Mar Biol (2016) 163:227
DOI 10.1007/s00227-016-2989-5

INVASIVE SPECIES - ORIGINAL PAPER

Using the critical salinity (S_{crit}) concept to predict invasion potential of the anemone *Diadumene lineata* in the Baltic Sea

Imke Podbielski[1,2] · Christian Bock[3] · Mark Lenz[1] · Frank Melzner[1]

Received: 3 March 2016 / Accepted: 10 September 2016

Abstract It is widely assumed that the ability of an introduced species to acclimate to local environmental conditions determines its invasion success. The sea anemone *Diadumene lineata* is a cosmopolitan invader and shows extreme physiological tolerances. It was recently discovered in Kiel Fjord (Western Baltic Sea), although the brackish conditions in this area are physiologically challenging for most marine organisms. This study investigated salinity tolerance in *D. lineata* specimens from Kiel Fjord in order to assess potential geographical range expansion of the species in the Baltic Sea. In laboratory growth assays, we quantified biomass change and asexual reproduction rates under various salinity regimes (34: North Sea, 24: Kattegat, 14: Kiel Fjord, 7: Baltic Proper). Furthermore, we used ^{1}H-NMR-based metabolomics to analyse intracellular osmolyte dynamics. Within four weeks *D. lineata* exhibited a fivefold population growth through asexual reproduction at high salinities (34 and 24). Biomass increase under these conditions was significantly higher (69 %) than at a salinity of 14. At a salinity of 7, anemones ceased to reproduce asexually, their biomass decreased and metabolic depression was observed. Five main intracellular osmolytes were identified to be regulated in response to salinity change, with osmolyte depletion at a salinity of 7. We postulate that depletion of intracellular osmolytes defines a critical salinity (S_{crit}) that determines loss of fitness. Our results indicate that *D. lineata* has the potential to invade the Kattegat and Skagerrak regions with salinity >10. However, salinities of the Baltic Proper (salinity <8) currently seem to constitute a physiological limit for the species.

Responsible Editor: E. Briski.

Reviewed by S. Wijnhover and an undisclosed expert.

This article is part of the Topical Collection on Invasive Species.

Electronic supplementary material The online version of this article (doi:10.1007/s00227-016-2989-5) contains supplementary material, which is available to authorized users.

✉ Imke Podbielski
imke.podbielski@posteo.de

[1] GEOMAR Helmholtz Centre for Ocean Research Kiel, Hohenbergstr 2, 24105 Kiel, Germany

[2] Leibniz Center for Tropical Marine Ecology (ZMT), Fahrenheitstraße 6, 28359 Bremen, Germany

[3] Alfred-Wegener-Institute Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

Introduction

In marine ecosystems, the distribution of organisms is regulated by their physiological characteristics, environmental factors, their dispersal potential and ecological interactions. Invasive species (*sensu* Goodwin et al. 1999) are often characterized by a high tolerance to abiotic stress that may also have predetermined their invasion success (Lenz et al. 2011). Geographical range expansions of marine animals into brackish waters are thought to be limited by the organism's ability to tolerate hypoosmotic conditions (Dahl 1956). The capability of tolerating a wide salinity range is especially important for sessile osmoconformers, since they cannot avoid unfavourable conditions and are incapable of actively adjusting their extracellular osmolarity.

The Baltic Sea is the largest brackish sea worldwide with a surface area of 0.4 million km^2 and is characterized by a steep salinity gradient from west to east (Janssen et al. 1999). It hosts over 100 non-indigenous species, of which

Published online: 11 October 2016

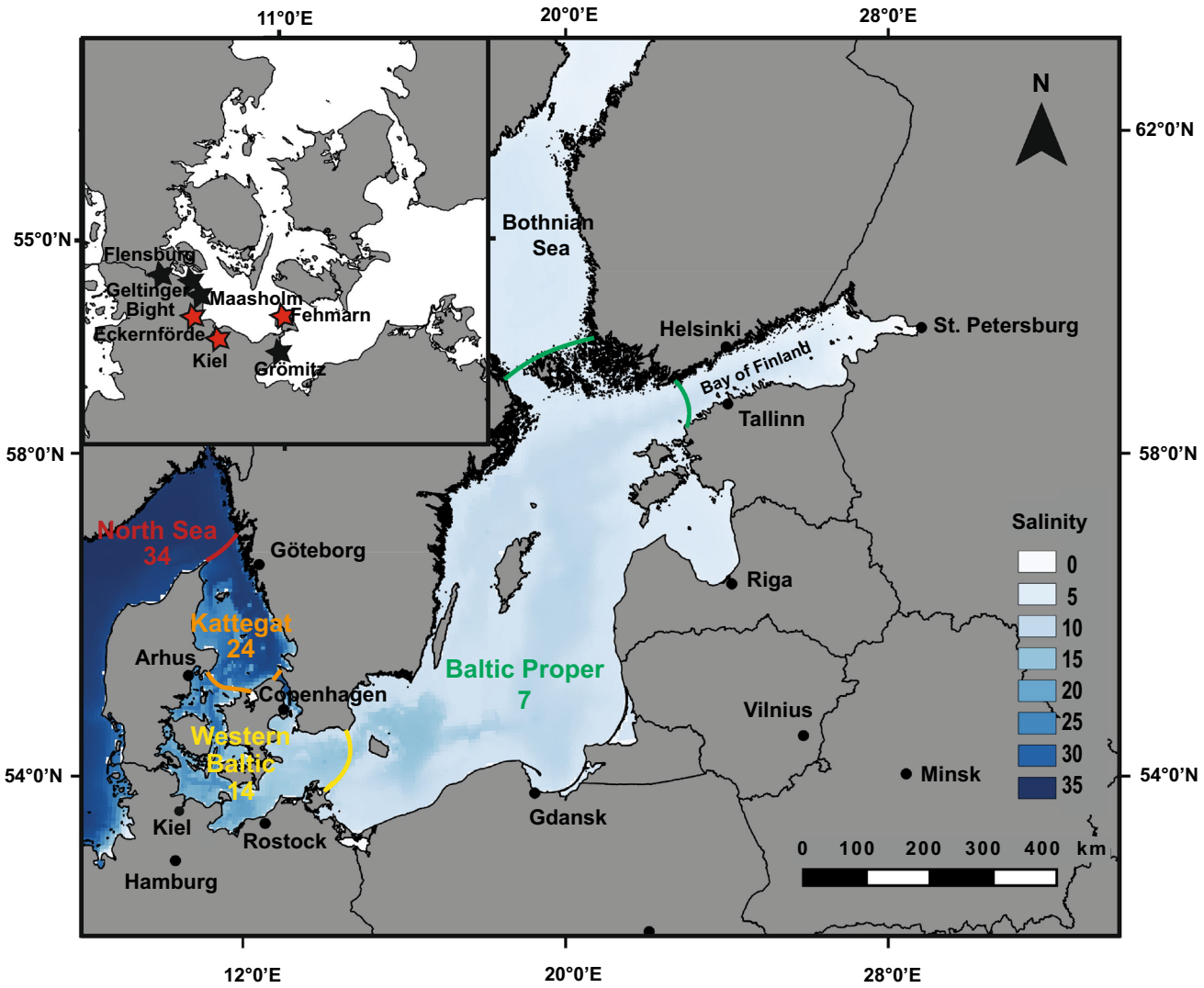

Fig. 1 Salinity regime of the Baltic Sea. The modelled mean bottom salinity of the Baltic Sea is depicted in blue tones (from: http://www.emodnet-seabedhabitats.eu). *Coloured lines* represent isolines of the respective experimental treatment. The small map shows the distribution of *Diadumene lineata* along the German Western Baltic Sea coast recorded in the rapid assessment survey including additional verified sightings (for coordinates see Table 1). *Black stars* mark the locations where the absence of the anemone was recorded, and *red stars* mark the presence of the species. A higher resolution of the Kiel Fjord area can be seen in the supplementary material (Fig. 3). Background map from: http://www.eea.europa.eu

approximately 70 % have established stable populations (Ojaveer et al. 2010). Salinity (next to temperature) has been described as the key limiting factor for the spread of marine organisms into the Baltic Sea (Bonsdorff 2006) and several taxa are restricted to the more saline waters of the Kattegat and Western Baltic Sea (WBS), including native anthozoans (sea anemones) (Leppäkoski et al. 2002). In addition, projected increases in precipitation and resulting desalination of the Baltic Sea will likely shift distribution boundaries of native and invasive organisms within the next decades (Gräwe et al. 2013) (Fig. 1).

The assessment of the (transgenerational) acclimation potential to environmental stress can provide an understanding of how abiotic factors affect the distribution of marine species (Munday et al. 2013). Deviations of environmental variables from the respective optimum are known to affect a species' metabolism, growth, and eventually fitness (Guppy and Withers 1999). Thus, determining the threshold at which hypoosmotic conditions impact fitness in a given marine species can help to estimate its expansion potential into brackish waters.

