The adaptive potential of early life-stage *Fucus vesiculosus* under multifactorial environmental change

Dissertation

to obtain the academic degree
Dr. rer. nat.
at the Faculty of Mathematics and Natural Sciences
of the Christian Albrecht's University of Kiel

Balsam Al-Janabi

Kiel, February 2016

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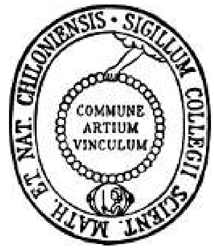
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Contents

1 Summary

Multiple global and local stressors threaten populations of the bladderwrack *Fucus vesiculosus* (Phaeophyceae). This brown macrophyte is an important foundation species in benthic hard-bottom communities. Therefore, environmental impacts on *F. vesiculosus* are an important concern in conservation ecology. Baltic *F. vesiculosus* populations presumably have a decreased genetic diversity compared to other, e.g. Atlantic populations, which may restrict their potential to adapt to environmental changes. Our present understanding of global change effects on brown macrophytes mostly relies on studies of single factors under constant conditions during only one season on the adult life-stage, while neglecting the potential for evolutionary adaptation. This was the starting point of my doctoral project, when I investigated the role of genetic diversity for the potential to adapt to multifactorial environmental change in the early life-stage *F. vesiculosus*.

To study the seasonal variation of global factors, warming and acidification were orthogonally crossed and their single and combined effects on *F. vesiculosus* germlings were analysed during one year (April 2013 – April 2014). The two factor levels "present" and "future" (according to predictions of the year 2110) were manipulated at the "Kiel Outdoor Benthocosms" (KOBs) by applying delta-treatments. Present conditions consisted of the natural fluctuations of the Kiel fjord (western Baltic Sea, Germany). For achieving future conditions, warming consisted of the increase of the present temperature plus 5 °C, while acidification consisted in the increase of present CO_2 concentration to 1100 µatm. Warming enhanced germlings' growth in early summer, but strongly induced germlings' mortality in late summer and in winter. In contrast to warming, acidification had only weak effects on germlings' performance. Subsequently, the interaction between global and local factors of the Baltic Sea was investigated. In the Baltic Sea, eutrophication will likely be enhanced in the near future. The two factors "combined ocean acidification and warming" (OAW) and nutrient enrichment were orthogonally crossed at the KOBs. *F. vesiculosus* germlings were exposed to the treatment level "present" and "future" of both factors during summer (July – September 2014). Nutrient enrichment strongly mitigated the negative effects of heat stress on germlings' performance, showing an antagonistic interaction between the factors temperature and nutrients. Besides simultaneous interactions, the sequential action of environmental factors was analysed. Therefore, germlings previously treated under the different combinations of the OAW x nutrient experiment were subsequently exposed to a hypoxic upwelling event at the KOBs. Germlings' sensitivity to hypoxia was enhanced by the previously experienced warming and acidification. Essentially, these results show that the performance of *F. vesiculosus* germlings is determined by the seasonal variation of global change factors and by the simultaneous and sequential actions of different environmental factors.

Furthermore, the role of intraspecific genetic diversity for the adaptation to environmental change was analysed. Therefore, experimental populations of genetically different *F. vesiculosus* sibling groups and diversity level were exposed to the KOB experiments. Strong differences between sibling groups' performance under warming, acidification, nutrient enrichment and hypoxia were observed. These differences demonstrate a genetic variation of traits which are relevant for the tolerance to the predicted environmental changes. This finding indicates an increased adaptive potential in populations of a relatively high genetic diversity.

Corroborating this, higher survival in high diversity level compared to the low diversity level was observed under warming and acidification. Evolutionary adaptation can be accelerated or dampened if the sensitivity of genotypes towards multiple environmental factors correlates or anticorrelates. To test this, different *F. vesiculosus* sibling groups were analysed regarding their sensitivity towards multiple factors. Sibling groups' sensitivity towards warming and acidification correlated positively indicating an accelerated rate of adaptation towards the tolerance to these factors, thus adaptation does not have to be acquired individually. Sibling groups' sensitivity towards OAW and hypoxia showed an anticorrelation pattern. This result demonstrates that *F. vesiculosus* genotypes previously selected under warming and acidification in summer are most sensitive to hypoxic upwelling events in autumn.

Furthermore, physiological and evolutionary responses of marine organisms to climate change were analysed through different levels of biological organisation. In a literature review, studies on the molecular, cellular, individual, population and community level of different marine taxa were integrated. The review showed that climate change has different effects on each single level of biological organisation.

Taken together, this thesis demonstrates that Baltic *F. vesiculosus* populations have the potential to adapt to environmental change in presence of a relatively high genetic diversity. This study highlights that global change research requires an upscaling approach with regard to multiple factors, seasons, natural fluctuations, different developmental stages and levels of biological organisation in the light of the adaptive potential. Global change research with a realistic assessment of stress sensitivity and the evolutionary response of Baltic *F. vesiculosus* populations allows for appropriate conservation strategies.

2 Zusammenfassung

Multiple globale und lokale Stressoren gefährden Populationen des Blasentang *Fucus vesiculosus* (Phaeophyceae). Dieser braune Makrophyt ist eine wichtige Schlüsselart der Artengemeinschaften benthischer Hartsubstrate. Daher sind Umwelteinflüsse auf *F. vesiculosus* im Bereich der Naturschutzökologie von großer Bedeutung. *F. vesiculosus* Populationen der Ostsee haben mutmaßlich eine verringerte genetische Diversität im Vergleich zu z.B. atlantischen Populationen. Daher könnte das Potential zur Anpassung an Umweltveränderungen eingeschränkt sein. Unser gegenwärtiges Wissen über die Effekte des globalen Wandels auf braune Makrophyten beruht größtenteils auf Studien einzelner Faktoren unter konstanten Bedingungen, einer Jahreszeit und adulter Lebensstadien. Dabei wurde des Potentials zur evolutiven Anpassung nur selten untersucht. An diesem Punkt setzte meine Doktorarbeit an, in der ich die Rolle der genetischen Diversität bezüglich des Anpassungspotentials an multifaktorielle Umweltveränderungen in *F. vesiculosus* Jungstadien untersucht habe.

Um die jahreszeitliche Dynamik globaler Faktoren zu untersuchen, wurden Erwärmung und Versauerung orthogonal gekreuzt und ihre einzelnen und kombinierten Auswirkungen auf *F. vesiculosus* Keimlinge im Laufe eines Jahres (April 2013 – April 2014) getestet. Die zwei Faktorlevel „Gegenwart“ und „Zukunft“ (entsprechend der Vorhersagen des Jahres 2110) wurden an den „Kiel Outdoor Benthocosms“ (KOBs) mithilfe von Delta-Behandlungen bestimmt. Der gegenwärtige Zustand bestand aus den natürlichen Fluktuationen der Kieler Förde (westliche Ostsee, Deutschland). Die zukünftige Erwärmung wurde durch die Erhöhung der gegenwärtigen Temperatur um 5 °C erreicht; Versauerung bestand aus der Erhöhung der gegenwärtigen CO_2 Konzentration zu 1100 µatm. Erwärmung verstärkte das Keimlingswachstum im Frühsommer, führte jedoch zur erhöhten Sterblichkeit der Keimlinge im Spätsommer und Winter. Im Gegensatz zur Erwärmung beeinflusste die Versauerung die Effizienz der Keimlinge nur gering. Anschließend wurde die Interaktion zwischen globalen und lokalen Faktoren der Ostsee untersucht. Die Eutrophierung der Ostsee wird voraussichtlich in naher Zukunft zunehmen. Daher wurden die zwei Faktoren „kombinierte Ozeanversauerung und -erwärmung“ (OAW) und Nährstoffanreicherung in den KOBs orthogonal gekreuzt. *F. vesiculosus* Keimlinge wurden den beiden Faktorstufen „Gegenwart“ und „Zukunft“ im Sommer (Juli – September 2014) ausgesetzt. Die Nährstoffanreicherung mäßigte den negativen Effekt des Hitzestresses auf *F. vesiculosus* Keimlinge und zeigte somit eine antagonistische Interaktion zwischen den Faktoren Temperatur und Nährstoffe. Neben simultanen Interaktionen wurden auch sequentielle Effekte verschiedener Umweltfaktoren untersucht. Keimlinge, welche die verschiedenen Kombinationen des OAW x Nährstoffexperimentes erfahren hatten, wurden anschließend einem hypoxischen Auftriebsereignis an den KOBs ausgesetzt. Die Sensitivität zur Hypoxie wurde durch die Vorbehandlung von Erwärmung und Versauerung verstärkt. Zusammenfassend zeigen die Ergebnisse die jahreszeitliche Dynamik des globalen Wandels und die simultanen und sequentiellen Effekte verschiedener Umweltfaktoren auf die Reaktion der *F. vesiculosus* Keimlinge.

Des Weiteren wurde die Rolle der intraspezifischen genetischen Diversität für das Anpassungspotential an Umweltveränderungen untersucht. Dazu wurden experimentelle Populationen genetisch unterschiedlicher Geschwisterscharen und genetischer Diversitätslevel von *F. vesiculosus* Keimlingen den KOBs Experimenten ausgesetzt. Die Geschwisterscharen unterschieden sich stark in ihrer Toleranz gegenüber Erwärmung, Versauerung, Nährstoffanreicherung und Hypoxie. Die Unterschiede zwischen Geschwisterscharen zeigen eine genetische Variation in Merkmalen von *F. vesiculosus*, welche für die Anpassung an Umweltveränderungen relevant sind. Dies weist darauf hin, dass *F. vesiculosus* Populationen einer relativ hohen genetischen Diversität ein erhöhtes Anpassungspotential haben. Passend dazu zeigten Gruppen der erhöhten genetischen Diversität ein erhöhtes Überleben im Vergleich zu Gruppen der niedrigen Diversitätslevel unter erwärmten und versauerten Bedingungen. Evolutive Anpassung kann beschleunigt oder verzögert werden, wenn die Sensitivität verschiedener Genotypen zu multiplen Umweltfaktoren korreliert oder antikorreliert. Zu dieser Untersuchung wurden verschiedene *F. vesiculosus* Geschwisterscharen bezüglich ihrer Sensitivität zu verschiedenen Faktoren untersucht. Die Sensitivität verschiedener Geschwisterscharen zeigte eine positive Korrelation hinsichtlich Erwärmung und Versauerung. Dies deutet auf eine beschleunigte Anpassung zur Toleranz der Erwärmung und Versauerung hin, da die Anpassung zu beiden Bedingungen nicht einzeln erlangt werden muss. Geschwisterscharen zeigten eine negative Korrelation hinsichtlich ihrer Sensitivität zu OAW und zur Hypoxie. Diese Ergebnisse zeigen, dass *F. vesiculosus* Genotypen, welche im Sommer unter erwärmten und versauerten Bedingungen selektiert wurden, höchst empfindlich zu hypoxischen Auftriebsereignissen im Herbst sind. Ferner wurden physiologische und evolutive Antworten mariner Organismen zum Klimawandel im Hinblick verschiedener biologischer Organisationsstufen untersucht. Dazu wurden in einer Literaturrecherche Studien der molekularen, zellulären, einzelner Individuen, Populationen und Lebensgemeinschaften verschiedener mariner Taxa zusammengefasst. Die Recherche ergab, dass der Klimawandel sich unterschiedlich auf jede einzelne biologische Organisationsstufe auswirkt.

Zusammenfassend zeigt diese Arbeit, dass *F. vesiculosus* Populationen der Ostsee das Anpassungspotential zu Umweltveränderungen haben, wenn eine relativ hohe genetische Diversität vorhanden ist. Die Ergebnisse unterstreichen, dass Forschung des globalen Wandels eine Hochskalierung hinsichtlich multipler Faktoren, jahreszeitlicher Dynamik, natürlichen Fluktuationen, verschiedenen Lebensstadien und biologischen Organisationslevel unter Berücksichtigung des Anpassungspotentials benötigen. Studien des globalen Wandels mit einer realistischen Einschätzung der Stress Sensitivität und des Anpassungspotentials der *F. vesiculosus* Ostseepopulationen ermöglichen geeignete Naturschutzstrategien.

