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Interactive effects of temperature and salinity on shell formation and general condition in Baltic Sea *Mytilus edulis* and *Arctica islandica*

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ABSTRACT: Stress often induces metabolically expensive countermeasures. Bivalve shell production is costly and can thus be indirectly impacted by environmental stress. Suboptimal salinity and temperature may constitute stressors that allocate energy away from shell production to cellular processes such as osmoregulation or to the repair of cellular damage. In the course of climate change, water temperatures of the Baltic Sea are predicted to increase, and salinity is predicted to regionally decrease. These shifts may lead to increased stress for temperate marine species adapted to relatively cool water temperatures and high salinity conditions. To better understand the importance of climate change-related stress, we assessed the isolated and interactive effects of salinity and temperature on shell increment (cumulative growth: shell), cellular oxidative stress (accumulation of oxidized lipids and proteins: lipofuscin), instantaneous physiological condition (condition index: CI), and mortality of young *Mytilus edulis* and *Arctica islandica* from the western Baltic Sea. Temperature and salinity interactively affected shell increment, lipofuscin accumulation, and mortality of *M. edulis* as well as shell increment of *A. islandica*. Shell increment of *M. edulis* was less affected by hyposalinity than shell increment of *A. islandica*. In both species the CI decreased and lipofuscin accumulation increased with increasing temperature. Lipofuscin accumulation negatively correlated with shell increment in *M. edulis*. We conclude that Baltic Sea populations of ecologically relevant bivalve species may experience severe stress by the predicted regional scenario of warming and desalination if evolutionary adaptation does not happen at a similar rate.

KEY WORDS: *Mytilus edulis* · *Arctica islandica* · Growth · Condition · Cellular stress · Mortality

INTRODUCTION

Bivalve growth rates may be affected by food availability, competition for space, wave exposure, light, pH, temperature, and salinity (Malone & Dodd 1967, Seed 1976, Bayne & Worrall 1980, Kautsky 1982, Wong & Levinton 2004, Berge et al. 2006). Bivalve growth is costly and includes shell production, which involves calcification and the formation of the organic matrix (Palmer 1992, Irie & Iwasa 2005). Stress was recently defined as 'the impact of any set of abiotic and/or biotic factors that adversely affects individual "performance" and ultimately impairs population growth rate through reduced individual survival, growth and/or reproduction' (Wahl et al. 2011, p. 39). If, under conditions of limited energy supply, resources allocated to stress response processes are no longer available for shell and soft tissue production, cumulative growth (including shell increment) and instantaneous general physiological condition (con-

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dition index: CI) of bivalves should be sensitive to physiological stress. Hyper- or hyposaline conditions may cause physiological stress as they force the animal to invest more energy into ion regulatory processes (Silva & Wright 1994, Deaton 2001). Furthermore, physiologically stressful conditions (e.g. salinities and temperatures outside the individual's optimum) can increase cellular damage in marine invertebrates due to an increased generation of reactive oxygen species (ROS: O_2^-, H_2O_2, OH^-) (Abele et al. 2002, Abele & Puntarulo 2004). Removal of the damaged cell structures by proteasomes and lysosomes requires energy, is often incomplete, and can result in waste accumulation (Terman 2001, Brunk & Terman 2002b). The 'aging pigment' lipofuscin represents such damaged cell structures, mainly oxidised proteins and lipids, which were incompletely degraded. Largely indigestible, lipofuscin accumulates in the lysosomes (Terman 2001, Brunk & Terman 2002b) and can be used as a biomarker for lifetime oxidative cell damage (Winston 1991, Brunk & Terman 2002a, Philipp et al. 2006). In marine molluscs, increased lipofuscin accumulation has been measured under physiological stress caused by pollution (Krishnakumar et al. 1997, Kagley et al. 2003, Aarab et al. 2008) or high temperatures (Abele et al. 1998).

Mytilus edulis is a eurythermal bivalve species that can survive transient high temperatures of 27°C (<10% mortality after 2 d) as well as freezing (Read & Cumming 1967, Williams 1970, Almada-Villela et al. 1982, Seed & Suchanek 1992). The species' geographical range includes areas with salinities of 4 to 5 (e.g. in the Baltic Proper, leading to dwarfism) and areas where salinity may exceed 40 (e.g. in rock pools on the Atlantic coast [Newell 1979]). The optimum temperature for shell increment (cumulative growth: shell) of *M. edulis* has been reported to be 20°C (Almada-Villela et al. 1982, Reuter 2004). The optimum salinity for shell increment of this species is ~24 (Almada-Villela 1984, Kossak 2006).