Osmoconformers allow fluctuations in their extracellular osmolarity that correspond to changes in the environment and achieve acclimation via cellular volume regulation (Pierce 1982). To prevent cellular swelling or shrinking, concentrations of compatible organic osmolytes

or inorganic ions are modulated (Yancey 2001). Usually, both substance classes are utilized by marine organisms, albeit at different time scales. Inorganic ions are typically used in response to short-term and rapid changes in external osmolarity, while organic osmolytes are utilized during long-term acclimation (Silva and Wright 1994). Compatible organic osmolytes have the advantage of not interacting with intracellular metabolism, whereas inorganic ions and certain amino acids can have strong perturbing effects on vital functions, such as cellular enzymatic reactions (Bowlus and Somero 1979). Thus, osmoconformers employ a variety of cellular regulatory mechanisms to maintain relatively constant concentrations of intracellular inorganic ions when external salinity fluctuates.

Cellular osmoregulation is an energy-dependent process. The physiological response to moderate abiotic stress often involves an increase in metabolic activity or a repartitioning of cellular energy allocation to compensate for the elevated energy demand induced by the stressor (Pan et al. 2015). Severe stress may, however, exceed the organism's capacity to supply ATP from food or internal storages to sustain routine metabolism. Many species subsequently reduce metabolism to conserve energy and extend survival time, although a down-regulation of metabolic processes cannot be maintained permanently (Guppy and Withers 1999).

Here, we report for the first time the presence of the anemone *Diadumene lineata* (Verrill 1871, scientific synonyms are *Haliplanella luciae* Verrill 1898 and combinations of the two genus and species names), a sessile osmoconformer, in the WBS. The distributional range of this invader in its new environment is yet unknown. In this study, we use organismal and cellular physiology to assess its potential for range expansion along the salinity gradient in the Baltic Sea. The small sea anemone *D. lineata*, which originates from the Northern Pacific (Uchida 1932), is extremely tolerant to abiotic environmental stressors (Shick 1976). Invasive populations of *D. lineata* are suspected to solely reproduce asexually and often occur in single sex populations, mono- and multi-clonal (Shick and Lamb 1977). This clonal reproduction can proceed rapidly under optimal thermal conditions (Minasian and Mariscal 1979). These characteristics presumably contribute to its invasion success. In terms of salinity tolerance, the lower limit of *D. lineata* lies between 8 and 12, although it can survive salinities down to freshwater conditions for short periods (Miyawaki 1951). Upon environmental stress, such as hypoosmotic conditions, thermal stress or desiccation, sea anemones are known to alter their behaviour, by contracting and producing a mucus coating (Shumway 1978). *D. lineata* is an osmoconformer and achieves cellular osmoregulation using intracellular osmolytes. Of the four classified groups of compatible osmolytes, only free amino acids (FAAs) and methylamines have been recorded in actinians (Yancey et al. 2010). Shick (1976) reports that taurine and glycine comprise over 70 % of the FAA pool in *D. lineata.* Methylamines have only rarely been investigated in anthozoans (corals: Yancey et al. 2010), albeit they are known as important intracellular osmolytes in other marine invertebrates (Silva and Wright 1994).

The extreme tolerance of *D. lineata* to environmental stress combined with its rapid clonal reproduction may determine its invasion success. The aim of this study was to investigate the salinity tolerance of *D. lineata* experimentally in order to assess whether novel *D. lineata* populations, which were recently found in the WBS (Kiel Fjord, salinity 12–20), have the potential for further geographical range expansion in the Baltic Sea. For this, we used two approaches: First, we studied metabolism, growth and asexual reproduction capacity (a proxy for evolutionary fitness) of *D. lineata* acclimated to different osmotic conditions representing different parts of the Baltic Sea as well as the North Sea. We hypothesized that fitness decreases with decreasing salinity. Second, we analysed intracellular osmolytes of the anemone from non-targeted metabolic profiles using ^{1}H-NMR spectroscopy to identify cellular mechanisms of osmoregulation. We aimed to identify the main organic osmolytes responsible for *D. lineata's* ability to acclimate to a wide range of salinities. We hypothesize that a depletion of the intracellular osmolyte pool causes a physiological stress response and defines the capacity for salinity tolerance. Combining these two approaches, we propose the concept of critical salinity (S_{crit}), which is defined as the critical low salinity threshold at which fitness as well as the intracellular osmolyte pool becomes zero. By using the S_{crit} concept, it may be possible to estimate the lower salinity limits and thus geographical boundaries of *D. lineata* based on organic osmolyte pool versus habitat salinity regressions.

Materials and methods

Animal collection and rapid assessment survey

Diadumene lineata specimens were collected in spring 2013 (05.03.13, 15.03.13, 10.04.13) from mussel aggregates located in the inner Kiel Fjord (coordinates: 54° 19.72′N, 010°8.88′E; depth: 1–3 m; temperature varied between 1 and 3 °C and salinity between 11 and 14 during the sampling period). After collection, anemones were transported within 5 min to the GEOMAR laboratory facilities. Of the ±60 animals, 20 were immediately frozen at −80 °C for DNA sequencing, while the remaining individuals were used for the acclimation experiment. DNA Sequencing was performed to ensure that the morphological identification of the introduced anemone as *Diadumene lineata* was correct.

Table 1 Locations of the rapid assessment survey for recording the distribution of *Diadumene lineata* in the Western Baltic Sea

Location	Coordinates		First record	Data source
Eckernförde	*54°28.42′N*	*009°50.01′E*	*2015*	*This study*
Fastensee, Fehmarn	54°30.69′N	011°2.18′E	2007	Pers. comm. P. Jonas (http://www.unterwasser-welt-ostsee.de)
Kiel Fjord				
Falckenstein	54°23.59′N	010°11.36′E	*2013*	*This study*
Holtenau	54°22.27′N	010°9.40′E	2013	Scientific Diving Guild Kiel
Kiellinie 1	*54°19.72′N*	*010°8.88′E*	*2012*	*This study*
Kiellinie 2	54°20.25′N	010°9.45′E	*2013*	*This study*
Mönkeberg 1	54°21.32′N	010°10.62′E	2013	Pers. comm. V. Sandow (CRM, Kiel)
Mönkeberg 2	54°20.59′N	010°10.42′E	2013	Pers. comm. V. Sandow (CRM, Kiel)
Mönkeberg 3	54°20.81′N	010°10.38′E	2013	Pers. comm. V. Sandow (CRM, Kiel)
Mönkeberg 4	54°20.44′N	010°10.49′E	2013	Pers. comm. V. Sandow (CRM, Kiel)
Schwedenkai	54°19.12′N	010°8.28′E	2013	Pers. comm. V. Sandow (CRM, Kiel)

Additional locations are based on anecdotal information about the presence of *Diadumene lineata* obtained from personal communications (I. Podbielski) with professional local divers

The regional distribution of *D. lineata* in surface waters (upper 3 m) was recorded at multiple locations along the German WBS coast (Table 1). Patchy distribution of *D. lineata* in the WBS makes a screening of its distribution via rapid assessment survey difficult, and thus we recorded potentially suitable natural and anthropogenic hard substrates in each location. For this, we used an Olympus Tough 8010 underwater camera that was fixed to a 2-m-long telescopic pole, ca. 0.5 m above a measuring frame (0.0625 m^2). The whole set-up was lowered to the ground to keep the distance from the frame to the recorded surface constant. Benthic communities were video-recorded for 10 s at randomly chosen mussel aggregates ($n = 5$–10) in 1–2 m water depth. The video material was visually analysed for the presence of *D. lineata*, and anemone density was determined. With this approach we were able to detect anemones >0.3 cm in diameter. Water temperature and salinity were recorded using a WTW cond 315i salinometer (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany).

Species determination

Frozen *D. lineata* (−80 °C) was ground under liquid nitrogen using mortar and pestle. RNA was extracted following homogenization of the tissue using QIAshredder and RNeasy kits (Qiagen, Hilden, Germany). cDNA was synthesized using SuperScript reverse transcriptase (Thermo Fisher). *D. lineata* primers for the 18S rRNA gene (accession number: JF832987.1) were designed using MacVector10 (MacVector Inc., Cary, USA; forward primer: 5′-ACGGCTACCACATCCAAGGAAG-3′, reverse primer: 3′-GGCATAAAGCAACAGTCTCCACT-5′) resulting in amplification of a 519-bp fragment. cDNA was amplified using Taq-Polymerase (Invitrogen, Karlsruhe, Germany) in the presence of 1.5 mM $MgCl_2$ (PCR conditions: 4-min denaturation at 94 °C, 45-s annealing at 53 °C and 45-s elongation at 72 °C, 35 cycles followed by a final amplification step of 8 min at 72 °C). PCR fragments were separated after electrophoresis in 1 % agarose gels. Extraction and purification of the PCR fragments was accomplished using the QIAquick gel extraction kit (Qiagen, Hilden, Germany). Samples were Sanger-sequenced with BigDye 3.1 ready reaction mix (Applied Biosystems) after BigDye XTerminator purification (Applied Biosystems) on an AB3130 genetic analyser. Five samples from five animals were sequenced, and all were identical with the reference sequence JF832987.1.

Individual stress response

To investigate changes in the performance of *D. lineata* in response to osmotic stress, animals were exposed to different salinities during a four-week acclimation experiment. The experiment was conducted using the GEOMAR climate chamber facilities in summer 2013. Anemones were cultured in covered aerated aquaria (0.2 L) containing filtered seawater (FSW, 0.2 μm) from Kiel Fjord (salinity = 12–16) at 19 °C and a 12/12 h dark-light cycle. Salinity was gradually adjusted at a rate of 2 units day^{-1} until the experimental treatment levels were reached (salinity = 7, 14, 24, and 34) by diluting the FSW with distilled water or by adding artificial sea salt (SeequaSal, Münster, Germany) to it. The timing of the dilution steps was stacked, so that all experimental groups reached their targeted salinity level on the same day (day 0 of the experimental period).