3 General Introduction

3.1 A changing global climate

The climate varied considerably over earth's history (Petit et al. 1999), but recent climate variabilities are much more rapid when compared to historical records from the industrial revolution in the 18th century. Atmospheric carbon dioxide (CO_2) has increased steeply from 280 ppm before the industrial revolution to current 400 ppm (Tans and Keeling 2015). CO_2 is one of the most important greenhouse gases (GHG) in the atmosphere (Crutzen 2002) trapping solar radiation and resulting in global warming. During the last century, the global average surface air temperature has already increased by ~1 °C (Thompson et al. 2008). If the CO_2 emissions continue unabated, the global temperature will increase 2.6 °C to 4.8 °C until the end of the 21st century (Collins et al. 2013)(Fig. 1). Ocean warming in the last half century was almost ubiquitous on earth, but varies in magnitude on a regional scale. Currently observed temperature increases are higher in the northern hemisphere and at higher latitudes (Hansen et al. 2006) than in lower latitudes and in the southern hemisphere. In the Atlantic Ocean, warming of the sea surface temperature (SST) during the 20th century was higher compared to the Indian and Pacific Ocean (Lee et al. 2011). Future projections of global warming vary between different scenarios of CO_2 emission (Fig. 1), as global temperature is predicted to increases by 0.3 °C to 4.8 °C until the end of the century (Collins et al. 2013).

In addition to warming of the atmosphere and oceans, higher concentrations of atmospheric CO_2 increase the CO_2 concentrations in the oceans (Raven et al. 2005), a process called ocean acidification. Before the industrial revolution in the 18th century the oceans were a source of CO_2, however this pattern changed after the 18th century due to increased burning of fossil fuels, deforestation and cement production since the industrialisation (Crutzen 2002): now, the ocean acts as a sink of atmospheric CO_2. In fact the actual atmospheric CO_2 concentration would be 55 ppm higher without the oceanic uptake (Sabine et al. 2004). As a consequence, an increase of the dissolved inorganic carbon (DIC) concentration is accompanied by changes in the carbon chemistry. After the dissolution in the oceans, dissolved CO_2 ($CO_{2\,(dis)}$) reacts with seawater (H_2O) forming carbonic acid (H_2CO_3) that dissociates to bicarbonate (HCO_3^-) and further to carbonate (CO_3^{2-}) with the release of one proton in each dissociation (Equation 1).

$$CO_2 + H_2O \Longleftrightarrow H_2CO_3 \Longleftrightarrow HCO_3^- + H^+ \Longleftrightarrow CO_3^{2-} + 2H^+ \qquad (1)$$

A decrease of seawater pH by 0.1 has already occurred since the pre-industrial time (Rhein et al. 2013). Climate models predict a reduction in pH of ~0.3 to 0.5 units by the end of the 21st century (Caldeira and Wickett 2005) (Fig. 1). The DIC increase and the pH decrease in seawater results in a shift of the relative contributions of the DIC species because the equilibrium of the reactions of dissociation depends on the pH (Zeebe and Wolf-Gladrow 2001).

At the current global mean pH of 8.04, the dissolved inorganic carbon is partitioned into 1 % $CO_{2\,(dis)}$, 91 % HCO_3^- and 8 % CO_3^{2-} (Raven et al. 2005). The projected pH of 7.66 results in an increase of $CO_{2\,(dis)}$ to 1.4 %, HCO_3^- to 93.8 % and in a decrease of CO_3^{2-} to 4.8 % until the end of the 21st century (Koch et al. 2013).

Marine autotrophs, *i.a.* phytoplankton and macrophytes, assimilate dissolved inorganic carbon (carbon dioxide and bicarbonate) and produce biomass through photosynthesis. Future ocean acidification and the shift of carbon species towards carbon dioxide and bicarbonate will increase the substrate availability for primary producers and may increase growth and photosynthesis of marine autotrophs (Riebesell et al. 2007; Koch et al. 2013).

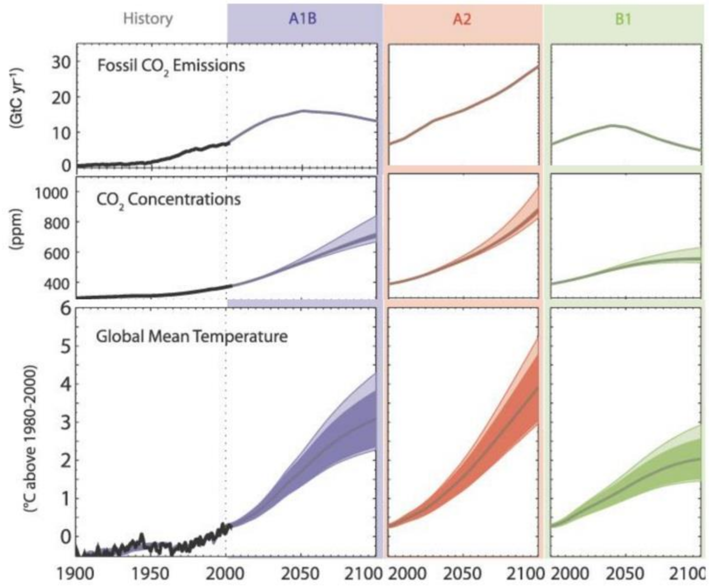

Figure 1 Historical records and predictions for the coming century of fossil CO_2 emissions, atmospheric CO_2 concentrations and the global mean temperature of 19 models. Projections are illustrated for the three different scenarios B1 (low emissions, including programs for the decrease of CO_2 emission), A1B (medium emissions, balanced use of energy resources), and A2 (high emissions, continuous human population growth). The figure was adapted from the IPCC WGI AR4 (Meehl et al. 2007) (Fig. 10.26)

On a global scale, ocean acidification and global warming have impacted marine ecosystems as reflected in shifts of ecosystem composition and function (Connell et al. 2013). The impact on marine species performance will further shape the geographical distribution of marine organisms (Pörtner 2008). Global warming enhances thermal stratification, oxygen depletion (Meier et al. 2011) and phenological shifts in marine organisms (Walther et al. 2002). Also, global warming enhances the evaporation that alters the precipitation pattern, enhancing the riverine input and freshwater content of many coastal areas (Rhein et al. 2013).

3.2 Multifactorial environmental impacts on ecosystems in the Baltic Sea

As one of the largest brackish water ecosystems of the world, the Baltic Sea features particular properties compared to other seas. The water exchange is restricted to the narrow and shallow transition area of the Danish strait region, leading to the consistent horizontal salinity gradient and strong terrestrial impacts in the Baltic Sea (Stigebrandt 2001). The strong salinity gradient determines marine ecosystems in the Baltic Sea: at the entry region of the Danish straits, the salinity concentration is higher (~25 psu) than in the northern region at the Gulf of Bothnia and the eastern Gulf of Finland, where the salinity conditions equal freshwater conditions (~1-2 psu) (Kullenberg 1981). In the western Baltic Sea, marine species immigrated from the Northeast Atlantic and the North Sea while the northern area is also shared by freshwater species that originated from rivers. Anthropogenic impacts, such as pollution and nutrient input, are enhanced by the high catchment area of 14 countries (HELCOM 2011). In the Baltic Sea, an increase by up to 5 °C (Elken et al. 2015) and a decrease in pH of 0.15 is predicted until the end of the century (Schneider et al. 2015). Besides global impacts, also regional environmental change affect Baltic ecosystem. Due to an increase of precipitations and river input, the salinity concentration of the Baltic Sea is predicted to decrease (Meier 2015). Regional climate models (RCM) simulate the hydrography of the Baltic Sea under the global change impact: the freshwater content will increase about 20 % until the end of the 21st century (Neumann 2010).

Another important regional factor in the Baltic Sea is eutrophication, defined as the enhanced inputs of nutrients (especially dissolved inorganic nitrogen and phosphorous) in water (Ærtebjerg et al. 2003). Anthropogenic activities, *i.a.* agriculture, cause the excessive input of nutrients in marine ecosystems. These are transported by rivers, atmospheric deposition of nitrogen and direct discharges from coastal sources and ships (HELCOM 2007 - 2011). Furthermore, the predicted increase of precipitation enhances the terrestrial runoff of nutrients (Neumann 2010) and may further aggravate eutrophic conditions in the Baltic Sea. At the moment, almost the entire Baltic Sea is affected by eutrophication with consequence for marine ecosystems. As a consequence, an increase of phytoplankton biomass, dissolved oxygen depletion and a reduction of the biodiversity have been observed (Ærtebjerg et al. 2003). Further consequences include increased plant growth, prolonged algal blooms and increased sedimentation followed by hypoxia in the bottom water (HELCOM 2007 - 2011) i.e. oxygen concentrations below 4 mg L^{-1} O_2 (Ærtebjerg et al. 2003). Further expansion of hypoxic and anoxic areas in the Baltic Sea has been predicted until the end of the 21st century (Meier et al. 2011). Salt water inflows into the Baltic Sea enhance stratification and the picnocline, which reduces the vertical mixing of oxygen maintaining hypoxic conditions in deeper water layers (Carstensen et al. 2014). Oxygen depletion in many coastal regions impacts several species leading to structural changes in benthic communities (Diaz and Rosenberg 1996).

Moreover, environmental change is caused by local upwelling predominantly occurring during late summer and autumn, bringing deeper waters to the surface. Upwelling can be caused by wind driven actions and by changes in hydrographic patterns (Lehmann et al. 2012). Consequently, temperature and oxygen concentration decrease while salinity, DIC and nutrients concentration increase in shallow waters (Saderne et al. 2013). Local upwelling events in coastal regions can occur over a period of days and can last for up to a few weeks (Melzner et al. 2013). In summary, multiple global and regional drivers in the Baltic Sea, such as global warming, acidification, local eutrophication and hypoxia, affect marine ecosystems (Jutterström et al. 2014).

3.3 Fluctuations of abiotic factors

The impacts of abiotic factors are determined by temporal variations that may be rhythmic (e.g. diurnal, seasonal) or stochastic (e.g. storms) (Wahl et al. 2011). The rate of change, the amplitude, frequency and durations of fluctuation of abiotic factors likely modulate the impact on marine organisms (Wahl et al. 2015a).

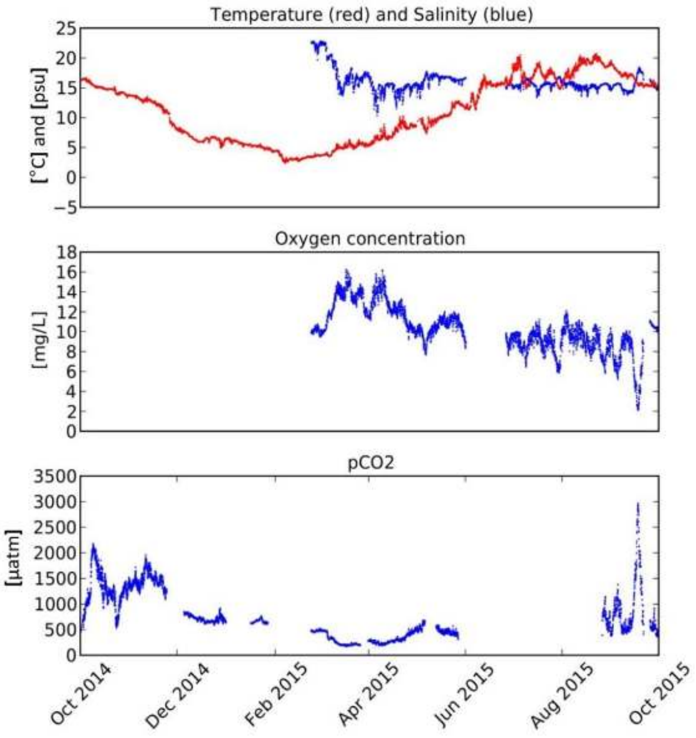

Figure 2 Temporal variation of the environmental variables temperature (°C), salinity (psu), oxygen (mg L^{-1}) and CO_2 (µatm) in 1 hour time intervals. Temperature was measured 0.5 meters depth by GEOMAR, Ocean circulation and Climate Dynamics - Marine Meteorology; pCO_2, O_2 and salinity were measured by CONTROS HydroC^{TM} in 1 meter depth in the inner Kiel Fjord by KIMOCC (Kiel Marine Organism Culture Centre). Temperature, salinity and oxygen were not measured in June and CO_2 was not measured in June and August when sensors were in service.