Arctica islandica reaches its eastern limit of distribution in the Baltic Sea at an average salinity of ~10 (von Oertzen & Schulz 1973, Darr et al. 2008). Young *A. islandica* from the Atlantic Ocean (salinity 35) tolerate hyposalinity of 15 for more than 27 d (Kraus et al. 1991) and temperatures ranging from 1 to 20°C (Merrill et al. 1969, Cargnelli et al. 1999). The optimal temperature for shell increment of *A. islandica* is 12°C (Witbaard et al. 1997). So far, no controlled salinity experiments have been conducted with *A. islandica*. While final maximum shell sizes of this species are higher in the fully marine North Atlantic than in the brackish Kiel Bight, shell increment rates reported for young Baltic *A. islandica* (Brey et al. 1990) are similar or higher than those reported for North Atlantic populations (Schöne et al. 2005, Begum et al. 2010, Ridgway et al. 2011).

Given the projected changes in temperature and salinity in the course of climate change and the gaps in knowledge about their single and combined effects on important Baltic bivalve species, we investigated the influence of both environmental factors on lipofuscin accumulation, CI, mortality, and shell increment in 2 bivalve populations from the western Baltic Sea. The investigated bivalve species, *Arctica islandica* and *Mytilus edulis*, live in the North Atlantic as well as in the Baltic Sea (Loosanoff 1953, Theede et al. 1969, Gosling 1992, Bers 2006). While *A. islandica* burrows into the sandy sea bottom, *M. edulis* is attached to hard substrata (Seed & Suchanek 1992, Witbaard & Bergman 2003) or forms loose beds on sandy bottoms. In the Baltic Sea, *M. edulis* often dominates communities of shallow hard-bottom habitats (e.g. Jansson & Kautsky 1977, Reusch & Chapman 1997), and *A. islandica* frequently is dominant in western Baltic soft-bottom habitats below the halocline (~15 m) (Brey et al. 1990, Zettler et al. 2001). The Baltic Sea populations of both species are spread out over an area with a pronounced large-scale salinity gradient and they experience strong local temporal fluctuations of both salinity and temperature (see 'Materials and methods' for typical ranges of temperature and salinity in Kiel Bight). Both bivalve species play important roles as biomass producers (Brey et al. 1990, Reusch & Chapman 1997), enhancers of benthо-pelagic coupling (Kautsky 1981, Brey et al. 1990), reducers of water turbidity (Kautsky & Evans 1987, Lozan et al. 1996), and ecosystem engineers (Lohse 1993, Borthagaray & Carranza 2007, Norling & Kautsky 2007). Environmental changes that affect survival or competitiveness of *M. edulis* or *A. islandica* can substantially alter regional ecosystem services.

The combination of stressors can cause antagonistic, synergistic, and additive effects (Wahl et al. 2011), making predictions from single-stress studies to climate change effects difficult. Therefore, a 2-factorial experimental design was chosen to assess isolated and interactive effects of the 2 putative stressors in various combinations on a series of response variables in the 2 bivalve populations. Both the regional habitat that *Mytilus edulis* was collected from and the distributional range of this species include a wider array of the 2 abiotic variables inves-

tigated here than the habitat and distributional range of *Arctica islandica*. Consequently, we hypothesized that *M. edulis* exhibits a broader stress tolerance than *A. islandica,* and we tested the bivalves' salinity tolerance over a range of temperatures that naturally occur in the habitats of both species (4, 10, 16, 20, and 25°C for *M. edulis,* and 4, 10, and 16°C for *A. islandica*).

MATERIALS AND METHODS

The Baltic Sea is characterized by a marked salinity gradient, with salinities decreasing from ~25 in the Kattegat region to ~16 in the Kiel Bight to <3 in the northeastern Gulfs of Bothnia and Finland (Hansson & Gustafsson 2011). Baltic Sea salinity strongly fluctuates around regional means as a consequence of water inflow from the North Sea, precipitation events, or upwelling (Hansson & Gustafsson 2011). Salinity in Kiel Bight may vary between ~10 and ~27 (continuous logging of the German Federal Maritime and Hydrographic Agency [BSH] between 2004 and 2006). While the central and northern areas of the Baltic Sea are dominated by continental climate conditions with cold winters, warm summers, and moderate precipitation, milder and moister marine west-coast climate prevails in most of the southwestern and southern areas (HELCOM 2007).