Salinity levels were chosen in order to represent the mean surface salinities of Baltic Sea regions: 34 = North Sea, 24 = Kattegat, 14 = Kiel Fjord, 7 = Baltic Proper (Fig. 1). Water was exchanged three times a week, and physiochemical water variables were controlled regularly (salinity, temperature, oxygen, ammonium). The sea anemones were fed ad libitum on a daily basis with *Artemia franciscana* nauplii of instar stage 2–3 (Sanders, Great Salt Lake Utah, USA). Each treatment level had 14 replicate tanks, with 3 animals (= subreplicates) per experimental unit. During the course of the experiment, fission rates, biomass change, feeding rates and behavioural variables, such as anemone activity, attachment rate and mucus production, were measured. After the experimental period of 4 weeks, anemones destined for metabolite analysis were shock frozen in liquid nitrogen and stored at −80 °C ($n = 8$). The remaining animals were used for assessing respiration rates ($n = 6$).

Fission rate and growth rate

Diadumene lineata's ability to reproduce is the most reliable fitness proxy, while biomass change allows to quantify the fraction of the energy budget that is allocated to growth processes. Clonal reproduction was recorded daily. An asexual reproduction event was viewed as completed when a dividing anemone appeared as clearly separated into two individuals. Fission rate was calculated as the number of fission events per day per experimental unit. To determine growth rates in *D. lineata*, initial fresh weight ($FW_{initial}$) was recorded on day 0. Animals were then assigned to the tanks in a way that aimed at having a similar mean biomass per tank. The final fresh weight (FW_{final}) and dry weight (DW_{final}) were obtained following the respiration trials (see below). For this, anemones were quickly and gently blotted dry with non-sticking tissues and FW was determined using a precision scale (LC220 s, Sartorius, Göttingen, Germany, 1 mg resolution). For the drying, anemone samples were transferred to small pre-weighed aluminium boxes. Anemones were dried at 80 °C for 14 h (U30 793 590 Memmert, Schwabach, Germany), and DW was measured subsequently using the same precision scale utilized for FW-measurements. Initial DW ($DW_{initial}$) was estimated using regression coefficients from a linear regression model based on the FW_{final} and DW_{final} data (see supplementary material Fig. 1). Total FW and DW per replicate tank were determined, and growth rates were calculated by substracting $DW_{initial}$ from DW_{final} and devided by the duration of the experiment.

Feeding rate

Feeding rates were assessed to estimate the energy intake of *D. lineata* under different salinity regimes. Feeding rates of *D. lineata* were recorded for six randomly chosen replicate tanks for each salinity level during the experiment. Anemones were fed ad libitum with a daily portion of 400 ± 50 nauplii per 3 anemones. Abundance of nauplii in an aliquot of the food slurry was counted prior to feeding, and food portions were adjusted to the number of anemones per tank. Food input per tank was regression-estimated by examining food subsamples. For this, absolute numbers of nauplii were counted under the microscope in known aliquots of the homogenously mixed food suspension. Furthermore, to assess nauplii DW, determined nauplii abundances were applied onto pre-weighed glass fibre filters (Whatman, type 693, retention 1.2 µm, size 24 mm, GE Healthcare UK Limited, Buckinghamshire, UK). Filters were rinsed with distilled water and dried at 80 °C overnight (U30 793 590, Memmert, Schwabach, Germany), and then nauplii DW was determined with a microscale (SC2, Sartorius, Göttingen, Germany, 1 µg resolution). Nauplii DW was then modelled as a function of nauplii abundance, and thus the weight of the food input could be regression-estimated from the known nauplii abundance in the food portion (see supplementary material Fig. 2). The portion of non-ingested food was determined three times per week. For this, tanks were carefully rinsed with FSW and all food debris was then filtered using a 25-µm mesh width sieve. The residuals were pooled per week and stored at −20 °C. At the end of the experiment, debris samples were defrosted and filtered onto pre-weighted glass fibre filters. Filters were then rinsed with distilled water and dried at 80 °C overnight, while their DW was determined. Feeding rates were calculated as the difference between food input and the amount of food debris collected per anemone over time.

Respiration rates

Oxygen consumption of *D. lineata* at different salinities was measured at the end of the 4-week acclimation period. Anemones were starved for 24–72 h before the measurement and were then transferred in groups of three into gas-tight glass respiration chambers of 15 mL volume (Eydam, Kiel, Germany) and allowed to settle. One measuring circuit comprised three replicate chambers (containing animals) and a control chamber (without animals) serving as a control for bacterial respiration (bacterial respiration <1 %). Chambers were filled with aerated, 0.2 µm filtered FSW and positioned in a circulating water bath (19 °C). The glass respirometers were equipped with oxygen sensor spots that were attached to their inner walls (Type PSt3 Sensorspots, Presens, Regensburg, Germany) and oxygen concentration was recorded every minute using fibre-optic light transmitters connected to the outside of the respiration vials directly above the sensor spots and a

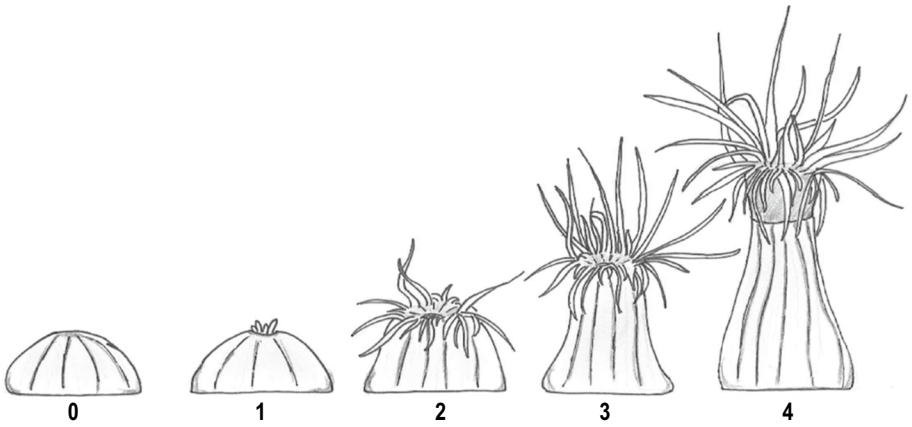

Fig. 2 Habitus of *Diadumene lineata* indicative for anemone activity: Stages 0–4. Stage 0: Complete contraction, no tentacle visible, column height reduced; Stage 1: Almost completely contracted, column height reduced, tentacle tips visible; Stage 2: Cylindrical column slightly contracted, tentacles moderately extended; Stage 3: Column erect, cylindrical, only scapus visible, tentacles widely extended; Stage 4: Column erect to maximum degree, scapus and capitulum visible, tentacles completely extended

multi-channel oxygen transmitter (OXY-4 mini, Presens, Regensburg, Germany). Each trial lasted for 2 h, with the exception that incubation was shortened in the case air saturation decreased to 80 %. In order to avoid disturbing the animals, experiments were carried out without stir bars. Pilot experiments assured that no oxygen gradient established throughout the trial period. To calculate oxygen consumption rates, the linear decrease in oxygen concentration was measured over an experimental interval of 15–120 min and respiration rates were calculated ($R^2 = 0.9022 \pm 0.1234$).

Behavioural observations

Sea anemones show various behavioural responses to stressful osmotic conditions. Mucus production in actinians can give information about the onset of mechanisms that help to maintain cellular homeostasis when exposed to osmotic stress, while contraction upon exposure to osmotic stress reduces the surface area that is in contact with the surrounding medium (Shumway 1978). Furthermore, individuals of *D. lineata* detach from their substratum under abiotic stress (Davenport 1904). However, it is unknown whether they actively detach to find a better location, or if attachment depends on the amount of energy that remains for basic physiological processes after coverage of the vital metabolic needs. In this experiment, mucus production was recorded daily. Presence or absence of mucus was noted and additionally assessed as a mean percentage of mucus production per tank. Mucus in this case was defined as the presence of a thick, slimy, but colourless coating on or around the scapulum of the anemones. An index of the anemone habitus was developed that classifies five categories which exemplify anemone activity shown in Fig. 2. The habitus was noted every second day. For analysis, the mean habitus index per experimental unit was calculated. The presence of detached anemones was noted daily. 'Attachment' was quantified as the mean time it took for 50 % of anemones to be attached.