Changes of pH can occur within one day due to biotic activities such as photosynthesis and respiration (Hofmann et al. 2011), due to changes of the currents or during several days to weeks due to local upwelling events (Saderne et al. 2013). Under certain conditions, high diurnal pCO_2 fluctuation caused by hydrodynamic forces may expose marine coastal ecosystems on a short-term to values that even exceed acidification predictions for the end of the 21st century (Waldbusser and Salisbury 2014). In autumn, local events such as upwelling decrease pH and oxygen and increase pCO_2 concentration in shallow waters, that are rather rare during other seasons (Melzner et al. 2013) (Fig. 2). A realistic assessment of global climate change hence requires an upscaling approach towards natural settings including the impacts of multiple stressors on marine communities under consideration of stochastic and rhythmic fluctuations (Wahl et al. 2015a).

3.4 Adaptive and physiological responses of marine organisms to global change

The response of marine organisms to anthropogenic environmental changes is of crucial concern to understand their sensitivities and further persistence (Davis et al. 2005). Under strong selection pressures imposed by climate change, evolutionary adaptation can occur rapidly enabling to withstand stressful conditions (Hoffmann and Sgro 2011).

The responses to global climate change differ among levels of biological organisation (molecular, cellular, whole-organism, population and community). However, the interactions among levels have been largely neglected (Harvey et al. 2014) (Fig. 3). To understand the interactions among levels under future conditions, the knowledge of populations, species, and community responses should be combined (Munday 2013). On the molecular level, epigenetic responses, i.e. the heritable change in gene regulation processes without changes in the DNA sequence, adjust the individual phenotype to environmental change (Turner 2009). The cellular stress response is based on changes in the biochemistry and metabolic pathways (e.g. ion regulation under acidic conditions) that maintain cellular homeostasis. On the individual level, changes on fitness traits, such as the development, reproduction, growth and life-span may occur due to trade-offs of energy allocation (Sokolova et al. 2012). Behavioural responses of single organisms (e.g. migration) have even been described as the pacemaker of evolution (Duckworth 2008). Responses of single individuals, such as migration, may reduce environmental stress and selection pressures. Evolutionary responses of single species to environmental changes may affect the ecological community (Schoener 2011). Responses of single species to global change may lead to complex changes of trophic levels in a whole community (Harvey et al. 2013). However, interactions between evolutionary and ecological factors have been rarely considered in the field of community ecology. The environmental impact on ecological systems requires the consideration of evolutionary and physiological processes (Hairston et al. 2005).

Populations under adverse environmental conditions prevent local extinction via (1) distributional range shifts by migrating towards habitats with more suitable environmental conditions (Thomas 2010), (2) adaptive evolution, i.e. genetic change due to natural selection that improves the fitness of a population in its environment and (3) phenotypic plasticity, i.e. variation in the phenotype of individuals with the same genotype due to their experiencing different environments (Frankham et al. 2010). Plasticity is enabled when a steep slope of the reaction along an increasing environmental factor maintains marine organisms above a critical threshold (Reusch 2014).

3.5 Genetic diversity confers potential for adaptation

The combined impact of multiple environmental factors may adversely affect marine organisms. The potential to adapt to a changing environment is potentially conferred by genetic diversity (Frankham et al. 2010).

Adaption to adverse environmental conditions, i.e. evolutionary rescue, is enabled in relatively big populations with an increased genetic variability (Bell and Gonzalez 2009). Populations with lower genetic diversity are likely limited in their response to rapid environmental changes (genostasis) when a genetic constrain may not provide the potential for adaptation (Bell 2012). Genetic erosion, i.e. the loss of genetic variability, can occur by genetic drift and inbreeding limiting the variation in individual fitness within a population (Bijlsma and Loeschcke 2012). In a changing environment, strong selection pressures may lead to increased mortality and subsequently to local adaptation via natural selection. This may decrease the genetic diversity and hence the potential for adapting to further environmental change (Pauls et al. 2013). Genetic variability contributes to the individual variance in stress sensitivity, the width of species niches and the functional diversity, i.e. the type, range and relative abundance of functional traits in a given community (Violle et al. 2012). Moreover, genotypic diversity (i.e. the number of distinct clones per area) may enhance ecosystem productivity and recovery from disturbance (Reusch and Hughes 2006). In conservation genetics and management, genetic diversity analyses have received increasing attention for population viability analysis (PVA) to improve the protection of threatened species (Frankham et al. 2014). Evolutionary adaptation to multifactorial environmental changes may be determined by different patterns of sensitivity correlation. However, correlation analyses have been so far widely disregarded in evolutionary biology. Within a population, genotypes tolerant to one stressor may also be tolerant to another stressor, described by Vinebrooke et al. (2004) as a positive co-tolerance relationship. The positive pattern of correlation may accelerate adaptive evolution (Sunday et al. 2014) when selection pressures of multiple stressors favour tolerant genotypes.

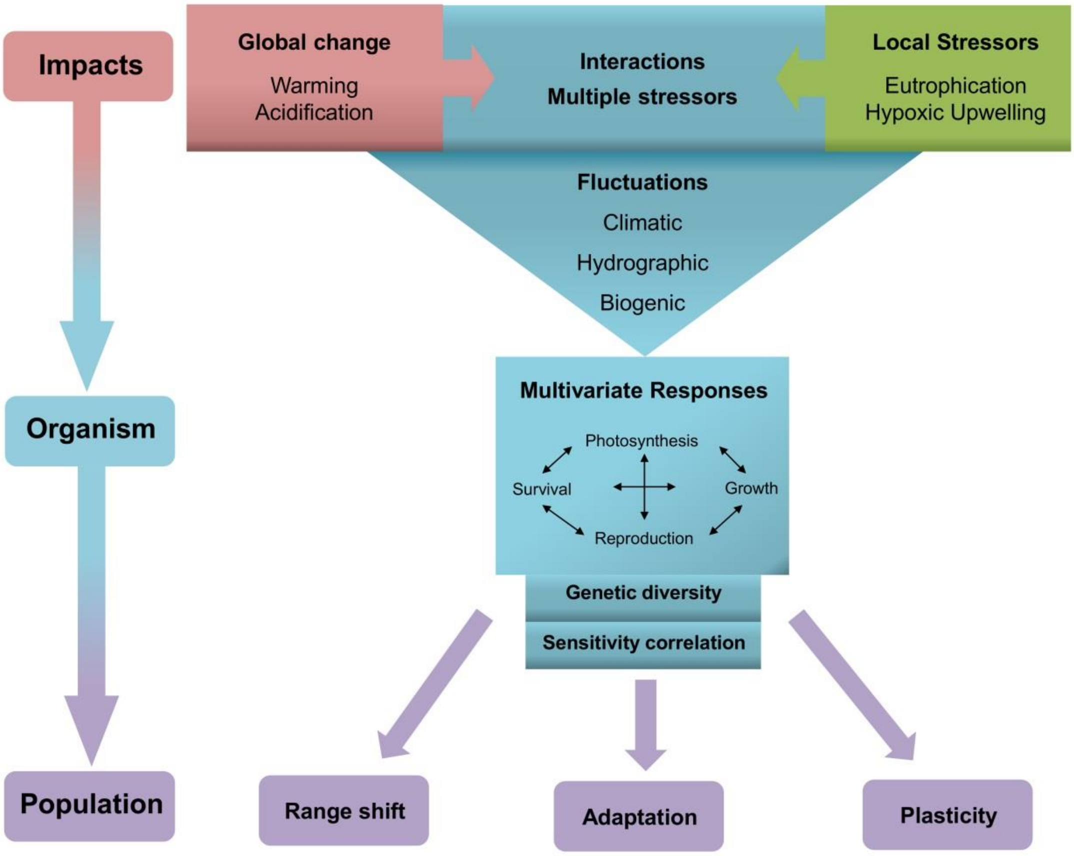

Figure 3 Environmental impacts on autotrophic organism and populations during summer. Global and local factors interactively affect organisms. The combined effect of multiple factors is modulated by fluctuations. Multivariate responses to environmental changes may be determined by trade-offs. Under environmental change, populations undergo range shifts, phenotypic plasticity or adaptation. Adaptation is conferred by genetic diversity and can be modulated by the pattern of sensitivity correlation.

Reasons for a positive correlation of sensitivities can be genetic pleiotropy, i.e. one single gene influences various phenotypic traits, or linkage disequilibrium, i.e. the non-random association of alleles of different genes at different loci (Conner 2002). Conversely, a negative correlation of sensitivities is given when increased tolerance towards one stressor is related to increased sensitivity towards another stressor. Genotypic sensitivity towards multiple factors can also be unrelated: in this case adaptation of tolerance to one stressor does not modify the sensitivity towards another stressor (Vinebrooke et al. 2004). The analysis of sensitivity correlation seems to be very important for macroalgae populations that are exposed to multifactorial environmental changes (Wahl et al. 2015b).

3.6 An important foundation species in the Baltic Sea: *Fucus vesiculosus*

The perennial brown algae *Fucus vesiculosus*, (Linnaeus 1753) belongs to the class Phaeophyceae, the order Fucales, the family Fucaceae and the genus *Fucus* (Strasburger et al. 2008). The etymology 'vesiculosus' derives from the characteristic gas filled bladders that confer buoyancy to the thalli. Bladder wrack grow on hard substratum of the intertidal and subtidal shores of coastal marine ecosystems and is often the dominant macroalgae in Baltic shallow macroalgal communities (Torn et al. 2006). *F. vesiculosus* is widely distributed in the artic and cold temperate Northern Atlantic down to southern Iberian shores (Lüning 1990). In the Baltic Sea, *F. vesiculosus* established approximately 6000 years ago and is widely distributed until the northernmost parts at the Bothnian Sea (Johannesson et al. 2011a), where it grows close to its lower salinity limit (Nygård and Dring 2008).

F. vesiculosus forms Baltic macrophyte communities by providing habitat, shelter and food for many associated organisms, such as the epibiotic bacterial community, macrobial epibionts, herbivores and carnivores (Wikström and Kautsky 2007). Important ecosystem services to the benthic community are provided by the oxygen production (Häder and Schäfer 1994). Moreover, the high carbon retention via photosynthetic carbon fixation (Schmidt et al. 2011) may temporally dampen high CO_2 concentrations that exceed the present mean conditions of 400 µatm CO_2. Macrophytes can act as "nutrient filter" in eutrophic coastal areas by increased uptake of nutrients (such as nitrogen and phosphorous). This capacity of nutrient retention favours the conditions for the benthic macrofauna under eutrophic conditions to some extent (Lloret and Marín 2011).

3.7 Reproduction and developmental stages of *Fucus vesiculosus*

As many other fucoid species, *Fucus vesiculosus* is a dioecious alga with separate male and female individuals. Male and female individuals become fertile when thalli tips form the reproductive receptacles. There are two reproductive periods of *F. vesiculosus*: the summer-reproducing population is present in some regions, whereas in some other regions the autumn-reproducing population dominates (Maczassek 2014). In some regions, also mixed reproductive periods are present, where algae are fertile in both seasons (Berger et al. 2001). The receptacles contain conceptacles that produce gametangia: oogonia in females and antheridia in males. Eggs and sperm are produced in the oogonia and antheridia, respectively, and released simultaneously into the environment (Pearson and Serrão 2006). Egg and sperm release peak at full moon and during calm conditions (Serrão et al. 1999). Fertilisation by sperm occurs close to the mother plant due to the fast sinking of eggs and the dispersal capacity (Tatarenkov et al. 2007). Zygotes settle on hard substratum, germinate and form rhizoids.