Young *Mytilus edulis* specimens were collected in the Kiel Fjord (southwestern Kiel Bight) where salinity is on average 16.3 (±2.4 SD; min: 10.6, max: 23.8) and surface water temperatures range from 0.15°C in winter to 23.4°C (mean 10.48 ± 6.13 SD) in summer (continuous logging of salinity and temperature by BSH between 2004 and 2006). The previous year's cohort of *M. edulis* was used in this experiment. Individual shell height (measured from the umbo [dorsal] to the opposite side of the shell [ventral]) of *M. edulis* ranged from 13.3 to 26.5 mm (mean 19.0 ± 2.23 SD).

Arctica islandica specimens were dredged at 24 m depth at the station Süderfahrt (54° 32.6′ N, 10° 42.1′ E) in central Kiel Bight. Here, salinity is on average 21.8 (±2.4 SD; min: 13.6, max: 27.1) and temperatures vary between 0.6 and 17.5°C (mean: 9.03 ± 4.23 SD; continuous logging of salinity and temperature by the BSH between 2004 and 2006). The height (dorso-ventral axis, see paragraph above) of *A. islandica* individuals ranged from 12.1 to 33.0 mm (mean 20.4 ± 2.23 SD), which corresponds to an age of approximately 1.5 to 5 yr in the western part of the Baltic Sea (Brey et al. 1990). *A. islandica* is the longest lived non-colonial animal, with maximum life spans >400 yr (Wanamaker et al. 2008). Since Baltic Sea *A. islandic*a can reach an age of at least 40 yr (Begum et al. 2010), we considered all sampled specimens as 'young' individuals.

Bivalve populations of the western Baltic experience narrower ranges of environmental parameters than are observable over the entire distributional range of the species, and they differ in some phenotypic features from north Atlantic populations. The shells of *Mytilus edulis* from the Baltic Sea decrease in size (age-corrected) and thickness along the salinity gradient from the North Sea to the Bothnian Bay (Kautsky et al. 1990), while *Arctica islandica* is shorter-lived in the Baltic Sea (~40 yr) than in the north Atlantic (>200 yr) (Begum et al. 2010).

Experimental design

In order to explore possible interactions between treatment factors, we conducted a 2-factorial, fully crossed experiment for each species separately with the factors temperature and salinity. Treatment levels were 4, 10, 16, 20, and 25°C for *Mytilus edulis* and 4, 10, and 16°C for *Arctica islandica* regarding temperature, and 15, 25, and 35 regarding salinity for both species. These treatment levels fall within the natural range of temperature and salinity in the distributional range of the 2 species, while the sampled populations of both species only rarely, if ever, experience salinities >25 and temperatures >23°C. Each treatment combination of temperature and salinity was replicated 4-fold, which required 60 (5 temperatures × 3 salinities × 4 replicates) *M. edulis* and 36 (3 temperatures × 3 salinities × 4 replicates) *A. islandica* independent experimental units.

Culture

Bivalves were kept in 96 temperature-insulated 4 l containers at the Helmholtz Centre for Ocean Research Kiel (GEOMAR), with 10 ind. of *Mytilus edulis*, and 7 ind. of *Arctica islandica* in each container (experimental unit). Since individual bivalves of one experimental unit are interdependent pseudo-replicates, single uni-variate values (mortality of all individuals, mean shell increment, lipofuscin content and CI of single individuals) representing all individuals of one container where assigned to the replicates (experimental units). Bivalves were fed 0.5 ml ind.$^{-1}$ d^{-1} of a con-

centrated living-phytoplankton suspension 5 times a week (DT's Premium Blend; DT's Plankton Farm) containing *Nannochloropsis oculata* (40%), *Phaeodactylum tricornutum* (40%), and *Chlorella* sp. (20%). Algal biomass was approx. 2.91 g (dry weight) l^{-1}, resulting in 1.5 mg ind. $^{-1}$ d^{-1}. One-eighth of the water volume per experimental unit (0.5 l) was exchanged twice a week. Temperature was recorded by loggers (HOBO® Onset Computer Corporation). Salinity was measured twice a week with a WTW conductometer (cond 330i; WTW). Both factors were kept constant for the experimental duration of 15 wk. Salinity levels were set by admixing freshly collected Baltic Sea water with either ion-exchanged water or artificial marine salt (SEEQUASAL). Individuals were allowed to slowly acclimatize to the respective treatments. Initial salinity corresponded to the salinity conditions in the habitat of provenance and was increased or decreased by 1 unit d^{-1}. Initial temperature represented temperature conditions in the habitat of provenance, and it was subsequently changed by max. 1°C d^{-1} until treatment conditions were reached. Dead bivalves were replaced by new specimens to keep the bivalve density constant. The newly introduced individuals were not considered for further analysis.