Cellular response

Non-targeted metabolic profiling was assessed via high-resolution magic angle spinning (HR-MAS) ^{1}H-NMR (nuclear magnetic resonance) spectroscopy at the NMR laboratory of the Alfred-Wegener-Institute for Polar and Marine Research in Bremerhaven, Germany. Frozen animal samples of *D. lineata* were weighed, and then animals of similar biomass were chosen for the measurements. Frozen animal tissue (9.78 ± 4.11 mg) was transferred directly (within 1 min) to a sample container (rotor) filled with deuterium oxide (D_2O) containing 1 % trimethylsilyl propionate (TSP) as an internal reference standard. The assembled rotor was injected into a triple tunable ^{1}H-^{13}C-^{31}P-HRMAS probe of a Bruker WB Avance III 400 (9.4T) NMR spectrometer operating at 400 MHz (Bruker Biospin GmbH, Germany). High-resolution one-dimensional (1D), one-pulse ^{1}H-NMR spectra were acquired at 4 °C and a spinning rate of 3 kHz to assess the metabolic profiles of *D. lineata* whole-body tissue using a composite pulse presaturation sequences with following acquisition parameters: Bruker protocol cpmgpr1d, ns = 64, TD = 70,656, SW = 8802 Hz; acquisition time of 4 s and a relaxation delay of 4 s. Field homogeneity was optimized using a standard shim protocol resulting in a typical line width of 2–4 Hz. Spectra post-processing, metabolite identification and analysis were performed using an evaluation license of

Chenomx Software (Chenomx NMR Suite 7.6, Chenomx Inc., Canada). Prior to Fourier transformation all data were automatically zero-filled to at least 128 k and processed with an exponential multiplication of 0.3 Hz. After phase- and baseline correction, spectra were calibrated to the internal standard TSP (at 0.0 ppm). This procedure yielded specific metabolic profiles visualizing possible changes in the osmolyte composition of *D. lineata* and their relative concentrations caused by salinity alterations. Specific metabolites were identified using the Chenomx Software database and chemical shift tables (Tikunov et al. 2010). Osmolyte signals of interest were analysed in more detail. To do so, relative metabolite concentrations were determined by integration of ^{1}H-NMR signals using the integration routine in Chenomx. All integrals were corrected for anemone weight. Portions of the major osmolytes are given as per cent of the total sum of the osmolytes of interest.

Statistics

Experimental aquaria were regarded as units of replication. For all response variables, normality of data and homogeneity of variances were tested graphically and with the Shapiro-Wilks-W Test as well as the Levene's Test, respectively. These assumptions were tested on base of the residuals of the applied ANOVA and regression models. Differences between the salinity levels were analysed using one-way ANOVA followed by Tukey's HSD post hoc tests. Biomass values were transformed reciprocally ($1\ x^{-1}$) prior to statistical analysis. Fission rates at the lowest salinity (7) showed no variance and were thus excluded from the analysis. Anemone habitus and attachment data were analysed with the rank-based Kruskal–Wallis ANOVA, which was followed by focused comparisons of the mean ranks between groups. Changes in relative osmolyte concentrations in relation to salinity were analysed using linear regression. All analyses were made using the free statistical computing software R! (R Development Core Team 2014). The threshold for significance was $p < 0.05$. The data in the text and figures are presented as the mean ± standard deviation (SD).

Results

Distribution and identification

Hitherto *D. lineata* was detected at nine locations in Kiel Fjord (2010–2015), Eckernförde harbour and in the Fastensee on Fehmarn in surface waters of maximal 3 m depth (Fig. 1 and supplementary material). The first record of the invader in the Baltic Sea was reported from the brackish Fastensee in 2007 (pers. comm. Peter Jonas, http://www.unterwasser-welt-ostsee.de), while the first sighting in Kiel Fjord was in 2010 (pers. comm. Verena Sandow, CRM, Kiel). In October 2013, *D. lineata* was found in highly variable densities of 939 (± 877) individuals m^{-2} (± SD) in the inner Kiel Fjord region and large, patchily distributed populations were observed again in the summers of 2014 and 2015 (Melzner unpub. obs.). The anemone was found in Holtenau, north of the Kiel Canal, but not further north than Falckenstein (Table 1 and supplementary material). It is commonly associated with blue mussels (*Mytilus edulis x trossulus*) beds. Sequencing of a fragment of the 18S rRNA gene from five anemone samples confirmed 100 % identity with *D. lineata* (accession #: JF832987.1).

Acclimation experiment

Fission rates (i.e. fitness proxy) of *D. lineata* were significantly influenced by salinity: At a salinity of 7, anemones did not reproduce asexually, while fission rates peaked between the salinity of 24 and 34 (0.30 ± 0.06 fission day^{-1} and 0.27 ± 0.09 fission day^{-1}, respectively) leading to a five- and four-fold increase in anemone abundance during the course of the experiment, respectively (Fig. 3b). Fission rates differed significantly between all salinity treatments, except between the levels 24 and 34. ANOVA results for this and the following response variables are shown in the supplementary material.

Growth rates revealed a pattern similar to the one that was seen for fission rates and differed significantly between all but the two highest salinity levels (Fig. 3a). The highest biomass increase (0.53 ± 0.09, 0.54 ± 0.15 mg day^{-1}, respectively) occurred at the two highest salinity levels (24 and 34). At a salinity of 7, anemone biomass decreased by -0.10 ± 0.02 mg day^{-1}.

Feeding rates of *D. lineata* also differed significantly between the salinity treatment levels (Fig. 3c), and food intake increased with increasing salinity. We measured reduced feeding rates at salinities of 7 (15 % reduction) and 14 (7 % reduction) when compared to the highest salinity treatment (34).

Respiration rates varied significantly between a salinity of 7 and all other treatment levels. Anemones that were acclimated to the lowest salinity (7) respired 57 % less than conspecifics at salinities of 14, 24, and 34. No significant differences in oxygen consumption emerged between the higher salinity levels (Fig. 3d).

The habitus was significantly influenced by salinity. Anemones acclimated to salinities of 7 and 14 varied significantly in their habitus from those that were reared at salinities of 24 and 34 (Fig. 3e). At the lower salinities, the column was contracted permanently and the tentacle crown was not as widely expanded as in those animals that were in the high salinity treatments. In these specimens, the