Subsequently, germlings grow to juveniles over a period of ca. 3-6 months and afterwards reach the adult life-stage at the age of ca. 1.5 years depending on the environmental conditions (L. Kautsky, pers. comm.) (Fig. 4). The mortality is particularly high at the early life-stages considering that less than 1 % of the released eggs develop to the adults, as shown in *Fucus distichus* (Ang 1991). Each life-stage presents a different sensitivity towards environmental conditions: adults are sensitive to epibiosis, UV stress, warming, light competition, grazing and hyposalinity. Zygotes, germlings and juveniles are additionally threatened by emersion, sedimentation and space competition (Wahl et al. 2011). Northern Baltic populations are particularly threatened by low salinity conditions that strongly reduce the viability of sperm (Serrão et al. 1999). In this region, population persistence is facilitated because *F. vesiculosus* also reproduces asexually: adventitious branches are formed, dispersed and attached to the substrate by forming rhizoids (Tatarenkov et al. 2005).

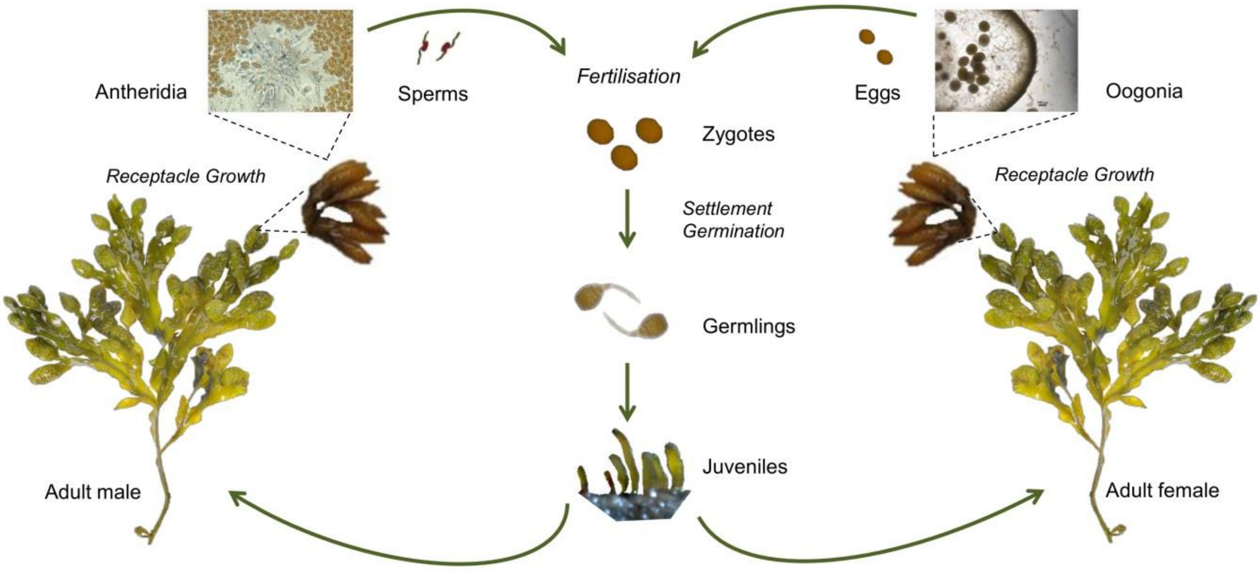

Figure 4 Life-cycle of *Fucus vesiculosus*. Adult male and female individuals develop receptacles containing conceptacles, with antheridia in males and oogonia in females. After gamete production in conceptacles, gametes are released to the environment. Eggs get fertilised by sperm and settle on hard substratum. After germination, zygotes grow to germlings, which become juveniles until reaching the adult and reproductive stage. [Pictures: www.istock.com juniorbeep, G. Bock, K. Maczassek, B. Al-Janabi]

3.8 Future threats of global and local impacts on seaweed

Various global and local environmental changes affect macroalgal populations in the Baltic Sea determining the response rather by the combined effects than by single effects (Wahl et al. 2015b).

Warming is an important abiotic factor determining the performance *F. vesiculosus*: the optimal range for Baltic populations is 4 - 10°C (Nygård and Dring 2008). High temperatures enhance the metabolism according to the Q10 rule leading to enhanced vegetative growth and to premature receptacle growth (Kraufvelin et al. 2012). At temperatures above the thermotolerance range, decreased performance was observed: growth was decreased at temperatures above 20 °C and photosynthesis was decreased at temperatures above 24 °C; while even higher temperatures (> 27°C) cause irreversible physical damage and lead to necrosis of algal tissue (Graiff et al. 2015a). Additional negative effects of heat stress are due to damages of active proteins involved in the photosystem II (e.g. D10 protein), and of enzymes involved in the ATP generating and carbon assimilation processes (Allakhverdiev et al. 2008). The early life-stages of seaweed show a different pattern in sensitivity to environmental stress than the adult life-stage (Coelho et al. 2000). At high temperatures (22 °C), the early life-stage of *F. serratus* is more sensitive compared to the adult life-stage, as reflected by the reduction of germlings' survival and growth (Nielsen et al. 2014). In addition, fertilisation of eggs and germination in *F. vesiculosus* was impeded by warming (>25 °C) (Maczassek 2014). Compared to global warming, the effects of ocean acidification on seaweeds' performance are generally less severe, as documented in the literature. High pCO_2 conditions enhanced the performance (growth and photosynthesis) in seaweed due to a fertilising effect (Nygård and Dring 2008) by an enhanced carbon fixation of the enzyme RuBisCO (Koch et al. 2013). As another example, gametogenesis in females and growth of sporophytes were enhanced under acidification in the brown algae *Laminaria hyperborea* (Olischläger et al. 2012).

In the Baltic Sea, *F. vesiculosus* is impacted by direct and indirect effects of eutrophication on different life stages. Nutrient enrichment caused direct positive effects on *F. vesiculosus*, as reflected by enhanced growth rate and photosynthetic efficiency (Nygård and Dring 2008). However, eutrophication has negative effects on *F. vesiculosus* mainly due to indirect effects. Growth of microalgae and filamentous algae is enhanced under eutrophication, shading thalli of *F. vesiculosus* (Rohde et al. 2008). Increased algal blooms in summer enhance the turbidity and limit the light penetration into deeper waters (Kautsky et al. 1986) causing light competition with ephemeral filamentous algae (Worm and Sommer 2000) and higher palatability (Hemmi and Jormalainen 2002). As a consequence of enhanced sedimentation under eutrophic conditions, bacterial re-mineralisation and oxygen depletion occur in the bottom water of the Baltic Sea (Meier et al. 2011). Decreased respiration rates due to hypoxic conditions may become particularly severe in macroalgae during night in the benthic ecosystem, when photosynthesis is downregulated, leading to a lower metabolism (Peckol and Rivers 1995).

Indirect effects of eutrophication in the Baltic Sea may be more severe regarding the early than the adult life-stage (Berger et al. 2004). Organic sedimentation originating from primary producers strongly reduced the recruitment success of germlings in the Baltic Sea by pre-emption of attachment and burial of small-sized germlings, endangering subsequent developmental stages (Eriksson and Johansson 2003). Enhanced concentrations of phosphate and nitrite reduced germination, growth and survival in the early life-stage of *F. vesiculosus* (Bergström et al. 2003). Also, the early life-stage is sensitive towards short term exposure (2 hours) to frost (Bird and McLachlan 1974), which has not been observed to that extent in the adult life-stage (own observation). Oil pollution has severe consequences by impeding fertilisation of gametes, demonstrating the high sensitivity to carbohydrates in the earliest stages (Thélin 1981). Also, the increase in storminess will likely enhance the detachment of newly settled zygotes, thereby decreasing the recruitment of seaweeds. Impacts of various environmental stressors on the weakest link of early life-stages limit the recruitment and determine the fate of subsequent developmental stages (Coelho et al. 2000).

The decrease of the biomass of Baltic *F. vesiculosus* during the 20th century by almost 95 % in populations of the Western Baltic Sea has been mainly attributed to the direct and indirect effects of eutrophication (Vogt and Schramm 1991). The past and present depth distribution was documented in the largest parts of the Baltic Sea: while bladder wrack grew in depth at 10 meters in the in the 1930´s, the present depth distribution goes down to 1-2 meters for the most part (Torn et al. 2006). This harsh decline has come to the attention of conservation ecologist. The aim is to better understand the causes of the decline and mechanisms of tolerance and adaptation in Baltic *F. vesiculosus* populations (Berger et al. 2004; Johannesson and André 2006).

3.9 Evolutionary responses of seaweeds to global climate change

Different responses of *F. vesiculosus* under environmental change have been documented. A poleward shift of *F. vesiculosus* distribution to habitats with optimal thermal conditions is a strategy to avoid thermal stress under warming. Local extinction along the warmer low latitude range and colonisation along the expanding colder high latitude edge lead to range shifts. In fact, the historical endpoint of the *F. vesiculosus* southern distribution in Southern Morocco (Khnifiss Lagoon) has already disappeared in the 1980s. At the current distributional limits of the western African and southern Iberian coasts, warming already caused range contraction (Jueterbock et al. 2013). A northward latitudinal shift of *F. vesiculosus* populations at the Atlantic Iberian Peninsula occurred during the last 30 years and was related with increasing shifts of the sea surface temperatures (SST) (Nicastro et al. 2013). The distribution ability was assumed to be relatively low due to the sessile adult life-stage of macroalgae. However, the dispersal ability and population connectivity is enhanced by floating *F. vesiculosus* (Rothäusler et al. 2015). Hence, range shift may be faster than assumed so far. Ecological niche models predict local extinctions of southern populations at the southern Iberian coasts while expansion to suitable habitats as Greenland, Northern Norway and other coasts of the Barents Sea will likely occur (Jueterbock et al. 2013).

Macroalgae also persist adverse environmental conditions if phenotypic plasticity is large. Marine plants can acclimatize to stressful conditions via phenotypic adjustment, when epigenetic mechanisms trigger the appropriate protein production or changes of the biochemical pathways. When the phenotypic performance is maintained under stressful conditions, phenotypic buffering, a type of phenotypic plasticity, allows for higher resistance (Reusch 2014). For instance, thermal stress resistance is given by acclimatisation due to changes in the photosynthetic efficiency and the upregulation of heat shock protein genes in *F. serratus* (Jueterbock et al. 2014).

A long-term solution of Baltic *F. vesiculosus* populations to avoid local extinction under environmental stress is local adaptation (Johannesson et al. 2011b). The Baltic Sea constitutes a marginal habitat with particular conditions, such as the decreased concentration in salinity. Bottleneck effects and isolation processes likely caused a decrease genetic diversity in Baltic *F. vesiculosus* populations compared to Atlantic populations (Johannesson and André 2006). Pairwise F_{ST} analyses revealed strong differences between Baltic compared to Atlantic populations of *F. vesiculosus*. Towards the northern region of the Baltic Sea, clonality of *F. vesiculosus* is higher than in southwestern populations, indicating higher degrees of inbreeding and the loss of genetic diversity (Johannesson et al. 2011a). Genetic differences between Baltic populations indicate that local adaptation in selected traits occurred; moreover the two reproductive modes of summer and autumn reproducing populations showed genetic differences (Tatarenkov et al. 2007). Specific conditions in the Baltic Sea, such as the low salinity, led to sympatric speciation where the new endemic species *F. radicans* diverged from *F. vesiculosus* a few thousand years ago (Pereyra et al. 2009). *F. radicans* presents a higher clonality than *F. vesiculosus* (Johannesson et al. 2011a) and is mainly distributed in the northern and eastern Baltic Sea (Ardehed et al. 2015). Since the genetic diversity of *F. vesiculosus* is likely decreased in the Baltic Sea, the consideration of genetic diversity is an important concern for resistance and resilience to environmental disturbances (Reusch and Hughes 2006).

3.10 The increasing plea for upscaling approaches

The assessment of the fate of marine populations under a changing environment requires the consideration of multiple factors caused by human impacts, which increase together with the growing human population (Crain et al. 2008). Anthropogenic factors, such as local (e.g. eutrophication and pollution) and global stressors (e.g. warming and acidification), do have an interactive and cumulative effect in marine coastal systems. The response to multiple factors can show the sum of individual factors (additive effects) or can interact by enhancing (synergism) or mitigating (antagonism) the common impact (Folt et al. 1999). The interconnection of different impacts determines the threat on marine organisms: in the Baltic Sea impacts as hypoxia, eutrophication and pollution should be analysed in combination with the global factors warming and acidification in management strategies (Jutterström et al. 2014).