Data collection

Bivalves were marked individually with numbered discs (Ø: 2 mm) glued to the shell after locally removing the periostracum to enhance adhesion. Shell height (dorso-ventral axis) was measured monthly after the acclimatisation phase to the nearest 0.02 mm using callipers. Mean shell increment rates of all bivalve individuals of one experimental unit were used as response variable for each replicate.

Best grown individuals of each experimental unit were chosen for measuring lipofuscin, because in the high temperature treatments (20 and 25°C) these were the only individuals providing enough soft tissue material for this measurement. After 15 wk of incubation under different temperature and salinity regimes, the whole soft tissue of these individuals was removed from the shells and deep-frozen at –80°C. Individual frozen soft tissues were weighed. Shells were air-dried (7 d at 20°C) and their dry weight recorded. The condition index was calculated as CI = soft tissue wet $\text{weight}_{(\text{frozen})}$ / shell dry weight. For comparison of different CI calculations see Davenport & Chen (1987).

Lipofuscin contents were determined by an extraction method following Vernet et al. (1988) and Sukhotin et al. (2002). Frozen soft tissue material of whole animals was ground in liquid nitrogen and homogenised (1:20 w/v) in a chloroform-methanol solution (2:1 v/v). The homogenate was mixed with 100 mM $MgCl_2$ solution (1 ml per each 4 ml of chloroform-methanol). After 15 min of centrifugation at 2000 × *g* and 0°C, the chloroform phase was collected and washed with distilled water (1 ml per 4 ml initial chloroform-methanol). Chloroform-water was centrifuged as above and the chloroform phase collected again and measured in a fluorometer. An emission spectrum was obtained at an excitation wavelength of 350 nm. Fluorescence intensity of each sample was then determined at the emission maximum of 480 nm. Following Hill & Womersley (1993), lipofuscin contents were expressed as relative fluorescent intensity (RFI) using 0.1 µg quinine sulphate per ml of 1 N H_2SO_4 as standard. As lipofuscin accumulates in the whole soft tissue over time (i.e. with age; Sukhotin et al. 2002, Philipp et al. 2005), and for reasons of better internal and external comparability, RFI values were corrected by the incubation time and height of the individual bivalves (as a proxy for age) at the experimental start: RFI_{corr} = RFI (incubation time × start shell height)$^{-1}$. True age was not determined but in young *Mytilus edulis* and *Arctica islandica* individuals age and size correlate well (e.g. Bayne & Worrall 1980, Kautsky 1982, Begum et al. 2010). Mortality was expressed as percentage of mussels that died in an experimental unit during the experimental phase.

Data analysis

Data representing percentage values, namely mortality values, were arcsine-transformed. Not normally distributed data or those of unequal variances were (square root-, 4th- root, or log-) transformed to meet the necessary assumptions for an ANOVA. For one data set no transformation was successful (*Mytilus edulis*: CI). Here, the significance level α was lowered from 0.05 to 0.01 to reduce the risk of type-1 errors (Glasby 1998). Data were analysed for significant differences between treatment levels by 2-factorial ANOVA. In the absence of interactive effects, differences between single treatment levels were identified by 1-factorial Tukey's HSD post hoc test. Correlations between the response variables shell increment, lipofuscin accumulation, CI and mortality were determined with Pearson's correlation test. All statistical tests were run using the Statistica 8.0 software package.

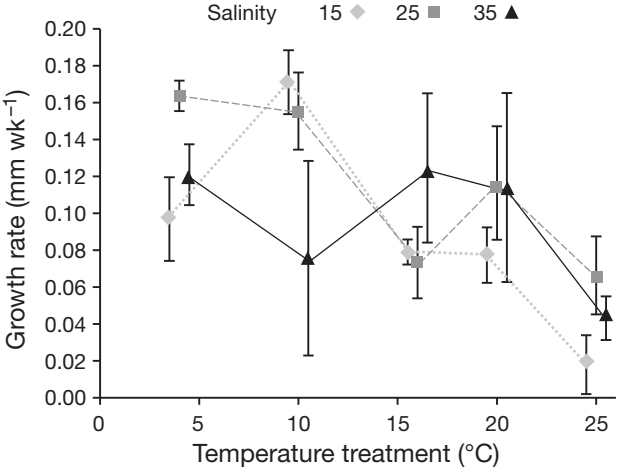

Fig. 1. *Mytilus edulis.* Shell increment rates (mm wk^{-1}) of all individuals in the experimental units at different temperatures. Data: mean ± 95% confidence intervals; n = 4