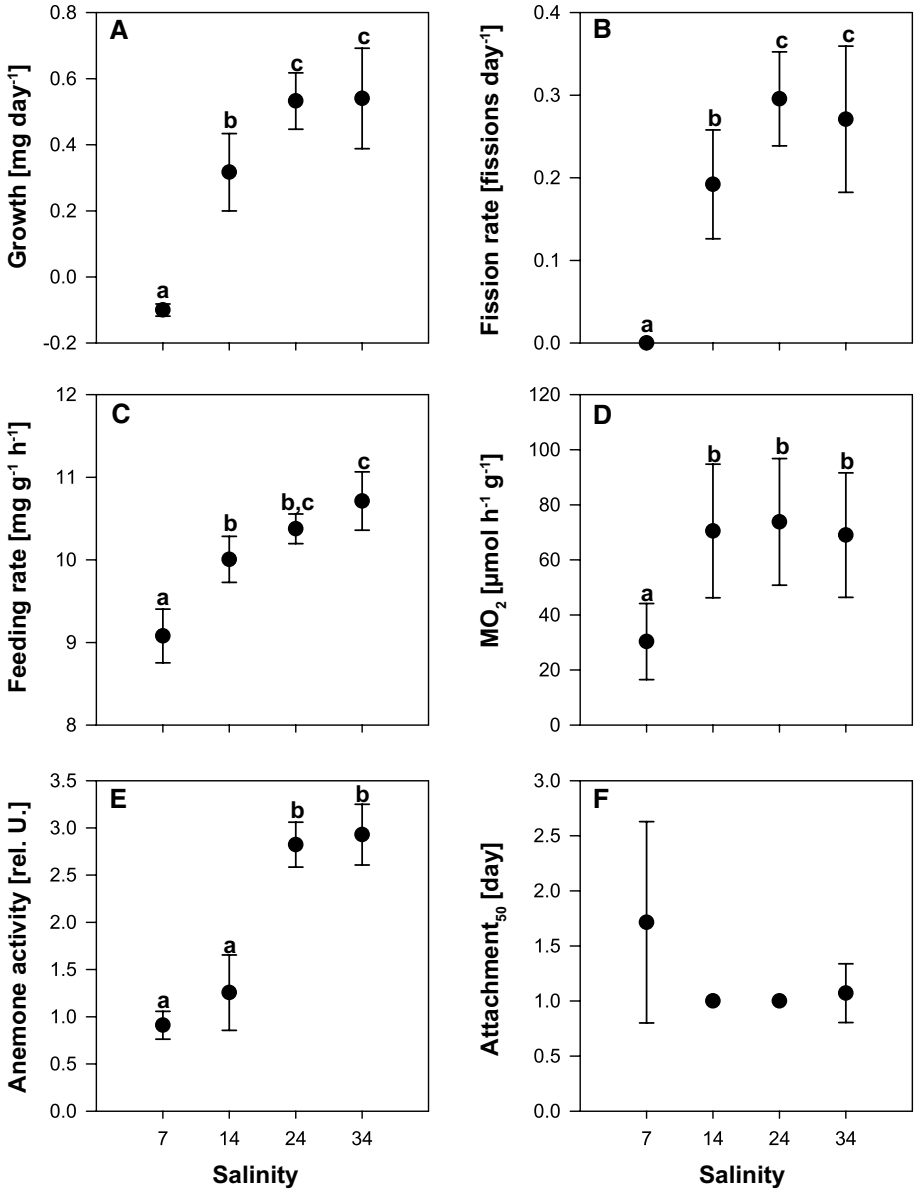

Fig. 3 Physiological response variables indicating salinity tolerance in *Diadumene lineata* individuals that were exposed to different salinities for four weeks. All values are depicted as means and standard deviation. Differences between the treatment levels were detected using one-way ANOVA for parameters **a**–**d** and with the Kruskal–Wallis test for parameters **e** and **f**. *Different letters* indicate significant differences between salinity levels identified by post hoc testing ($\alpha = 0.05$). **a** Growth (Tukey HSD, 14–7: $p < 0.001$, 24–7: $p < 0.001$, 34–7: $p < 0.001$, 24–14: $p = 0.005$, 34–14: $p = 0.005$, 24–34: $p = 1.000$; $n = 6$), **b** Fission rate (Tukey HSD, 24–14: $p = 0.001$, 34–14: $p = 0.016$, 24–34: $p = 0.638$; $n = 14$), **c** Feeding rate (Tukey HSD, 14–7: $p < 0.001$, 24–7: $p < 0.001$, 34–7: $p < 0.001$, 24–14: $p = 0.052$, 34–14: $p = 0.002$, 24–34: $p = 0.430$; $n = 6$), **d** Respiration rate (Tukey HSD, 14–7: $p = 0.039$, 24–7: $p = 0.05$, 34–7: $p < 0.017$, 24–14: $p = 0.994$, 34–14: $p = 0.999$, 24–34: $p = 0.980$; 7 & 14: $n = 5$; 24 & 34: $n = 6$), **e** A habitus index of *Diadumene lineata* represents a measure for anemone activity (Focused comparisons of the mean ranks between groups: 14–7: False, 24–7: True, 34–7: True, 24–14: True, 34–14: True, 24–34: False, $n = 14$), **f** Attachment of *Diadumene lineata* (Tukey HSD, 14–7: $p = 0.001$, 24–7: $p = 0.001$, 34–7: $p = 0.004$, 24–14: $p = 1.000$, 34–14: $p = 0.979$, 24–34: $p = 0.979$; $n = 14$). Attachment is depicted as the time 50 % of the anemones took to attach to substrate. The ANOVA outputs for this and the following parameters can be seen in the supplementary material

tentacle crown was usually fully extended and the column erected.

Speed of anemone attachment to the substratum, after transfer to the experimental units, increased with increasing salinity. At a salinity of 7, 50 % of the individuals needed approximately a day longer to attach to the walls or the bottom of the experimental units than anemones at higher salinity treatments (Fig. 3f). Focused comparisons did not

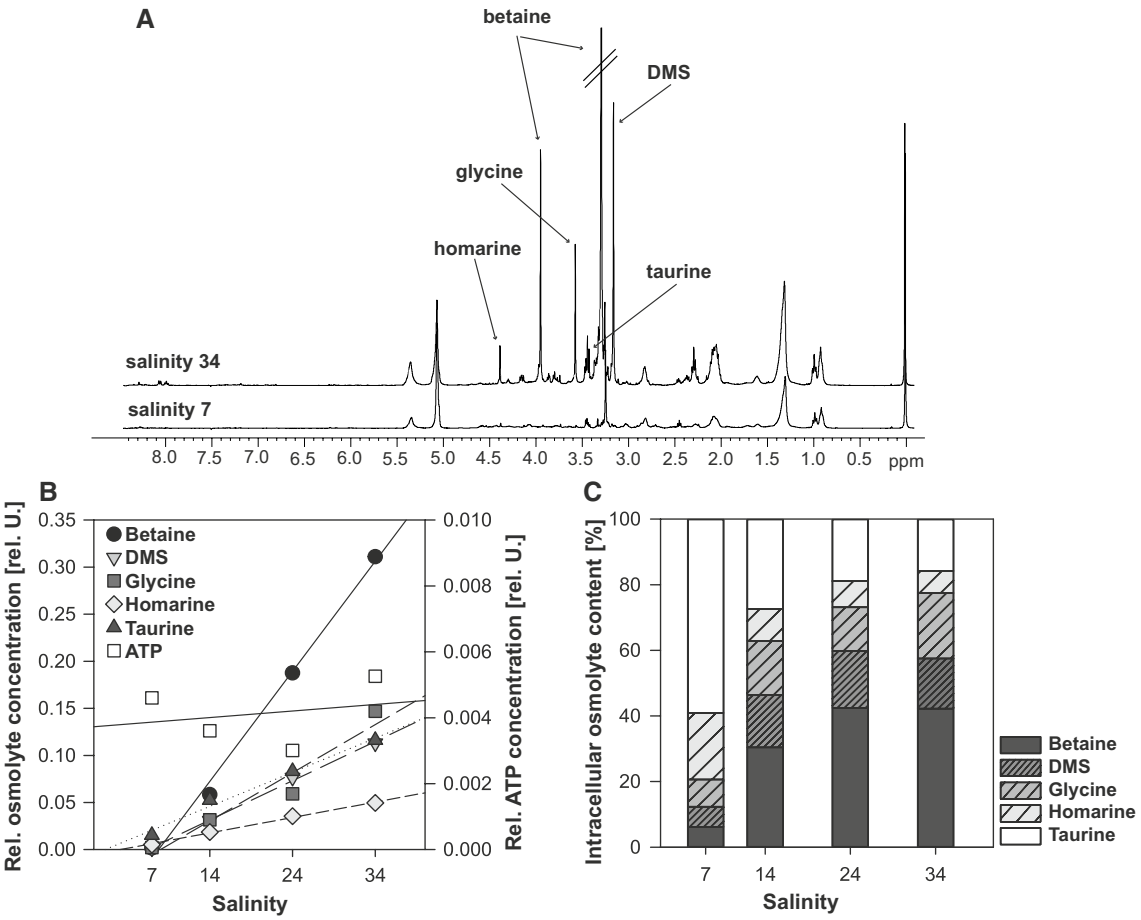

Fig. 4 **a** Representative one-dimensional 400 MHz ^{1}H–NMR spectra depicting the metabolite composition of whole-body tissue of the sea anemone *Diadumene lineata* at salinities of 7 and 34. *x*-axis = frequency [ppm]. **b** Relative osmolyte concentration corrected for anemone body mass is represented as a function of salinity for betaine, DMS, glycine, homarine and taurine. ATP values, shown on the second *y*-axis, were not affected by salinity changes, serving as control. Data are presented as mean values per metabolite ($n = 8$) including a regression line. Betaine: $R^2 = 0.7534$, $F(1,30) = 91.67$, $p < 0.001$. DMS: $R^2 = 0.5132$, $F(1,30) = 31.63$, $p < 0.001$. Glycine: $R^2 = 0.5461$, $F(1,30) = 36.09$, $p < 0.001$. Homarine: $R^2 = 0.5594$, $F(1,30) = 38.09$, $p < 0.001$. Taurine: $R^2 = 0.6041$, $F(1,30) = 45.77$, $p < 0.001$. ATP: $R^2 = 0.013$, $F(1,30) = 0.405$, $p > 0.05$. **c** Composition of the osmolyte pool of *Diadumene lineata* including the main five osmolytes along a salinity gradient ($n = 8$)

show significant differences, despite the highly significant result of the Kruskal–Wallis ANOVA.

Visible mucus production was exclusively observed in anemones acclimated to the lowest salinity level (7) ($n = 14$). 44.30 ± 9.41 % of all animals in this group produced a thick mucus coating. At higher salinities, no mucus production could be detected. No statistical test could be applied to these data due to a lack in variability.

All data have been deposited in Pangea database (https://doi.pangaea.de/10.1594/PANGAEA.861221).

Cellular response: analysis of osmolytes

We identified 17 signals from several metabolite classes in the specific NMR-profiles of *D. lineata* whole-body tissue: amino acids (alanine, glutamate, glutamine, glycine, homoserine, isoleucine, leucine, proline and valine), methylated ammonium and sulfonium compounds (betaine, carnitine, dimethyl sulfone (DMS), homarine, o-acetylcholine, taurine), lactate and glycogen. Betaine, DMS, homarine and taurine were identified as key osmolytes with the highest relative concentration (see Fig. 4a) and decreased in their relative concentrations strongly with decreasing salinity (Fig. 4b). The intracellular pool of organic osmolytes at marine conditions (salinity = 34) was dominated by betaine comprising 40 % of the organic osmolyte pool (Fig. 4c), and among all osmolytes, the betaine concentration increased most strongly with salinity. At a salinity of 34, relative betaine concentration was 4.9-times higher compared to all other identified intracellular metabolites. At the lowest salinity level (7), all major osmolytes were depleted and their

relative concentrations were 8–190 times lower than at marine conditions (salinity = 34).

Discussion

Distributional ranges and local abundances of species are regulated by abiotic and biotic limiting factors (Brown et al. 1996). Salinity is one major environmental stressor that can determine range boundaries in aquatic organisms (Berger and Kharazova 1997). Our preliminary survey and acclimation experiment revealed that: (1) *Diadumene lineata* invaded regions of the WBS and is capable of tolerating the ambient brackish conditions (salinity 12–20) that lie below the tolerance threshold of most anthozoans; (2) *D. lineata* shows a high phenotypic plasticity when exposed to osmotic stress. However, at a salinity of 7 a physiological limit is reached which is characterized by metabolic depression as well as inhibition of growth and reproduction; (3) salinity tolerance in this species is regulated via the intracellular osmolyte pool, which mainly consists of betaine, but also comprises glycine, taurine, dimethyl sulfone (DMS) and homarine. (4) Consistent with the constraints in metabolic performance, cellular volume regulation seems to reach an acclimation limit at a salinity of 7, characterized by organic osmolyte pool depletion.