Moreover, the frequency and magnitude of a fluctuating stress regime may enhance species acclimatisation and adaptation to different conditions, such as to low pH and oxygen conditions (Frieder et al. 2012). The impact of environmental change has mostly been regarded for one season only (Wahl et al. 2015a), although the effect may strongly differ among seasons. Warming predictions in the Baltic Sea at the end of the 21st century are season dependent (Neumann 2010). Furthermore, the effect of warming may be beneficial in colder but harmful in warmer seasons, when temperatures exceed the tolerance range of most marine species. A populations' ability for evolutionary adaptation to environmental changes is crucial in conservation ecology, however studies on genetic adaptation under global change are still relatively scarce (Merilä 2012). Hence, populations' genetic parameters, such as genetic diversity likely determine the predisposition to adapt to adverse environmental conditions and should be more widely included in conservation management (Frankham et al. 2014).

The interactive impact of multiple stressors has been rarely considered ecological research. Currently, multiple stressor applications receive an increasing demand in conservation ecology (Crain et al. 2008). So far, most research (< 70%) has been limited to the adult life-stage, one single factor, one season, and constant laboratory conditions, as reviewed for the field of ocean acidification research by Wahl et al. (2015a). This leads to an increasing plea for an upscaling approach in ecology (Queirós et al. 2015). Global change science increasingly request joint investigations by interdisciplinary approaches (Riebesell and Gattuso 2015) via the inclusion of fluctuations, different life-stages, multiple drivers and seasons in the light of adaptive responses.

To achieve higher realism, mesocosm approaches have been demanded with climate change simulations and the maintenance of natural fluctuations (Stewart et al. 2013). The novel mesocosm concept "Kiel Outdoor Benthocosms" (KOB) has been developed in the inner Kiel fjord, Baltic Sea, Germany (N 54°19.8´; E 010°09.9´) (Wahl et al. 2015c). Experiments were performed within the consortium 2 (benthic consortium) of the BIOACID II project (www.bioacid.de); an interdisciplinary research programme analysed the effects of ocean acidification and other global change factors on marine organisms and their habitats. Different working groups cooperated in order to analyse the future re-structuring and re-functioning of macrophyte communities at the KOBs during 2013 and 2014. Global change conditions of the end of 21th century were simulated during all seasons, while maintaining natural fluctuations of Kiel fjord. A continuous flow-through of seawater (1 tank-volume per day, i.e. 1500 L/ 24 h) into the experimental tanks allowed for maintaining natural in situ fluctuations. In a "delta-treatment", warming was achieved by adding +5 °C to the actual environmental temperature of the Kiel Fjord. Acidification was achieved by increasing the pCO_2 concentration to 1100 ppm within a hooded head space above the experimental tanks. The atmosphere-water exchange was enhanced by waves that were generated by the regular emptying of a continuously refilled water chamber. The CO_2 treatment was supported by the air injection from the headspace into the water of each tank. Environmental parameters were measured by continuous logging of temperature, pH, oxygen and salinity. The regulation of abiotic parameters occurs automatically by specialised software and the computer controlled application of heaters and coolers. Furthermore, nutrient enrichment and local upwelling were manipulated experimentally. The KOB infrastructure, a near-natural scenario, appropriately enables for experiments with the upscaling approach regarding multiple factors, seasons and multiple species under natural fluctuations.

References cited in the general introduction are listed below the general discussion.

3.11 Hypotheses

(1) The impact of global change factors on early life-stage *F. vesiculosus* differ between seasons
  - Effects of warming and acidification on the phenotypic performance on *F. vesiculosus* germlings differ among the four seasons
  - Interaction between warming and acidification depends on seasons

(2) Global stressors interact with local stressors
  - there are antagonistic or synergistic interactions between global and local stressors when occurring simultaneously
  - The sequential exposure to a new local stressor may be modulated by previous exposure to environmental change

(3) Phenotypic responses to environmental change differ between sibling groups and higher level of genetic diversity show a better performance
  - Genetically different sibling groups differ in their phenotypic responses to multifactorial environmental change
  - The phenotypic performance under environmental stress is better in higher diversity level than in lower diversity level

(4) There are correlation patterns in genotypes' sensitivity towards multiple stressors
  - The sensitivity of genetically different sibling groups towards multiple stressors correlate or anticorrelate

(5) Responses to global climate change depend on the level of biological organisation
  - Physiological and evolutionary responses to global climate change differ among the levels of biological organisation (molecular, cellular, individual, population and community)

4 Paper

Parts of this doctoral thesis have been published or submitted

Paper I Harvey B, Al-Janabi B, Broszeit S, Cioffi R, Kumar A, Aranguren-Gassis M, Bailey A, Green L, Gsottbauer C, Hall E, Lechler M, Mancuso F, Pereira C, Ricevuto E, Schram J, Stapp L, Stenberg S, Rosa L (2014) Evolution of Marine Organisms under Climate Change at Different Levels of Biological Organisation. Water 6: 3545-3574, DOI: 10.3390/w6113545

Statement of authorship: B. Harvey designed the original concept of the multi-author-article; B. Al-Janabi was the team leader of the chapter "population-level responses", which was also written by E. Hall and J.B. Schramm.

Paper II Al-Janabi B, Kruse I, Graiff A, Karsten U, Wahl M (2016) Genotypic variation influences tolerance to warming and acidification of early life-stage *Fucus vesiculosus* L. (Phaeophyceae) in a seasonally fluctuating environment. Marine Biology 163: 1-15 doi 10.1007/s00227-015-2804-8

Statement of authorship: B. Al-Janabi, I. Kruse and M. Wahl designed the study; B. Al-Janabi collected the data; B. Al-Janabi and A. Graiff analysed the data; B. Al-Janabi wrote the manuscript and all co-authors commented and corrected the manuscript.

Paper III Al-Janabi B, Kruse I, Graiff A, Winde V, Lenz M, Wahl M (under review in Plos One) Buffering and amplifying interactions among OAW (ocean acidification & warming) and nutrient enrichment on early life-stage *Fucus vesiculosus* L. (Phaeophyceae) and their carry over effects to hypoxia impact

Statement of authorship: B. Al-Janabi and M. Wahl designed the study; B. Al-Janabi and V. Winde collected the data; B. Al-Janabi, M. Lenz and V. Winde analysed the data; B. Al-Janabi wrote the manuscript and all authors commented and corrected the manuscript.

Paper IV Al-Janabi B, Wahl M, Karsten U, Graiff A, Kruse I (in preparation for submission in Ecology Letters) Genotypic correlation in response to global change parameters in seaweed germlings indicates potential for modulation of adaptation success

Statement of authorship: I. Kruse and M. Wahl designed the research; B. Al-Janabi collected and analysed the data; B. Al-Janabi, I. Kruse and M. Wahl wrote the manuscript.

Paper I

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Evolution of Marine Organisms under Climate Change at Different Levels of Biological Organisation

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Keywords: ocean acidification; climate change; acclimation; evolutionary potential; adaptation; biological organisation; biologically-relevant scales

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Review

Evolution of Marine Organisms under Climate Change at Different Levels of Biological Organisation

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Abstract: Research to date has suggested that both individual marine species and ecological processes are expected to exhibit diverse responses to the environmental effects of climate change. Evolutionary responses can occur on rapid (ecological) timescales, and yet studies typically do not consider the role that adaptive evolution will play in modulating biological responses to climate change. Investigations into such responses have typically been focused at particular biological levels (e.g., cellular, population, community), often lacking interactions among levels. Since all levels of biological organisation are sensitive to global climate change, there is a need to elucidate how different processes and hierarchical interactions will influence species fitness. Therefore, predicting the responses of communities and populations to global change will require multidisciplinary efforts across multiple levels of hierarchy, from the genetic and cellular to communities and ecosystems. Eventually, this may allow us to establish the role that acclimatisation and adaptation will play in determining marine community structures in future scenarios.

Keywords: ocean acidification; climate change; acclimation; evolutionary potential; adaptation; biological organisation; biologically-relevant scales

1. Introduction

Evolutionary processes play a fundamental role in the organisational structure of biological systems and the diversity of life [1]. It is possible for evolution to occur on a rapid ecological timescale, that may allow organisms to avoid extinction following environmental change [2]. One environment which is arguably changing faster than others is the marine environment [3], where increasing levels of atmospheric CO_2 are causing the seawater temperature and carbonate chemistry of surface waters to change at geologically unprecedented rates [4]. Future warming and altered ocean chemistry (broadly termed climate change throughout the present review) are recognised as pervasive and detrimental anthropogenic influences on marine life [5–9]. Climate change is expected to impose strong selection pressure on fitness-related traits, impacting on populations and ecosystems [10–14], and yet most future projections of community dynamics and population persistence in marine organisms do not consider the role of evolution and adaptive capacity [15–17].

The potential for genetic adaptation in response to climate change has been acknowledged [17,18], and adaptive evolution may represent a critical mechanism which could alleviate some of the negative consequences expected with future climate change [19]. However, the relatively limited number of studies means that evidence is still somewhat scarce [20]. A number of recent reviews outline the role of adaptive evolution in the face of climate change, including the need for determining species' capacity for evolutionary adaptation and physiological acclimatisation, the distinctions between evolutionary and phenotypically plastic responses, and summaries of the different experimental approaches (e.g., molecular tools, quantitative genetics, standing genetic variation, and experimental evolution). They also outline possible directions for future research (for reviews, see [16,17,19–23], and references therein). A glossary for some of the terms commonly used in this review is given in Box 1.

Studies investigating biological responses to climate change will often be carried out with a particular focus, whether that be physiology, evolutionary biology or community ecology. Such focus naturally means that other interacting facets of eco-evolution are often neglected [24]. Clearly, there are many important inter-disciplinary studies that do bridge this gap (e.g., [25–27]), however, there are still often disparities in the extent (if at all) that adaptive evolution is considered by different disciplines when determining a species' response under a changing environment. Inter-disciplinary work that links eco-evolution through biological hierarchies is not a new concept having been raised by numerous influential comparative physiologists in the 1950s, such as C. Ladd Prosser [28]. We believe this idea bears reiterating, and consider modern science to possess the necessary advancements in technology and communication required to begin incorporating this concept into future research.

There are a number of factors that mediate evolutionary processes, but their effects are highly dependent on the level of biological organisation that is considered (e.g., intra-individual, whole-organism, population, community and ecosystem, see Figure 1). The underlying mechanisms of how these levels of hierarchy will interact to influence fitness in the face of climate change are poorly understood, but are important in determining whether individual populations and communities will persist at levels comparable to the present day [16].

This review will focus on factors that can modulate adaptive evolution at different levels of biological organisation, by considering the response of marine organisms at these different levels in terms of the consequences for fitness traits (*i.e.*, lifetime reproductive success). We discuss: (1) what molecular and cellular mechanisms exist that can influence fitness and drive adaptive evolution; (2) how changes in life history and behavioural characteristics of organisms can influence lifetime reproductive success; (3) how demographic processes (gene frequencies, population size and turnover) and genetic architecture (heritability, imprinting, genetic correlations and diversity) of the population will influence adaptive evolution; and (4) how changes in species interactions and community composition influence the magnitude and direction of adaptive evolution of populations.

Box 1. Glossary for terms used in this article.

Acclimation: Reversible process of an organism to adjust to experimental conditions. When the process is induced by natural environmental changes, it is called **acclimatisation**.

Bottleneck effect: Reduction in population size due to environmental events, leading to a strong reduction of the variation in the gene pool.

Effective population size: Size of a hypothetical ideal population with random mating that corresponds to population genetic processes within the focal wild population.

Epigenetics: Heritable changes in gene regulation processes that are not caused by changes in the DNA sequence.

Evolution: Genetic changes in a population over generations. It is said to be microevolution when these changes occur over relatively short timescales, rather than on geological scales (macroevolution).

Evolutionary rescue: Genetic adaptation of populations that allows them to recover from demographic effects and avoid extinction.

Experimental evolution: Controlled experiment that exposes populations to new environmental conditions for multiple generations to observe for genetic adaptation.

Fitness: The potential for individuals of a given genotype to survive and pass their genes to future generations by influencing either their own reproductive success or that of related individuals.