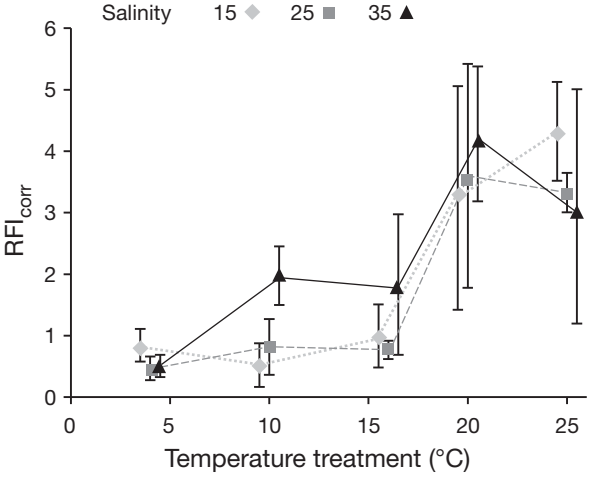

Fig. 2. *Mytilus edulis.* Relative fluorescence intensity (RFI$_{corr}$) of lipofuscin in measured individuals' soft tissue. Original values were corrected by incubation time and shell height at the start of the experiment. Data: mean ± 95% confidence intervals; n = 4

RESULTS

Mytilus edulis

Growth (shell increment). Temperature and salinity affected shell increment of *Mytilus edulis* (Fig. 1) interactively (2-way ANOVA, $F = 6.08$, $p < 0.001$). Trends of these interactive effects were a reduction of shell increment with increasing temperatures (especially at 25°C), and at low (15) and high (35) salinity.

Stress. Temperature and salinity affected lipofuscin accumulation (RFI$_{corr}$) in *Mytilus edulis* interactively (2-way ANOVA, $F = 2.74$, $p = 0.015$; Fig. 2). RFI$_{corr}$ increased strongly at temperatures >16°C. At salinity 35 the RFI$_{corr}$ increased already at 10°C causing the interaction. Lipofuscin contents were negatively correlated to shell increment ($r = -0.40$, $p = 0.001$).

Fitness parameters. CI of *Mytilus edulis* was not significantly affected by salinity (2-way ANOVA, $F = 2.61$, $p = 0.085$) but decreased with increasing temperatures (2-way ANOVA, $F = 27.4$, $p < 0.001$; Fig. 3A). The CI was positively correlated to shell increment ($r = 0.37$, $p = 0.003$) and negatively to lipofuscin accumulation ($r = -0.58$, $p < 0.001$).

Mortality of *Mytilus edulis* was interactively affected by temperature and salinity (2-way ANOVA, $F = 3.09$, $p = 0.007$; Fig. 3B). Low mortality was observed at salinity 25 and high mortality at treatment combinations salinity 35/10°C and salinity 15/25°C. Mortality in the experimental units was negatively correlated to the shell increment of living mussels in the units ($r = -0.44$, $p < 0.001$) but was neither correlated to the CI ($r = 0.06$, $p = 0.64$) nor to RFI$_{corr}$ ($r = 0.05$, $p = 0.69$).

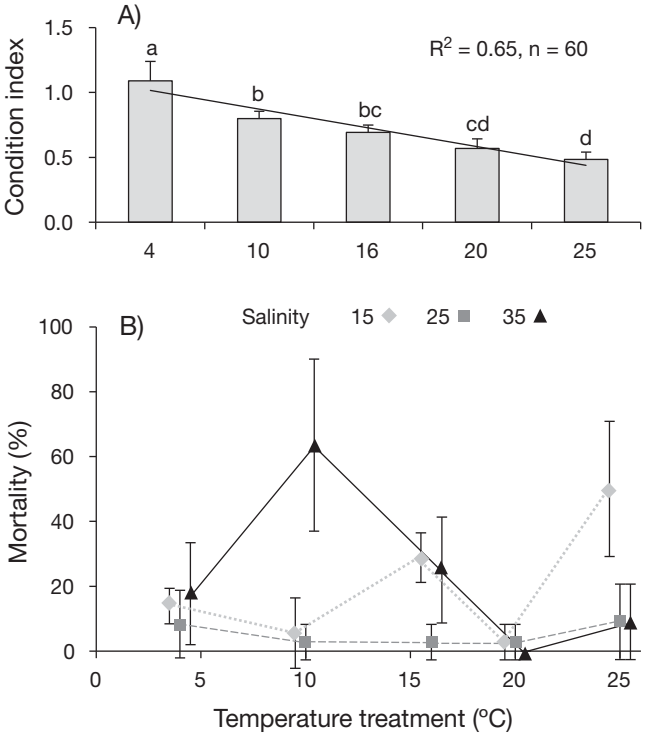

Fig. 3. *Mytilus edulis.* Fitness parameters. (A) Condition index (soft tissue weight / shell weight) of measured individuals and (B) interaction graph of mortality (%) in the experimental units. Equal letters in (A) designate group comparisons for which statistically significant differences were not detected. Data: mean ± 95% confidence intervals; (A) n = 12; (B) n = 4

Arctica islandica

Growth (shell increment). Temperature and salinity affected shell increment of *Arctica islandica* interactively (2-way ANOVA, $F = 3.12$, p = 0.031; Fig. 4A). While at 4°C the shell increment rate was highest at salinity 35, the shell increment rate at 10 and 16°C was already high at salinity 25.