Present distribution of the invasive sea anemone *D. lineata*

Our rapid assessment survey coupled with information about earlier findings indicates that *D. lineata* already invaded different parts of the WBS. We, for instance, found *D. lineata* to be already abundant in Kiel Fjord. Since the species has never been reported for the German Baltic Sea coast before 2007, although there are earlier reports of non-recurring findings in the German North Sea and the port of Hamburg (Gollasch and Riemann-Zürneck 1996), this suggests a rapid settlement and population expansion of the sea anemone in the WBS since 2007. Albeit this, there are also known cases in which neozoa persisted in relatively low numbers for decades before they exhibited a population explosion (Crooks 2005).

There are conflicting reports about the resilience of the species once it has arrived in a novel environment. Monoclonal populations have been described to be prone to population collapse (Shick and Lamb 1977), but other studies emphasized the extreme physiological tolerance of this species (Shick 1976). However, the recurrent observation of this anemone in Kiel Fjord (2010–2015) supports the theory that the species is able to successfully establish permanent populations under the ambient osmotic and thermal conditions in the WBS. This is presumably facilitated by the high growth and asexual reproduction rates the species exhibits particularly in summer (I. Podbielski, pers. obs.).

Our study is the first to report the presence of *D. lineata* in the Baltic Sea. We assume an earlier introduction into Kiel Fjord to be unlikely, as one of us (F. Melzner) has been sampling local mussel beds extensively without noting the species prior to 2011. Therefore, we assume that we found the initial population in Kiel Fjord, which could be the source for a prospective expansion north and south of the Kiel Fjord. However, there is little knowledge about the mode and time course of the introduction of *D. lineata* into the Baltic. Generally, ship traffic is one of the main vectors that transport non-native species into the Baltic Sea (Leppäkoski et al. 2002) and Kiel and Fehmarn are situated nearby areas of heavy commercial ship traffic (HELCOM 2009, www.kielcanal.org). *D. lineata* has been introduced to port areas via ship hull fouling before (Gollasch and Riemann-Zürneck 1996), but other vectors, such as imported oyster cultures or attachment to floating seaweed, could likewise play a role for the global dispersal of this species (Williams 1973). Another possibility is the introduction via ballast water. Unidentified actiniaria have been found in ship ballast tanks before (Briski et al. 2011). Especially for locations nearby Kiel Canal, where deballasting water is a typical ship operation before entering the lock, this could be a significant vector. Furthermore, Kiel harbour is an important hub for a variety of commercial and recreational ships with often prolonged layovers providing enough time for attachment to ship hulls. Busy hub locations are known to facilitate the spread of invaders (Floerl et al. 2009). It is thus possible that anemones could be transported from here to other potential habitats in the Baltic Sea and further. This offers the possibility to establish baseline knowledge and monitor range expansion in marine benthic invertebrates. For this, we would need a more detailed and international survey along the Baltic Sea coast line, while an assessment of the trophic impact of the species on Baltic Sea benthic ecosystems is needed to gauge the ecological consequences of this invasion.

Considering the large population densities of *D. lineata*, this anemone could have significant ecological and economical impacts on the benthic ecosystems of the WBS. So far nothing is known about the anemone's impact on ecosystems, but if high population densities, as observed in the Kiel Fjord become the rule, we would expect an impact on biochemical fluxes of the benthic ecosystem. For example, dense polyp mats could significantly influence the native benthic hard bottom communities, which are commonly dominated by blue mussels, by competition for space and food with other epibionts (Lages et al. 2011). Furthermore, little is known about the food spectrum of the animals: *D. lineata* feeds mainly on small crustaceans (Williams 1972), but according to Baker et al. (2004) the anemone might

also prey on larvae of economically important taxa such as blue mussels and oysters. It is unknown whether there are local predators of the anemone in the WBS, such as nudibranchs, seastars or fish (Ottaway 1977) that could prevent a massive population expansion.

Extreme salinity tolerance of *D. lineata*

Diadumene lineata populations investigated in this study showed a high acclimation potential to the hypoosmotic conditions of the WBS. This high salinity tolerance likely causes, at least partly, the anemone's invasion success, but our results also show that its range expansion into the Baltic Sea is limited. Survival rates, growth rates and asexual reproduction rates served as indicators for individual fitness of *D. lineata* under osmotic stress. Maximum somatic and population growth were observed at marine conditions (salinity = 24 and 34), whereas at a salinity of 14, fitness was moderately reduced. A total reduction in the anemone's fitness was then recognized at a salinity of 7, when asexual reproduction ceased and biomass decreased significantly over time. Previous findings report a high degree of euryhalinity in this species with a limit at salinities between 8 and 12 (Miyawaki 1951). This corresponds very well to our results. Other studies described a short-term tolerance of up to 2 weeks towards freshwater conditions, while at temperatures <10 °C hyposalinity tolerance is even higher (Shick 1976).

Even though size and biomass reduction caused by a sudden and substantial decrease in salinity is well known in other invertebrates (Westerbom et al. 2002), our study is the first one to test the effect of salinity on growth and reproduction of *D. lineata*. Fission rates observed here under marine conditions are comparable to those obtained for *D. lineata* by Minasian and Mariscal (1979). In addition to salinity, it is known that fission rates and body size in *D. lineata* depend on temperature and are inversely correlated (Minasian 1982). In this study, a temperature equivalent to those found in the WBS was used (19 °C, Janssen et al. 1999). When water temperature drops below a thermal threshold (usually 10 °C), *D. lineata* switches to a state of dormancy with no reproductive activity (Shick and Lamb 1977). Chia (1976) suggested that stressful conditions promote asexual reproduction in sea anemones in general. In invasive populations of *D. lineata* asexual reproduction seems to be the primary, if not the only way of reproduction (Hand 1955), while sexual reproduction so far has only been reported from native populations in Japan at water temperatures above 25 °C (Fukui 1991).

Energy metabolism is an essential part of an organism's ability to tolerate environmental stress (Dahlhoff 2004). Under optimal conditions an organism invests only a part of the available energy into maintenance, whereas the remaining energy serves to support somatic and reproductive growth (Guppy and Withers 1999). When exposed to osmotic stress, less energy can be invested into growth because a substantial amount is needed for cellular ion homeostasis. For example, acclimation of marine *Mytilus* sp. to low salinities (8) has been shown to increase Na–K-ATPase (NKA) activity by 63 % (Willmer 1978). This suggests that osmotic regulation can be associated with high energetic costs. In our study, anemone fitness increased with salinity, but reached a plateau at 24–34, indicating that only salinity stress <24 significantly disturbs the species' energy budget (Fig. 3b). This was reflected in a significantly reduced feeding activity as well as metabolic depression at the lowest salinity tested. Additionally, we detected behavioural changes such as enhanced mucus production, contraction and slower rate of attachment at salinities of 14 and 7. In our study, 45 % of the specimens reared at low salinities produced a visible mucus coating, which usually goes along with contraction (Shumway 1978). These two behavioural changes can be indicators of encystment and a protection against extreme environmental stress, such as hypoosmotic conditions, thermal stress or desiccation (McManus et al. 1997). Furthermore, attachment is also affected by low salinities, even leading to complete detachment and floating of anemones in the water column until conditions improve, indicating a complete loss of scope for activity (Davenport 1904).

Anemone feeding rates and growth rates both declined with decreasing salinity, while oxygen consumption remained constant at salinities of 34, 24 and 14. Similar observations of reductions in the scope for growth due to stable respiration rates while feeding rates decreased simultaneously have been made in other marine invertebrates during exposure to environmental stress (Stumpp et al. 2012; Appelhans et al. 2014). Thus, saving energy by reducing food intake and digestion as well as the subsequent anabolic processes (protein turnover for growth) seems to be a common strategy in marine invertebrates exposed to stress. However, the observation that growth rates decline more strongly than feeding rates could also be due to an overestimation of the latter. Feeding rate determinations in anemone growth trials are prone to error (Stumpp et al. 2012), and it is possible that we have overestimated feeding rates by not taking into account the dissolved material that is lost via "sloppy feeding" and the non-ingested food particles that were too small or fragile to be recorded (Zamer 1986). In actinians the degree of overestimation mostly depends on the absorption efficiency of the anemone as well as the prey assimilability (Lesser et al. 1994). The absorption efficiency of *D. lineata* under optimum conditions was estimated to be around 93 % (McManus et al. 1997). Thus 7 % overestimation of feeding rate can be considered a minor systematic error, unless absorption efficiency changes with salinity.

'Critical salinity' defined by organic osmolyte depletion

In order to understand more about the intracellular organic osmolyte pool of *D. lineata*, we used HR-MAS ^{1}H-NMR spectroscopy, which has the advantage to assess the metabolic profiles of intact cells and tissues within small sample sizes and which provided a qualitatively thorough evaluation of the organic osmolytes in our samples. Using a non-targeted metabolic profiling approach, we were able to identify all organic osmolyte groups in *D. lineata*. *D. lineata*'s organic osmolyte pool consists of five major osmolytes with betaine accounting for the largest portion of the intracellular osmolyte pool, similar to other anthozoans and marine invertebrates such as blue mussels or gastropods (Pierce et al. 1984; Silva and Wright 1994; Yancey et al. 2010). In contrast to this, glycine and taurine are known osmolytes of *D. lineata* (Shick 1976), whereas homarine has only been reported for other actinians (Mathias et al. 1960) and DMS is a known metabolite of corals (Yancey et al. 2010). Furthermore, intraspecific differences in osmolyte composition may also be due to an organism's diet or its metabolism and starvation can change osmolyte composition (Webb et al. 1972). In our study no intraspecific differences in osmolyte composition were observed.