Genetic adaptation: A process of transgenerational selection of genes to maximise or maintain the relative fitness of a population in a given environment.

Phenotypic buffering: Type of phenotypic plasticity, in which no difference in the response of a trait to a given environment might be observed because plasticity in a physiological process allows an organism to maintain fitness.

Phenotypic plasticity: Phenotypic adjustment to the environment without any genetic change.

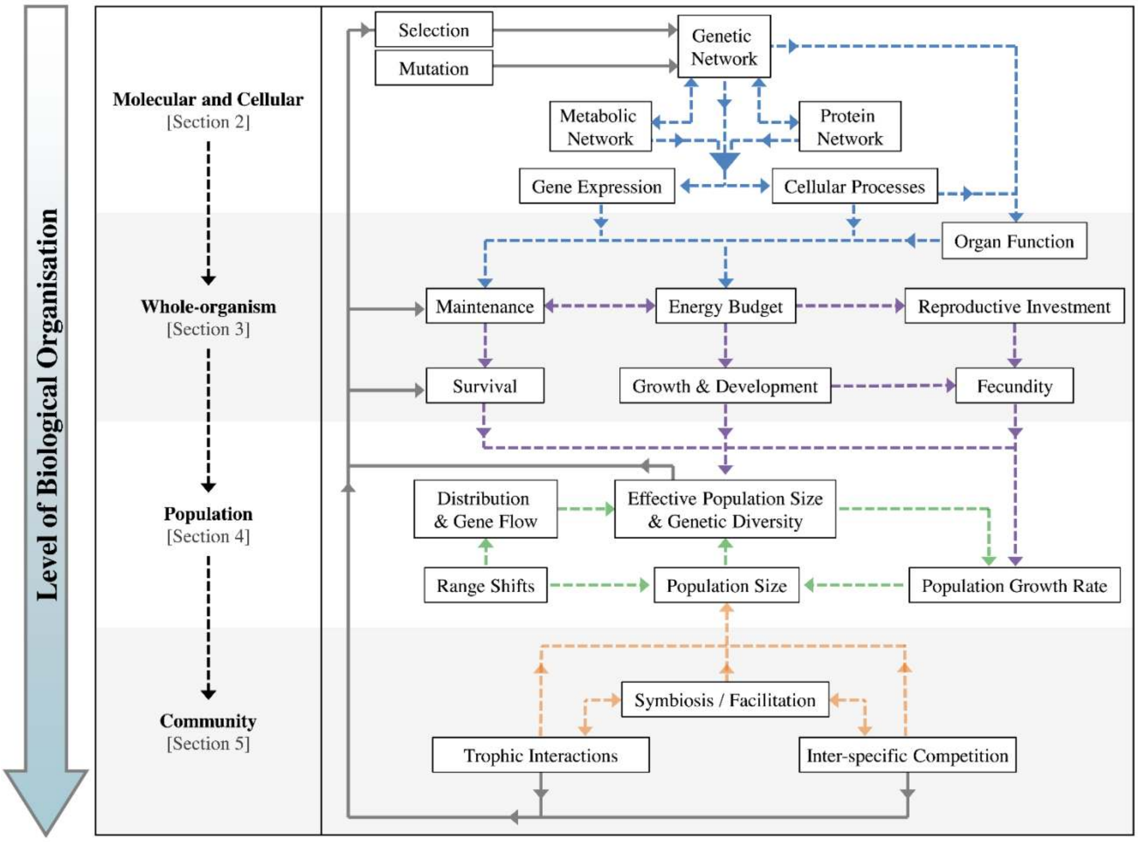
Quantitative genetics: Method to partition the observed phenotypic variance among relatives (of known genetic relatedness) into their environmental and genetic components.

Selection: Non-random reproduction or survival of individuals of a particular phenotype.

2. Role of Molecular and Cellular Processes in Evolutionary Responses

Molecular and cellular level studies can provide several approaches for improving our understanding of the potential for adaptation in response to climate change. These can include characterising an organism's capacity to acclimatise to changing environmental conditions, as well as establishing a more mechanistic understanding of the response of organisms to abiotic factors at different levels of intra-individual biological organisation, such as the nature of sub-lethal cellular stress [29]. Eventually this might enable us to investigate whether genetic adaptation can occur at a sufficient rate to maintain the physiological functioning required for survival and reproduction, and gain important insights into energy allocation and physiological responses due to climate change, as well as other biotic and abiotic stressors [22]. However, the distribution of a species is shaped by both a species' physiological limits and biotic interactions with co-existing species, and therefore, cellular and molecular studies alone may only provide part of the picture.

Figure 1. Conceptual diagram of the factors modulating evolution at different levels of biological organisation (molecular, cellular, whole-organism, population and community), that will determine the response of marine organisms to future climate change. The arrow on the left represents the increased biological complexity (going from top to bottom). Single-headed arrows indicate the direction of the effect with the level of biological organisation indicated by different colours. Effects originating from the molecular and cellular (dashed blue), whole-organism (dashed purple), population (dashed green) and community (dashed orange). Double-headed arrows indicate that there is feedback between two factors, as well as the effect, and the solid grey arrows indicate a feedback loop. Note that the depiction of factors is conceptual and not comprehensive.



2.1. Biochemical Reactions and Gene Expression

Within the organism, protein activity is often thought to underlie variations in fitness (for discussion, see [30]). Fitness at the biochemical level could be simply considered as the ability of proteins to function (within their respective intra- and extra-cellular setting) in order to integrate the diverse functions of cells and organelles [31]. Proteins are responsible for crucial functions in all biological processes [31], and evolutionary changes can occur through changes in the proteins themselves (e.g., post-translational modifications), the encoding gene(s) of those proteins, or the transcription of those encoding genes [31].

Fitness-related traits can be influenced through genetic variation in these proteins, such as the collinearity of gene mutations, whereby the point mutations in the DNA sequence will correspondingly change the sequence of amino acids in a protein [32]. These biochemical consequences can influence protein function and in turn, tolerances to environmental conditions [33]. For example, a minor mutation (only two amino-acids out of 334) in a dehydrogenase enzyme in the temperate mussel *Mytilus galloprovincialis* (Lamarck, 1819) resulted in higher thermal tolerance towards warm conditions [34]. Alternatively, enzymes possessing alternative alleles, such as for lactate dehydrogenase-B in cold- and warm-adapted populations of the killifish (*Fundulus heteroclitus* L., 1776) [35], may be able to confer adaptation potential for thermal tolerance through variable allele frequencies.

In order to produce adaptive phenotypes, changes may be required in multiple combinations of alleles [36]. Allelic changes are embedded within genetic networks and hence, will not occur independently to other changes, since any allelic changes at a particular locus will influence only one aspect of a genetic network [37]. These genetic networks essentially consist of the genes which encode the transcription factors as the input for each coding gene, and the *cis*-regulatory modules that control the appropriate phases of expression of these genes [38]. Gene regulatory networks control the expression of genes in any given developmental process [39], including fitness-related traits, and therefore, any changes in the networks could play an important role in adaptive evolution and climate change responses [37].

Environmental effects may cause changes either in specific genes within the network, influencing their gene expression, or affect the gene regulatory network as a whole [40]. Genetic networks will primarily be influenced by current environmental conditions and maternal effects (the latter described in Section 2.3), and these changes will, in turn, alter the protein and metabolic networks that influence gene regulation (*via* a feedback loop reaction) [37]. Changes in genetic networks may influence plastic responses and facilitate adaptive evolution by providing a rapid response to the changing environmental conditions. However, if the genetic regulatory network is influenced by other factors that do not follow the changing environmental conditions, such as photoperiod [41] or even biotic interactions [42] (discussed further in Section 5), then adaptive evolution might require a restructuring of the genetic network in order to conform to the novel environmental conditions [37].

Currently it remains unclear whether the few examples that demonstrate observable adaptive evolution of traits in response to climate change (e.g., body size [43], migration timing [44], thermal responses [45]) are dictated by various independent genes (within their respective genetic networks), or by fewer key regulatory genes within their genetic or metabolic networks. This is important to consider since any changes in the ‘upstream’ network genes could have extensive and numerous effects on traits [37], and yet the network itself may also provide some redundancy and buffering against perturbations, whereby changes to regulatory genes do not influence the genes they regulate [46]. Eventually, it may be possible to identify common genetic (e.g., collinearity in the gene order between genomes [47]) and physiological mechanisms underlying species responses [17]. However, studies demonstrating a clear link between the genetic variation and phenotypic variation for the majority of traits are scarce (but for example, see [48]). Therefore, any studies of genetic variation should focus on traits with more straightforward or measurable relationships to fitness [17,21].

Establishing the evolutionary significance of cellular-level plasticity (*i.e.*, the changes in the expression levels of stress-related genes, e.g., [49]) requires demonstration of a heritable component of expression variation, or allelic variation in the coding genes themselves [17,50]. Accurately estimating selection responses requires the genetic component of this variation (in regulatory responses) to be related to the fitness of the organism [51,52] in order to ascertain the fitness-related consequences for the individual and the population. This highlights the need to investigate transcriptome profile responses in terms of survival, fecundity, or other ecologically important traits that determine lifetime reproductive success (but see [30] for a discussion on the limitations in the link between the transcriptome and the phenotype), and importantly, ascertain whether sufficient genetic variation exists in that trait [53].

2.2. Cellular Processes and Organ Function

Cellular and organ functioning during stressful conditions will primarily be dictated by changes at the genomic and biochemical level (Section 2.1). The principal factor determining the underlying cellular stress response (a universally conserved mechanism to protect macromolecules within cells from damage [54]) depends on the extent of stress-induced disturbances (reviewed by [55]). During moderate stress, resources may be shifted from anabolic (e.g., protein biosynthesis) towards vital processes for cellular homeostasis (e.g., ion regulation; [55]) to maintain cellular integrity and ensure short-term survival. However, on longer time scales such shifts may not be feasible and might lead to a reduction in organism performance (e.g., reduced growth rates or fecundity) since the organismal energy budget can be considered as the sum of all cellular energy budgets [55].

Such trade-offs in physiological functions could have important fitness consequences, but may not be apparent when only observing the whole organism level. For instance, a study on the effects of ocean acidification on the reef-building coral, *Acropora millepora* (Ehrenberg, 1834), reported major changes in gene expression and cell physiology long before phenotypic effects were observed, in this case, a decrease in calcification rates [54]. Thus, cellular functioning might play a central role in linking environmental conditions to an organism's fitness [56], and the plasticity and adaptive evolution of cellular processes may be an important influence on species resilience towards changing environmental conditions.

Adaptive evolution in cellular function may be possible through gene duplication [57–59], whereby paralogous genes (*i.e.*, gene copies) that perform a particular function either increase their expression (increased gene dosage) or diverge their functions through mutation [60]. This divergence can be achieved by one of the copies acquiring a new function, or through a partial loss-of-function mutation of both copies that complement each other [61], while retaining the full set of functions (termed sub-functionalisation [60]). This sub-functionalisation is a relatively common mechanism for functionally related proteins [61], such as components of cell signalling pathways, and may facilitate evolution of advantageous traits: e.g., a changed pH optima of proteins [62], a beneficial trait for maintaining acid-base homeostasis in response to ocean acidification.

2.3. Epigenetics and Trans-Generational Plasticity

The environment experienced by an organism can shape the phenotype of their offspring, and is termed trans-generational plasticity (e.g., [63]). Trans-generational plasticity can be due to maternal or paternal effects, genomic imprinting, gene expression or other epigenetic processes. These epigenetic effects (whether a gene is being expressed or not) can be transmitted through the germ line [64], which can allow for transmission through meiosis to the succeeding generation, constituting a heritable, epigenetic change [65]. For example, five weeks exposure to elevated $p\mathrm{CO}_2$ during the reproductive conditioning of Sydney rock oysters (*Saccostrea glomerata*, Gould 1850) reduced the development time and increased the body size of their larvae through trans-generational plasticity [66].

Mechanisms exist that should allow these epigenetic changes to result in localised changes in the DNA sequence, such as changes in the activity of chromatin-modifying enzymes [65]. Providing they exert the same functional effect, any epigenetic effects can potentially become a genetic change, and exert a selectable phenotypic response [65]. During climate change, the environmental conditions that induce these epigenetic effects (like temperature) will persist (albeit progressively increasing) and therefore with each successive generation, the epigenetic response could actually result in continued DNA change in selected regions of the genome [65].