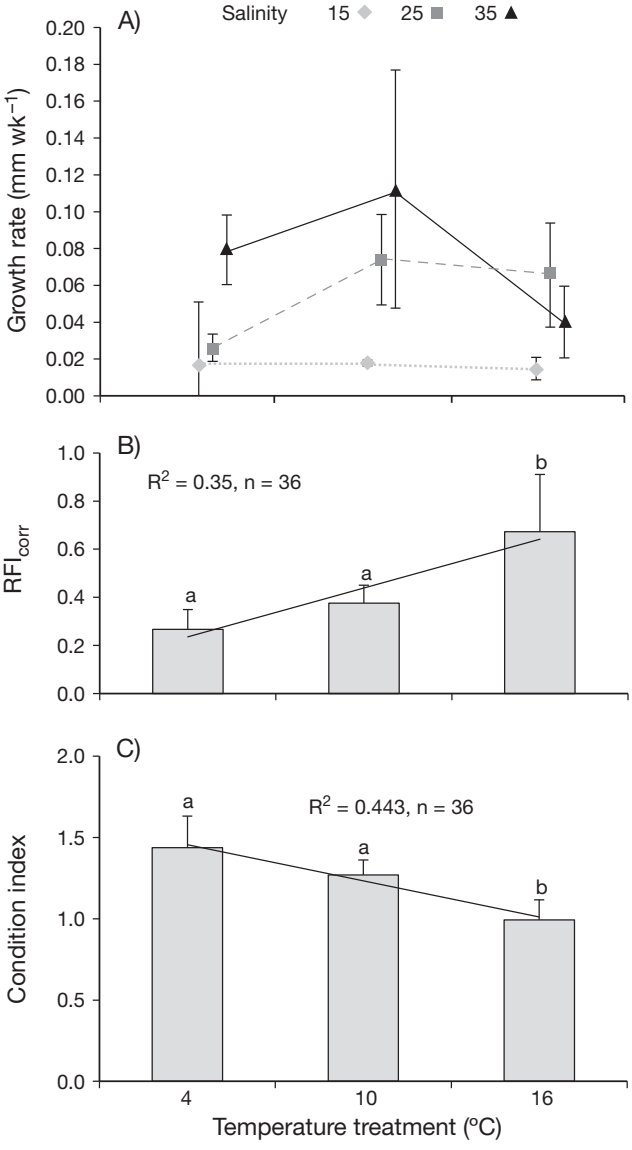

Fig. 4. *Arctica islandica.* (A) Mean shell increment rates (mm wk^{-1}) of all individuals in the experimental units, (B) relative fluorescence intensity (RFI$_{corr}$) of lipofuscin in measured individuals' soft tissue (original values were corrected by incubation time and shell length at the start of the experiment), and (C) condition index. In (B) and (C) equal letters designate group comparisons for which statistically significant differences were not detected. Data: mean ± 95% confidence intervals. (A) n = 4; (B) n = 12; (C) n = 12

Stress. When comparing lipofuscin content (RFI$_{corr}$) in the soft tissue of bivalves grown at temperatures 4 to 16°C, the RFI$_{corr}$ in *Arctica islandica* (0.44 ± 0.08 SE) was on average more than 50% lower than the RFI$_{corr}$ in *Mytilus edulis* (mean RFI$_{corr}$ of all measured *M. edulis* grown at temperatures 4 to 16°C: 0.97 ± 0.20 SE, *t*-test: t: 3.72, p < 0.001). RFI$_{corr}$ in *A. islandica* increased with temperature (2-way ANOVA, F = 14.5, p < 0.001; Fig. 4B) but no statistically significant relation was detected between salinity and lipofuscin content in soft tissues of *A. islandica* (2-way ANOVA, F = 0.08, p = 0.92). RFI$_{corr}$ was not correlated to shell increment of *A. islandica* (r = –0.21, p = 0.30).