Free amino acids (FAAs) and methylamines are the two organic osmolyte groups in marine invertebrates. Shick (1991) suggests that FAAs contribute 12–16 % to the change of the total intracellular osmotic concentration in *D. lineata*. FAAs have often been identified as major osmolytes in actinians (Pierce and Minasian 1974). In contrast, we discovered that the largest portion of the organic osmolyte pool consists of methylamines (>65 %) and not FAAs. This is in accordance with findings from Yancey et al. (2010). In *Diadumene leucolena*, a close relative of *D. lineata* (Rodríguez et al. 2012), the degree of euryhalinity is suggested to be directly related to the size of the intracellular FAA pool (Pierce and Minasian 1974). Kube et al. (2006) compared two bivalve species characterized by different geographical distributions and different habitat salinities and found differences in the total size of the intracellular FAA pool between the two species. Especially glycine, taurine and alanine concentrations were positively correlated with salinity (Kube et al. 2006), which is in accordance with our findings of salinity induced modulations of the intracellular osmolyte pool.

Our observations suggest that energy expensive osmoregulation in *D. lineata* cannot be sustained under the osmotic conditions of the Baltic Proper. With a decline in salinity, osmolyte concentrations decreased gradually (Fig. 4b), while an almost complete depletion of the intracellular osmolyte pool was observed at the lowest salinity level. Organic osmolytes are responsible for long-term salinity acclimation (Yancey 2001), but if the capacity of this mechanism is exhausted or if changes in seawater osmolarity are rapid, the ion regulatory system can also support osmoregulation (Silva and Wright 1994). However, under these circumstances cellular homeostasis cannot be maintained for long and hypoosmotic conditions are ultimately lethal (Shick 1976). We therefore conclude that the depletion in organic osmolytes determines the limits of low salinity tolerance in *D. lineata* as it correlates with a critical reduction in fitness. A salinity of 7 seems to pose a critical threshold for *D. lineata*, where relative osmolyte concentration is nearly zero, metabolism is depressed and growth as well as reproduction is inhibited. Braby and Somero (2006) studied salinity tolerance in blue mussels and defined a lower critical salinity (S_{crit}) as the point at which the organisms heart rate drops significantly. We hypothesize that a depletion of the intracellular osmolyte pool and subsequent intracellular ion disturbances are the ultimate cause for such a stress response. Therefore, we bring forward a modified concept of critical salinity (S_{crit}) defined as the salinity at which the intracellular osmolyte pool size and fitness become zero. However, it needs to be established for other species, whether the tight correlation observed between osmolyte pool state and fitness holds true. If so, it may be possible to estimate the lower salinity limits of marine osmoconformers based on organic osmolyte pool versus habitat salinity regressions (see Fig. 5).

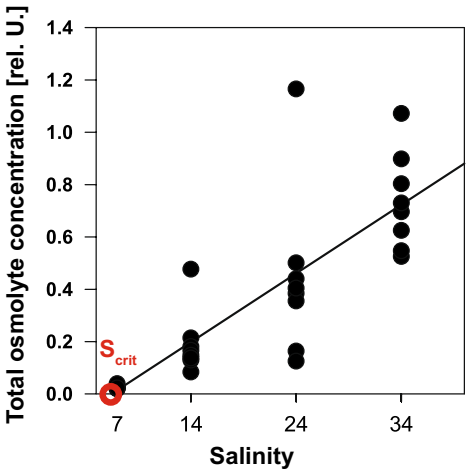

Fig. 5 Critical salinity (S_{crit}) is defined as the salinity where the organic osmolyte pool is fully depleted, thereby impairing cellular volume regulation. The total osmolyte concentration of the five main osmolytes (betaine, DMS, glycine, homarine and taurine) in relative Units (*y*-axis) is depicted in relation to salinity (*x*-axis). A linear model shows the decline of relative osmolyte concentration with decreasing salinity. According to the linear model $y = 0.026 \times x - 0.170$ ($R^2 = 0.684$) S_{crit} will be reached at a salinity of 6.5

Implications of salinity tolerance for potential invasion success and range expansion

Salinity and temperature are considered the key environmental stressors affecting arriving invaders (Lee et al. 2003), while our results highlight the extreme phenotypic plasticity that *D. lineata* exhibits in response to osmotic stress. The anemone shows a strong tolerance to brackish salinities, but reveals a clear physiological limit at salinities between 14 and 7. This acclimation potential will further determine its invasion success and geographical range expansion. In the case of the Baltic Sea, this means that *D. lineata* will most likely continue to thrive in the Western Baltic Sea (salinity 12–20) and there is a high probability of an invasion into the Kattegat and Skagerrak area (mean salinity 25 and 30, respectively) where osmotic conditions possibly facilitate settlement and are even more favourable for population growth (Figs. 1, 3b). Furthermore, *D. lineata*'s high acclimation potential likely allows a further southward invasion into the Arkona Sea (mean salinity 10). However, the species' current physiological limit restricts a range expansion into the Baltic Proper (mean salinity 7) and further eastwards.

In addition to phenotypic plasticity, genetic adaption can increase salinity tolerance (Ghalambor et al. 2007). According to Lee et al. (2003) invasive species that expand into areas that have a salinity that is lower than that in their native habitats are prone to strong selective pressures that lead to local adaptations. The salinity gradient of the Baltic Sea promotes strong selection and rapid evolution in species coming from fully marine habitats (Sanford and Kelly 2011). Considering the short generation times that invasive anemones such as *D. lineata* can have under favourable conditions, evolutionary shifts in tolerance and performance should be expected (Ayre 1995). However, the capacity of *D. lineata* for genetic adaptation to low salinities remains to be tested.

From our results, we conclude that salinity tolerance can play an important role for the successful establishment of non-native marine invertebrates and may ultimately determine their capacity to expand into brackish waters. More studies on other osmoconforming species are needed to further elucidate the role of organic osmolytes for the setting of geographical range boundaries in species that occur along salinity gradients.

Acknowledgments We would like to thank U. Panknin for her technical support, J. Thomsen and R. Kiko for advice on experimental design, methodology and data analysis, and L. Stapp for help with the anemone culture. We would like to thank E. Rickert for supplying the food for our anemone culture. The authors would further like to thank W. E. Zamer for his advice on anemone respiration trials.

Compliance with ethical standards

Conflict of interest Imke Podbielski declares that she has no conflict of interest. Christian Bock declares that he has no conflict of interest. Mark Lenz declares that he has no conflict of interest. Frank Melzner declares that he has no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

Appelhans Y, Thomsen J, Opitz S et al (2014) Juvenile sea stars exposed to acidification decrease feeding and growth with no acclimation potential. Mar Ecol Prog Ser 509:227–239. doi:10.3354/meps10884

Ayre DJ (1995) Localized adaptation of sea anemone clones: evidence from transplantation over two spatial scales. J Anim Ecol 64:186–196. doi:10.2307/5754

Baker P, Baker SM, Fajans J (2004) Nonindigenous marine species in the greater Tampa Bay ecosystem. Tampa Bay Estuary Program Tech. Publ, (02–04)

Berger VJ, Kharazova AD (1997) Mechanisms of salinity adaptations in marine molluscs. In: Naumov AD, Hummel H, Sukhotin AA, Ryland JS (eds) Interactions and adaptation strategies of marine organisms. Springer, New York, pp 115–126

Bonsdorff E (2006) Zoobenthic diversity-gradients in the Baltic Sea: continuous post-glacial succession in a stressed ecosystem. J Exp Mar Biol Ecol 330:383–391. doi:10.1016/j.jembe.2005.12.041

Bowlus RD, Somero GN (1979) Solute compatibility with enzyme function and structure: rationales for the selection of osmotic agents and end-products of anaerobic metabolism in marine invertebrates. J Exp Zool 208:137–151. doi:10.1002/jez.1402080202

Braby CE, Somero GN (2006) Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus Mytilus). J Exp Biol 209:2554–2566. doi:10.1242/jeb.02259

Briski E, Bailey SA, MacIsaac HJ (2011) Invertebrates and their dormant eggs transported in ballast sediments of ships arriving to the Canadian coasts and the Laurentian Great Lakes. Limnol Oceanogr 56(5):1929–1939

Brown JH, Stevens GC, Kaufman DM (1996) The geographic range: size, shape, boundaries, and internal structure. Annu Rev Ecol Syst 27:597–623

Chia F-S (1976) Sea anemone reproduction: patterns and adaptive radiations. In: Mackie GO (ed) Coelenterate ecology and behaviour. Plenum Press, New York, pp 261–270

Crooks JA (2005) Lag times and exotic species: the ecology and management of biological invasions in slow-motion1. Écoscience 12:316–329. doi:10.2980/i1195-6860-12-3-316.1

Dahl E (1956) Ecological salinity boundaries in poikilohaline waters. Oikos 7:1–21. doi:10.2307/3564981

Dahlhoff EP (2004) Biochemical indicators of stress and metabolism: applications for marine ecological studies. Annu Rev Physiol 66:183–207. doi:10.1146/annurev.physiol.66.032102.114509

Davenport G (1904) Variation in the number of stripes in the sea-anemone *Sagartia luciae*. Mark Anniv 137–146

