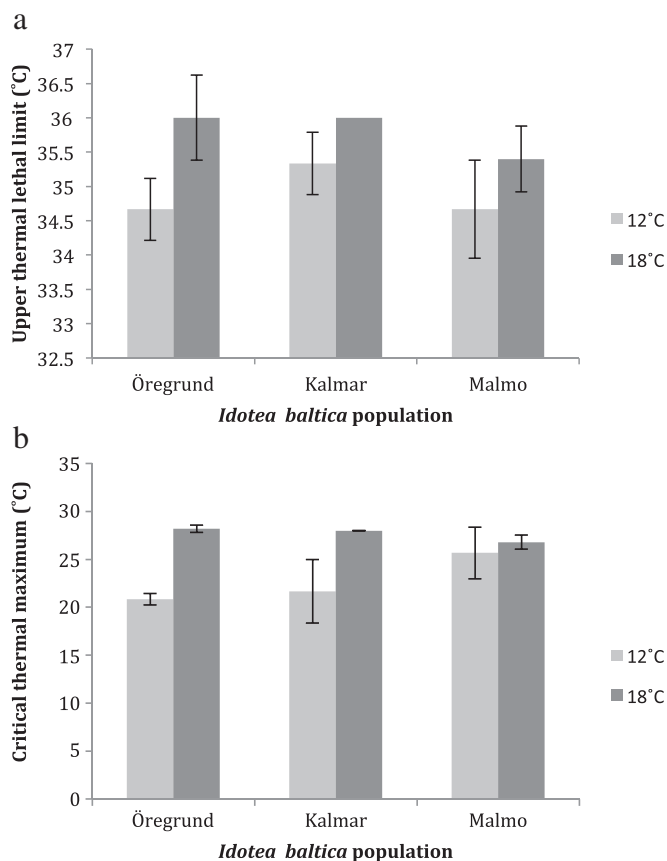


Fig. 8. a. Upper thermal lethal limit (°C) and b. critical thermal maximum (°C) of *Idotea baltica* from three geographic locations along the Swedish coast (mean \pm 95% confidence intervals). Limits shown for animals acclimated to 12 °C and 18 °C.

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