Fitness parameters. The CI of *Arctica islandica* was not influenced by salinity (2-way ANOVA, F = 1.07, p = 0.365) but decreased with increasing temperature (2-way ANOVA, F = 10.32, p = 0.001; Fig. 4C). Additionally, the CI was negatively correlated to lipofuscin accumulation (r = –0.68, p < 0.001) but was not correlated to shell increment (r = 0.03, p = 0.88).

Mortality of *Arctica islandica* was not influenced by temperature (2-way ANOVA, F = 0.15, p = 0.86) but decreased with higher salinity (2-way ANOVA, F = 3.61, p = 0.04; Fig. 5A,B). Mortality of *A. islandica* was not correlated to any of the other parameters (shell increment: r = –0.13, p = 0.45; RFI$_{corr}$: r = 0.22, p = 0.27; CI: r = 0.18, p = 0.38).

DISCUSSION

Temperature and salinity interactively affected shell increment rates, lipofuscin accumulation, and mortality of *Mytilus edulis* as well as shell increment rates of *Arctica islandica*. In both species, lipofuscin accumulation increased while the instantaneous general physiological condition (CI) decreased with increasing temperature. Lipofuscin accumulation of *M. edulis* was negatively correlated to the bivalve's shell increment rates.

The higher lipofuscin accumulation at higher temperatures in *Mytilus edulis* and *Arctica islandica* was expected since metabolic rates increase with increasing temperature in ectothermal organisms (e.g. Clarke 2003), which bears the risk of an elevation in free radical production leading to increased oxidative cellular damage (e.g. Abele 2002). The strong increase in lipofuscin accumulation further indicates that at higher temperatures the free radical defence and cellular repair mechanisms were not sufficient to prevent or reverse cellular damage (Terman & Brunk 2004, Philipp et al. 2006).

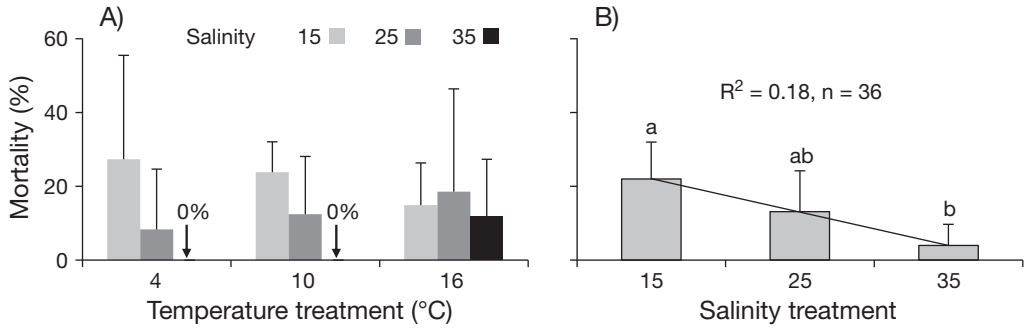

Fig. 5. *Arctica islandica.* Mortality (%) in experimental units in (A) dependence on salinity and temperature and (B) pooled over all temperatures in dependence on salinity. In (B) equal letters designate group comparisons for which statistically significant differences were not detected. Data: mean ± 95% confidence intervals; (A) n = 4; (B) n = 12

The inverse relationship between the CI and lipofuscin accumulation appears to be due to positive feedbacks of both effects: reduced growth of soft tissue reduces the dilution of lipofuscin by cell proliferation (Sukhotin et al. 2002, Philipp et al. 2005). Furthermore, increasing lipofuscin contents reduce the functionality of body cells. Therefore, negative correlations of lipofuscin accumulation with shell increment rates and the CI corroborate the assumption that temperature stress may force *Mytilus edulis* to invest additional energy into cellular homeostasis, energy that then is lacking for animal's maintenance and shell production. For *Arctica islandica,* energy partitioning between cellular homeostasis and basal maintenance or growth only incompletely explains the results since shell increment and lipofuscin accumulation are affected by different abiotic factors (salinity and temperature, respectively). As lipofuscin content and the CI were influenced only by temperature and, as in *M. edulis*, inversely related to each other, the bivalves indeed suffered at higher temperatures. Yet, they suffered not so severely that shell increment of *A. islandica* was reduced.