The gene regulatory network, responsible for many fitness-related traits (Section 2.1), is initiated by maternal transcripts and proteins, which cascade into subsequent gene regulatory interactions [67]. Early genes that function during development (such as for larval morphology) can be influenced by the fitness traits of the maternal parent (e.g., by changes in egg size or provisioning [67]), and therefore it may be possible that parental exposure to climate change can cause DNA (or heritable, epigenetic) changes that promote adaptive evolution in key regulatory genes, or the genetic network as a whole.

3. Role of Whole-Organism Physiological and Behavioural Responses

Marine organisms possess a range of reproductive and developmental strategies that have important implications for their fitness [68]. Different reproductive modes, life histories, and demographic processes can influence these strategies [69–71]. In this section we focus on how climate change, specifically ocean acidification and warming, can influence the physiology and behaviour of the individuals, affecting their survival and fitness. It is important to consider the factors that influence selection at this level of biological organisation in order to link individual phenotypes, which are in turn driven by transcriptional and cellular processes, to population-level effects.

3.1. Maintenance and Energetic Trade-Offs

The capacity to maintain metabolic processes under environmental stress may support (or promote) the retention of particular life history traits (such as reproductive output) that may ultimately determine a species' biogeography [72,73]. A recent study using an *in situ* transplant experiment with polychaetes, found that species capable of maintaining their metabolic rates (under stress) were able to migrate into or even colonise areas characterised by chronically elevated levels of $p\mathrm{CO}_2$ [26]. This high-CO_2 tolerance was achieved in the polychaetes *via* acclimatisation for *Amphiglena mediterranea* (Leydig, 1851) and by adaptation for *Platynereis dumerilii* (Audouin & Milne-Edwards, 1834) [26]. However, such resilience often comes at a cost [74]. The individuals of *P. dumerilii* were smaller in body size compared to nearby populations in lower $p$$CO_2$ conditions, attributed to increases in maintenance costs due to a higher mean metabolic rate under chronic exposure to elevated $p$$CO_2$. Since the size (in several polychaete species) can determine the maximum numbers of eggs that a female produces, this resilience could result in reduced reproductive output [26]. Although the study did not empirically test this, any reallocation of energy away from reproduction would clearly have important implications for lifetime reproductive success.

Fitness-related traits can be genetically correlated to each other and, depending on strength and direction of selection, influence the potential for adaptive evolution (for more detail, see [17]). Briefly, a positive correlation could include a co-tolerance to multiple stressors (e.g., developing sea urchin larvae obtaining tolerance to low pH and therefore also temperature [75]), or a selection for a particular trait providing tolerance for another trait (e.g., growth and disease resilience in Sydney rock oyster (*S. glomerata*) providing tolerance to high $p$$CO_2$, [66]). If the intra-individual physiological mechanisms (Section 2) and an organism's response during climate change are nonlinearly related, then there is a need to understand what physiological trade-offs are occurring that are influencing their fitness related traits. Fitness trade-offs will certainly influence potentially selected traits, if other energetically maintained traits are selected over survival or reproductive output.

3.2. Life-History Stages

Research into physiological responses to climate change has demonstrated that fitness traits, such as reproduction and development, are likely to be disproportionally affected [76–78]. Since natural selection acts upon lifetime reproductive success, climate change can reduce fitness through impacts on early life-history stages, such as an increase in developmental duration or number of defects [79–81]. However, many marine species have complex life histories, and despite early life history stages being considered to be particularly vulnerable to climate change [82], there is increasing evidence that selection pressures act on each life stage differently (e.g., [83]). Phenotypic carry-over effects can also occur between life history stages (as well as trans-generationally, Section 2.3) that could exacerbate or alleviate the impacts on fitness-related traits. For example, exposure to stressful conditions during the larval stage can reduce the juvenile fitness if those conditions continue (e.g., [84]). This may be particularly important given that different stages of ontogeny may utilise different habitats (e.g., [85]) or exhibit different behaviour. Hence, impacts considered on individual life-stages may not accurately estimate the fitness response of a given species [86].

3.3. Behavioural Responses

Organismal behaviour is mediated by multiple external and internal sensory inputs that may be changed directly and indirectly by climate change [87]. The plastic behavioural responses observed in organisms are largely a direct physiological response to a changing environment, since the nervous system is under biochemical and physiological control [88]. Hence, changes in the underlying physiological condition (see Section 2) could influence behavioural performance by constraining an ecologically-relevant behaviour, such as swimming activity [89]. A study in coral reef fish found that small temperature increases (<3 °C) contributed to changes in animal personality (activity, boldness, aggression), thought to be linked to individual responses in energy metabolism [90].

Changing environments can also modulate behaviour by interfering with sensory inputs and neural functioning. For example, elevated levels of $p\mathrm{CO}_2$ are hypothesised to remodel the sensory pathway of the GABA-A system of marine organisms, including the larval clownfish (*Amphiprion percula* Lacepède, 1802), damselfish (*Neopomacentrus azysron* Bleeker, 1877), and gastropod *Gibberulus gibbosus* (Röding, 1798) [91,92], causing sensory and behavioural impairment, including learning ability [93]. This phenomenon is thought to be associated with ion regulatory mechanisms during high CO_2 exposure (accumulation of intracellular HCO_3^- and Cl^-), which interfere with neurotransmitter functions (for more details, see [92]). Impaired learning regarding the identity of predators during high $p\mathrm{CO}_2$, or diminished detection of the olfactory cues for settlement (for instance) influence fitness by negatively affecting the survivorship of the individual [93,94]. Sensory pathways occur in differing complexities with receptors and messenger systems of different adaptive potential [95]. Hence, knowing the mechanistic pathway of a behavioural response is important for determining the evolutionary potential of an organism or indeed a trait. Linking these pathways with their genes is important for finding out if organisms can adapt, in order to cope behaviourally with environmental stressors [96]. Behavioural traits may be more evolutionary labile than other traits [97], and may contribute to or hinder adaptation [19,98].

4. Role of Population-Level Responses

Focusing on population-level organisation is crucial for connecting the fitness responses of lower levels (individual/population) to changes in higher levels (species/community). The analysis of microevolution in populations requires an understanding of how environmental changes influence evolutionary processes such as gene flow, mutation, genetic drift and natural selection [99]. Historically, the concept and investigation of population level adaptation in the marine environment was largely dismissed; it was assumed that marine connectivity would maintain high levels of gene flow between populations via adult and larval dispersal [100], and so impede local adaptation. However, new evidence compiled by Sanford and Kelly [101] shows that microevolution is not restricted to organisms with low dispersal abilities. Through a literature survey Sanford and Kelly [101] found that 66% of marine invertebrates with planktonic life stages for dispersal, *i.e.*, meroplankton, present highly adaptive differentiation at the population level (e.g. *Haliotis rufescens*, Table 1). Depending on the taxa investigated, the planktonic dispersal stages of the identified (66%) invertebrates experienced brief (up to a few days as with some corals, sea anemones or ascidians) to prolonged (several weeks to longer, some crustaceans and gastropods [100]) planktonic larval durations.

4.1. Demographic Processes

Populations can respond to environmental pressures more rapidly through range shifts and phenotypic plasticity rather than through evolutionary adaptation [102]. Evolutionary responses are likely to vary depending on the cost of adaptation, timescale, life-history and dispersal ability in addition to other factors [19]. Different evolutionary responses have been previously investigated and require a variety of techniques (for a survey of selected reference studies see Table 1). Understanding genetic variation, as well as specific population dynamics, is crucial to explore the potential for evolutionary rescue [103]. For example, populations in isolated environments, such as the Baltic Sea, may also undergo isolation and develop genetic endemism as a result of local extinctions or adaptation by evolutionary rescue [104]. Therefore, population size and genetic variation in the context of the intensity and duration of environmental selection pressures must be considered [105] to identify what part of the population (*i.e.*, the effective population size [106]) contributes to the next generation.

Table 1. Published studies investigating population level evolutionary responses to climate change (including ocean acidification) in marine species.

Taxonomic Affiliation	Response Variable(s)	Driver	Method(s)	Evolutionary Response	Ref.
Spermatophyta: *Zostera marina*	Growth rate Survival	T	F	Genotypic complementarity	[107]
Coccolithophyceae: *Emiliania huxleyi*	Growth rate Production rate: (PIC)	OA	LS	Selection of genotypes Direct positive adaptation	[108]
Gephyrocapsa oceanica	Growth rate Carbon fixation	OA	LS	Selection of genotypes (Adaptation)	[109]
Diatomophyceae: *Thalassiosira pseudonana*	Phytosynthetic efficiency	OA	LS	No adaptation	[110]
Anthozoa: *Acropora millepora*	Thermal and physiological tolerance	T	F	Natural selection	[111]
Pocillopora damicornis	Coral bleaching (thermal tolerance)	T ES	CG	Local adaptation or acclimation	[112]
Bivalvia: *Mytilus trossulus*	Growth rate Survival	T	TE	Possible thermal adaptation	[113]
Gastropoda: *Haliotis rufescens*	Genetic polymorphism	T	SNP	Local adaptation Genetic differentiation	[114]
Polychaeta: *Platynereis dumerilii*	Body size	OA	TE	Genetic adaptation	[26]
Amphiglena mediterranea	Body size	OA	TE	Physiological plasticity	[26]
Amphipoda: *Orchestia gammarellus*	Growth Thermal tolerance	T	LS	Selection	[115]
Cirripedia: *Semibalanus balanoides*	Genetic polymorphism	T D	TE	Balancing selection Local adaptation	[116]
Copepoda: *Tigriopus californicus*	Survival (LT_{50}) Thermal plasticity	T	LS	Low adaptation potential	[117]
Decapoda: *Uca pugnax*	Developmental rate	T	CG	Selection on variation Local adaptation	[118]
Echinoidea: *Heliocidaris erythrogramma armigera*	Hatching success	T	QG	Genotype-by- environment interaction	[119]
Strongylocentrotus purpuratus	Gene expression: thermal resistance	T	CG	Selection of thermally sensitive genes	[120]
Strongylocentrotus purpuratus	Larval body size	OA	CG	Heritability correlates with high-pCO$_2$	[121]
Centrostephanus rodgersii	Cleavage and gastrulation stage	T OA	QG	Heritable genetic variation for sires	[75]
Centrostephanus rodgersii	Embryonic development	T OA	CG	Varying expansion of population	[122]
Teleostei: *Gadus morhua*	Body shape	T	CG	Counter-gradient variation	[123]
Fundulus heteroclitus	Thermal tolerance	T	LS	Selection Regulation of heat shock proteins	[124]

Notes: Selective driver: abbreviated as T - temperature; OA - ocean acidification; ES - environmental stability; D - desiccation. Method: F - field experiment; LS - laboratory selection experiment; CG - common garden experiment; TE - transplant experiment; SNP - outlier SNP analysis; QG - quantitative genetics.

Populations may have an increased chance of persistence if they react to changing climatic conditions with higher phenotypic plasticity. Should this plasticity occur in a fitness-related trait, then this may present a heritable variation for selection to act upon (e.g., [23,125]). This mechanism would thereby allow for a faster non-mutational selection [126]. Populations that are maladapted to climate change will likely experience an initial decline and thus, a reduced effective population size [127]. Phenotypic buffering, a type of phenotypic plasticity, represents an important mechanism for maintaining population performance under stressful conditions until adaptive evolution can “catch up” and sufficiently improve population fitness [23,128]. For example, genetically diverse populations of the seagrass *Zostera marina* (L., 1758) showed quicker recovery following sub-lethal temperature stress when compared to less diverse populations [107]. This buffering effect was expressed due to the complementarity of different genotypes (e.g., facilitation) that maintained ecosystem functioning, and may promote adaptive evolution [107].