Floerl O, Inglis GJ, Dey K, Smith A (2009) The importance of transport hubs in stepping-stone invasions. J Appl Ecol 46:37–45. doi:10.1111/j.1365-2664.2008.01540.x

Fukui Y (1991) Embryonic and larval development of the sea anemone *Haliplanella lineata* from Japan. In: Williams RB, Cornelius

PFS, Hughes RG, Robson EA (eds) Coelenterate biology: recent research on cnidaria and ctenophora. Springer, New York, pp 137–142

Ghalambor CK, McKay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct Ecol 21:394–407. doi:10.1111/j.1365-2435.2007.01283.x

Gollasch S, Riemann-Zürneck K (1996) Transoceanic dispersal of benthic macrofauna: *Haliplanella luciae* (Verrill, 1898) (Anthozoa, Actiniaria) found on a ship's hull in a shipyard dock in Hamburg Harbour, Germany. Helgolander Meeresunters 50:253–258. doi:10.1007/BF02367154

Goodwin BJ, McAllister AJ, Fahrig L (1999) Predicting invasiveness of plant species based on biological information. Conservation Biol 13:422–426. doi:10.1046/j.1523-1739.1999.013002422.x

Gräwe U, Friedland R, Burchard H (2013) The future of the western Baltic Sea: two possible scenarios. Ocean Dynam 63:901–921. doi:10.1007/s10236-013-0634-0

Guppy M, Withers P (1999) Metabolic depression in animals: physiological perspectives and biochemical generalizations. Biol Rev Cambridge Philos Soc 74:1–40

Hand C (1955) The sea anemones of central california part III. The acontiarian anemones. Wasmann J Biol 13:189–251

HELCOM (2009) Biodiversity in the Baltic Sea—an integrated thematic assessment on biodiversity and nature conservation in the Baltic Sea. Baltic Sea Environment Proceeding No 116B. Helsinki: HELCOM

Janssen F, Schrum C, Backhaus JO (1999) A climatological data set of temperature and salinity for the Baltic Sea and the North Sea. De Hy Z 51:5–245. doi:10.1007/BF02933676

Kube S, Gerber A, Jansen JM, Schiedek D (2006) Patterns of organic osmolytes in two marine bivalves, *Macoma balthica*, and *Mytilus* spp., along their European distribution. Mar Biol 149:1387–1396. doi:10.1007/s00227-006-0303-7

Lages B, Fleury B, Menegola C, Creed J (2011) Change in tropical rocky shore communities due to an alien coral invasion. Mar Ecol Prog Ser 438:85–96. doi:10.3354/meps09290

Lee CE, Remfert JL, Gelembiuk GW (2003) Evolution of physiological tolerance and performance during freshwater invasions. Integr Comp Biol 43:439–449. doi:10.1093/icb/43.3.439

Lenz M, da Gama BAP, Gerner NV et al (2011) Non-native marine invertebrates are more tolerant towards environmental stress than taxonomically related native species: results from a globally replicated study. Environ Res 111:943–952. doi:10.1016/j.envres.2011.05.001

Leppäkoski E, Gollasch S, Gruszka P et al (2002) The Baltic: a sea of invaders. Can J Fish Aquat Sci 59:1175–1188. doi:10.1139/f02-089

Lesser MP, Witman JD, Sebens KP (1994) Effects of flow and seston availability on scope for growth of benthic suspension-feeding invertebrates from the gulf of maine. Biol Bull 187:319–335

Mathias B, Ross DM, Schachter M (1960) The distribution of 5-hyfroxytryptamine, tetramethylammonium, homarine and other substances in sea anemones. J Physiol 151:296–311

McManus MG, Place AR, Zamer WE (1997) Physiological variation among clonal genotypes in the sea anemone *Haliplanella lineata*: growth and biochemical content. Biol Bull 192:426–443

Minasian LL (1982) The relationship of size and biomass to fission rate in a clone of the sea anemone, *Haliplanella luciae* (Verrill). J Exp Mar Biol Ecol 58:151–162. doi:10.1016/0022-0981(82)90126-5

Minasian LL, Mariscal RN (1979) Characteristics and regulation of fission activity in clonal cultures of the cosmopolitan sea anemone, *Haliplanella luciae* (Verrill). Biol Bull 157:478–493

Miyawaki M (1951) Notes on the effect of low salinity on an actinian, *Diadumene luciae*. J Fac Sci Hokkaido Univ, Ser 6(10):123–126

Munday PL, Warner RR, Monro K et al (2013) Predicting evolutionary responses to climate change in the sea. Ecol Lett 16:1488–1500. doi:10.1111/ele.12185

Ojaveer H, Jaanus A, MacKenzie BR et al (2010) Status of biodiversity in the Baltic Sea. PLoS ONE 5:e12467. doi:10.1371/journal.pone.0012467

Ottaway JR (1977) Predators of sea anemones. Tuatara 22:213–220

Pan T-CF, Applebaum SL, Manahan DT (2015) Experimental ocean acidification alters the allocation of metabolic energy. PNAS 112:4696–4701. doi:10.1073/pnas.1416967112

Pierce SK (1982) Invertebrate cell volume control mechanisms: a coordinated use of intracellular amino acids and inorganic ions as osmotic solute. Biol Bull 163:405–419

Pierce SK, Minasian LL (1974) Water balance of a euryhaline sea anemone, *Diadumene leucolena*. Comp Biochem Physiol A Mol Integr Physiol 49:159–167. doi:10.1016/0300-9629(74)90551-9

Pierce SK, Edwards SC, Mazzocchi PH et al (1984) Proline betaine: a unique osmolyte in an extremely euryhaline osmoconformer. Biol Bull 167:495–500

Rodríguez E, Barbeitos M, Daly M et al (2012) Toward a natural classification: phylogeny of acontiate sea anemones (Cnidaria, Anthozoa, Actiniaria). Cladistics 28:375–392. doi:10.1111/j.1096-0031.2012.00391.x

Sanford E, Kelly MW (2011) Local adaptation in marine invertebrates. Annu Rev Mar Sci 3:509–535. doi:10.1146/annurev-marine-120709-142756

Shick JM (1976) Ecological physiology and genetics of the colonizing actinian *Haliplanella luciae*. In: Mackie GO (ed) Coelenterate ecology and behavior. Springer, New York, pp 137–146

Shick MJ (1991) Functional biology of sea anemones. Chapman & Hall, London

Shick JM, Lamb AN (1977) Asexual reproduction and genetic population structure in the colonizing sea anemone *Haliplanella luciae*. Biol Bull 153:604–617

Shumway SE (1978) Activity and respiration in the anemone, *Metridium senile* (L.) exposed to salinity fluctuations. J Exp Mar Biol Ecol 33:85–92. doi:10.1016/0022-0981(78)90044-8

Silva AL, Wright SH (1994) Short-term cell volume regulation in *Mytilus californianus* gill. J Exp Biol 194:47–68

Stumpp M, Trübenbach K, Brennecke D et al (2012) Resource allocation and extracellular acid–base status in the sea urchin *Strongylocentrotus droebachiensis* in response to CO_2 induced seawater acidification. Aquat Toxicol 110–111:194–207. doi:10.1016/j.aquatox.2011.12.020

Tikunov AP, Johnson CB, Lee H et al (2010) Metabolomic investigations of american oysters using 1H-NMR spectroscopy. Mar Drugs 8:2578–2596. doi:10.3390/md8102578

Uchida T (1932) Occurrence in Japan of *Diadumene luciae*, a remarkable actinian of rapid dispersal. J Fac Sci, Hokkaido Univ, Ser 6(2):69–82

Webb KL, Schimpf AL, Olmon J (1972) Free amino acid composition of scyphozoan polyps of *Aurelia aurita*, *Chrysaora quinquecirrha* and *Cyanea capillata* at various salinites. Comp Biochem Physiol, Part B: Comp Biochem 43:653–663. doi:10.1016/0305-0491(72)90150-2

Westerbom M, Kilpi M, Mustonen O (2002) Blue mussels, *Mytilus edulis*, at the edge of the range: population structure, growth and biomass along a salinity gradient in the north-eastern Baltic Sea. Mar Biol 140:991–999. doi:10.1007/s00227-001-0765-6

Williams RB (1972) Chemical control of feeding behaviour in the sea anemone *Diadumene luciae* (Verrill). Comp Biochem Physiol, Part A: Mol Integr Physiol 41:361–371. doi:10.1016/0300-9629(72)90067-9

Williams RB (1973) Are there physiological races of the sea anemone *Diadumene luciae*? Mar Biol 21:327–330. doi:10.1007/BF00381089

Willmer PG (1978) Sodium fluxes and exchange pumps: further correlates of osmotic conformity in the nerves of an estuarine bivalve (*Mytilus Edulis*). J Exp Biol 77:207–223

Yancey PH (2001) Water stress, osmolytes and proteins. Amer Zool 41:699–709. doi:10.1093/icb/41.4.699

Yancey PH, Heppenstall M, Ly S et al (2010) Betaines and dimethylsulfoniopropionate as major osmolytes in cnidaria with endosymbiotic dinoflagellates. Physiol Biochem Zool: Ecol Evol Approaches 83:167–173. doi:10.1086/644625

Zamer WE (1986) Physiological energetics of the intertidal sea anemone *Anthopleura elegantissima*. Mar Biol 92:299–314. doi:10.1007/BF00392670