Generally, a species' geographical distribution is not defined by single extrinsic factors that cause physiological stress. The reason is that any environmental factor can be modulated by biotic interactions as well as its interactions with other abiotic factors (e.g. CO_2), which may cause unexpected effects (Wahl 2008, Wahl et al. 2011). The general increase of lipofuscin accumulation in *Mytilus edulis* with increasing temperature is more pronounced at salinity 35 as compared to the lower salinity treatments. Any departure from optimal salinity conditions (24 in *M. edulis;* Kossak 2006) requires osmoregulation (regulation of intracellular ion concentrations and organic osmolytes, especially free amino acids; Silva & Wright 1994, Deaton 2001, Kube et al. 2006). This can require energy allocation away from antioxidant defence and cellular repair mechanisms, and may cause a further increase in metabolic rate, both mechanisms increasing lipofuscin accumulation (Resgalla et al. 2007). The up-regulation of intracellular organic osmolytes in response to increasing salinity can consist of breaking down of endogenous proteins, dietary assimilation, de novo synthesis, or uptake from the external media (Hawkins & Hilbish 1992, Deaton 2001). Osmoregulation in response to an increase of salinity from 25 to 35 may therefore be energetically more expensive and may cause higher metabolic rates than down-regulation (decomposition, excretion, leaking-out) of osmolytes with decreasing salinity from 25 to 15 (Silva & Wright 1994). This could explain why the threshold at which free radical production exceeds cellular defence and repair capacity (causing increased lipofuscin accumulation) is reached at lower temperatures at salinity 35 compared to salinities 25 (species' optimum) and 15 (habitat condition).

Shell increment and mortality of *Mytilus edulis* were impacted by non-optimal salinity conditions at 4, 10, and 25°C. In contrast, at 15 and 20°C there were no, or only small, salinity effects, suggesting that this temperature range may be most favourable for the mussels. Kossak (2006) showed that salinity and temperature effects on North and Baltic Sea *M. edulis* shell increment rates depend on nutrition levels. She found increasing shell increment with higher temperatures only at very high phytoplankton concentrations (double of typical present-day concentration in Kiel Bight). In field observations, Page & Hubbard (1987) also observed that temperature and shell increment rates of *M. edulis* in California waters correlated due to an indirect nutrition effect via higher phytoplankton production at higher temperatures. In our experiment, we deliberately ex-

cluded any nutrition effect by equal feeding at all treatment levels. Having now shown that the 2 environmental stressors directly and additively interact with each other, it is important to take salinity effects into account when considering effects of non-optimal temperatures on mussel performance and vice versa. Due to our non-ad-libitum nutrition routine, however, food limitation could have contributed to the reduction of shell increment of *M. edulis* at 25°C.

Interacting effects of temperature and salinity on shell increment of *Arctica islandica* at 4°C in salinity 25 corroborate field observations of Begum et al. (2010), who found that the combination of reduced salinity (25) and low temperature (4°C) is an especially harsh environment for *A. islandica*. Possibly, increased metabolic rates at temperatures 10 and 16°C observed in the present study compensated for shell increment decelerating effects of sub-optimal salinity (25). Such a positive temperature effect, however, may be too weak to compensate for reduced shell increment when salinity is reduced to 15. At fully oceanic conditions (salinity 35), we found that shell increment increased from 5 to 10°C, which is in accordance with findings from North Sea *A. islandica* (Witbaard et al. 1997). A further warming to 16°C led to reduced mean shell increment indicating thermal stress. Indeed, *A. islandica*'s southern distribution in the North Sea coincides with the 16°C summer isotherm (Cargnelli et al. 1999, Witbaard & Bergman 2003).

In summary, the responses of the 2 studied bivalve species to the applied stressors differ in several aspects. Performance (shell increment and the CI) of *Mytilus edulis* was mainly controlled by temperature and less by salinity. Future scenarios predict Baltic Sea temperatures to increase by 3 to 6°C and salinity to decrease regionally by up to 5 units with climate change (HELCOM 2007, Lehmann et al. 2011). This may cause additional physiological stress for the investigated bivalve populations of Kiel Bight, since both species already live below their salinity optimum year-round, and at or above their temperature optimum during summer. Unless *M. edulis* can adapt to the predicted conditions, our results imply that *M. edulis* may be less competitive due to increased cellular stress and food limitation if surface water temperatures in summer exceed 20°C more frequently but productivity and/or eutrophication does not increase. The expected desalination of the Baltic Sea would result in lower shell increment rates and higher mortality, especially of *Arctica islandica*. Warming will increase cellular damage, which would weaken the condition of *A. islandica* independently of, or in addition to, salinity stress. If the populations do not adapt fast enough, warming and desalination of the Baltic Sea may lead to a retreat of the 2 species, especially at their eastern boundary ranges, where salinity is most stressful. How severely organisms are impacted by warming and desalination will also depend substantially on the responses of interacting species and concurrent shifts in biotic interactions. These interactions must be investigated before sound predictions can be made about future distribution limits (Sareyka et al. 2011, Wahl et al. 2011).

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