4.2. Environmental Variability

The potential for adaptation under naturally low or fluctuating pH can be studied in regions of upwelling along the continental coast of (Western) North America [129]. A transcriptomic analysis of sea urchin larvae (*Strongylocentrotus purpuratus* Stimpson, 1857) collected from a naturally variable low pH upwelling site revealed that larvae under present day conditions initiated a robust transcriptional response, but only a muted response to near future conditions [130]. These exposures to transient extreme conditions may be sufficient to provide populations with a selection for tolerance (e.g., [131]). However, the question then becomes whether selection for one stressor will provide increased tolerance to another. Quantitative genetics is a technique that may help answer this type of question because it allows partitioning of the observed phenotypic variance of a population among relatives (with known genetic relatedness) into their environmental and genetic components [132], in a synchronic approach (*sensu* [23]). Numerous studies have demonstrated evolutionary adaptive capacity using quantitative genetics (as reviewed in [71]).

In the absence of mutations, adaptive evolution relies on the genetic variation in physiological tolerances [133], this is because in turn, the variation of physiological tolerances influences the likelihood of extinction [121,134]. These tolerance traits in natural populations are termed standing genetic variation, and arguably the most important influence maintaining this adaptive variation is spatially varying selection [135]. For most species, the temperature gradient across their distribution (e.g., 30 °C difference between the pole and equator [121]) will greatly exceed the expected future temperature change (3.7–4.8 °C, [136]). In contrast, pH gradients are often relatively homogenous when compared to predicted change (0.3–0.5 pH units by 2100 [136]; but see [129,137,138]). Therefore, populations may possess greater adaptive variation for temperature tolerance, but have less adaptive variation for pH tolerance [117].

It is crucial to distinguish between microevolutionary (genetic) and phenotypic (plastic) responses at the population level. Many past studies have lacked this focus, but identifying the drivers responsible for changes in fitness traits should be given more attention in future studies (e.g. [19,139]). Non-genetic evidence can also be lacking, missing potential patterns, such as in situations of counter gradient variation whereby genetic and environmental influence can oppose each other [140]. This was the case for the genetic divergence of body shape between two populations of juvenile Atlantic cod (*Gadus morhua* L., 1758), in which phenotypic differences were mitigated by environmental influences [123]. Even the positive, negative or neutral correlation between two fitness traits may accelerate, slow down, or not impact adaptive evolution [17]. As such, local environmental variability must be considered when determining population responses.

4.3. Modes of Population-Level Response

Examination of time series data reveals evolutionary responses to climate change, such as direct allochronic studies which include a mixture of populations that are on their way to adaptation or extinction (reviewed in depth by [102]). These studies can show that the selection of genotypes is an immediate mechanism of population-level adaptation. Multi-generational analysis of selection of the coccolithophore (*Emiliania huxleyi* (Lohmann) Hay & Mohler, 1967) has provided evidence for evolutionary adaptation responses detected by selection of genotypes and direct positive adaptation to increased $p\mathrm{CO}_2$ by mutation [108]. However, it is important to emphasise that the rate of adaptation for single-celled organisms, due to their fast generation times, will likely differ along with the mechanisms utilised when compared to multi-cellular organisms. Future studies should be optimised by an interdisciplinary approach, including abiotic changes driven by climate change, biological networks, and the relationship between the phenotypic and genetic analysis, for a better understanding of future climate change impacts on the evolution of populations.

5. Community Composition and Interactions

While studies of evolution on single species and populations are already underway (either *in situ* or in the laboratory), the potential of communities and ecosystems to evolve as a unit in response to changing environments has not yet received as much attention. This is partially due to the complex nature of communities. Another important reason is that for several decades, ecological and evolutionary time scales were thought to diverge widely and this has led to very different thought models of evolution and ecology [141]. In particular, it was thought that evolution takes place in time frames that cannot influence ecology, while the effect of ecology on evolution has been studied in some prominent examples. For instance, in the Atlantic cod (*Gadus morhua* L., 1758) fishing pressure led to earlier age at maturation [142]. However, the dynamic effect of evolution on ecology is an emerging field of study since it was recognised that evolution of ecologically relevant traits can influence contemporary communities [143,144].

If community composition is altered, the coevolution between interacting species will be driven and/or modified by their interactions within the community [145,146]. This diffuse coevolution means that the selection of a specific trait in one species may depend on the presence of another species

[147], making species identity and uniqueness a plastic response in community-level responses [148]. Therefore, the effects of future climate change on communities will likely be complex [149], and influence the outcomes of competition, facilitation (e.g. [150]) and trophic interactions (e.g., predator-prey [151,152], and plant-herbivore [153,154]).

5.1. Changes to Community Dynamics

The fast population turnover of single-celled phytoplankton represents a great opportunity to study experimental evolution and to quantify evolutionary and plastic responses of populations to future climate change [155]. Phytoplankton communities represent a pivotal role in marine ecosystem functioning [155], forming the base of the marine food web and crucial for global biogeochemical cycles [156]. Under current conditions of dissolved inorganic carbon, many phytoplankton species are not fully saturated for growth and photosynthesis, and therefore, will benefit from the addition of CO_2 (e.g., [157–159]). However, any selection for fast growth, despite providing competitive ability through size (but see [160]), may come at the cost of reduced resilience to pCO$_2$ [161]. This was shown by a study ([161]) that used genetically distinct isolates of phytoplankton species (sixteen strains of the diatom *Skeletonema marinoi*, Sarno & Zingone 2005 and eight strains of dinoflagellate *Alexandrium ostenfeldii* (Paulsen) Balech & Tangen, 1985) and found that slow-growing cultures generally responded positively to elevated pCO$_2$, while fast-growing cultures either showed neutral or negative responses. Hence, the effects of climate change need to be considered holistically in terms of both ecological performance as well as physiological tolerance.

The enormous diversity of phytoplankton and the variety of environmental stressors makes it unthinkable to experimentally test all the possible trait responses in every phytoplankton group. The difficulty lies in establishing whether this evolutionary potential can be realised, and whether results from laboratory experiments can be related to natural populations (see [155]). Therefore, understanding the mechanistic effects of future climate change on key functional groups (e.g., [162]) will require a deeper understanding, across biological hierarchies, of the direct effects on their physiology (molecular and cellular), basic biology (whole-organism), as well as estimates of gene flow, population size, and recombination rates (population) [155].

In order to extrapolate from the organism and individual species' responses to the community level, we also need to understand the response of the ecological interactions within the community. For example, any increased biomass associated with higher atmospheric CO_2 may be indirectly mediated by the presence of grazers (indirect trophic interactions, e.g., [163]), or regulated by heterotrophs of the same community (e.g., [164]). Similarly, phytoplankton responses associated with climate change can lead to bottom-up control (e.g., [165]), or, due to sufficient food availability to marine organisms may provide physiological homeostasis (e.g., *Mytilus edulis* L.; 1758 [166]). As such, if the effects of climate change differ between similar co-existing species (e.g., [167]), it may indirectly influence selection by causing ecological release; reducing the need for competitive traits.

In addition to the direct effects, future climate change may have indirect effects on other communities. Where CO_2 is a resource for organisms, it can play an important role leading to changes in community competition (e.g., [168]). For example, opportunistic turf- and mat-forming algae have been demonstrated to inhibit other taxa (e.g., [169]) and outcompete kelp recruitment (e.g., [170]), inducing phase shifts. Species in diverse communities tend to have lower effective population sizes compared to when they are in isolation due to the competitive interactions [171]. This typically increases the role of genetic drift compared to selection, and might reduce the rates of adaptive evolution [172]. Climate change might reduce those inter-specific interactions (e.g., bottom-up control releasing resource limitation [165]) and thereby enhance the potential for adaptation, through reductions in genetic drift. Alternatively, climate change may increase competition (e.g., [173]) and amplify changes in mean population size, increasing extinction risks, as well as decreasing adaptation rates (Figure 1). This may be further exacerbated through co-extinctions, due to increased habitat and biodiversity loss, whereupon one species is dependent on another that is already extinct [174].

The presence of co-occurring species might enable adaptation by initiating coevolutionary interactions (e.g., [175]), however, it has also been suggested that increasing biodiversity may begin to inhibit that subsequent adaptation (e.g. [171]). This is due to an increased number of species in an assemblage, increasing the chance that a current species will possess traits that would predispose the species towards favourable selection under future environmental conditions, and could restrict the opportunity of other co-occurring species to adapt. Species-specific adaptation mechanisms could ultimately feedback to influence ecosystem functioning [143]. For example, three bacterial species that were raised together had higher productivity compared to the same species that adapted in isolation [176]. This was due to the inter-specific competition that caused them to select for specialisations in their resource use (niche partitioning [177]), leading to a complementary adaptation [176]. Hence understanding whether the evolutionary potential can be realised will require investigations that utilise realistically diverse assemblages (e.g., [169,173]). It does however, also raise the challenge of understanding whether future ecosystems will become sustained ecosystems (with fewer species that are selected for their favourable traits), or more evolved ecosystems as a whole. This is crucial given the extensive research regarding biodiversity and ecosystem multi-functionality in present day communities (for more details, see [178]).

5.2. Habitat Fragmentation and Biological Invasions

Anthropogenic climate change is expected to reorganise patterns of species diversity [179,180]. One possible approach for investigating the selection response using naturally assembled communities is through the use of natural analogues for future climate change, such as CO_2 vents [26], or coastal upwelling sites [114,181]. These areas provide long-term chronic exposure to novel environmental conditions, and allow experimental work to capture an organism's response in fitness-related traits [26, 182], such as reproductive success. Moreover, organism responses will include carry-over effects (between life-history stages and trans-generationally), as well as being influenced by other ecological interactions, such as competition and trophic interactions. Yet, (a caveat) for those species that are not direct-developing, these sites may be confounded by larvae received from outside of the site, with different environmental conditions, likely reducing selection pressure.

For long-lived sessile foundation species, such as reef-building corals, evidence suggests that acclimatisation and adaptation will be essential for population persistence in the face of climate change [25], given that any range shifts are likely to be slow [183]. A recent transplant experiment utilising the table top coral (*Acropora hyacinthus* Dana, 1846) found that acclimatisation and adaptive responses (mirrored in the patterns of gene expression) allowed this faster-growing coral to inhabit areas of the reef that far exceeded their expected temperature tolerances [25]. This tolerance to elevated temperature might be associated with either the coral host (e.g., [48,184]), or their associated *Symbiodinium* (e.g., [185]). In contrast, experimental work investigating the coral reefs at the shallow volcanic CO_2 seeps (in Papua New Guinea) found an overall reduction in diversity and recruitment in the coral communities pre-acclimated to high $p\mathrm{CO}_2$, thought to be associated with shifts in competitive interactions [173]. This highlights that the adaptive evolution of coral reef communities is possible and driven by abiotic factors (Court Jester hypothesis, [186]), however, community-level interactions (such as the increased competition in high $p\mathrm{CO}_2$) may equal or exceed these fitness-related responses (*i.e.*, survival), and lead to adaptive evolution being driven by biotic factors (Red Queen hypothesis, [186]) Clearly, the relative roles of biotic and abiotic factors will be stressor-specific reaffirming the need to investigate the adaptive evolution responses with multiple stressors in realistic communities.

Biological invasions are important drivers of change in marine communities, particularly coastal communities (e.g., [187,188]). Increases in temperature may facilitate species' range shifts, thereby aiding invasion [188]. One particular example of this is the 'tropicalisation' of the Mediterranean Sea, where, invasions and establishments have been made possible due to increasing annual mean temperatures all year around [189]. The integration of novel species may influence evolutionary processes by altering existing interactions (e.g., [190]) or population growth rates (see [191]). Alternatively, both the native and non-native species may be able to achieve coevolution if their co-existence can maximise their habitat use [192]. Although native species might be able to overcome the invasion of some non-natives, some may become less adapted to the new conditions and be out competed by invasive organisms, which exhibit greater adaptability or the ability to demonstrate strong fitness effects [188].

6. Future Directions

It is inevitable that increasing ocean acidification will be accompanied by changes in other abiotic factors, and therefore interactions with other stressors (*i.e.*, temperature, nutrients, hypoxia or salinity) are extremely likely [193]. For both single and multiple stressors, there is a crucial need to incorporate the potential for adaptation to future climate change, to reliably determine the sensitivity and mechanisms for adaptation of marine organisms.

Adaptation capacity will be driven through a number of mechanisms with different taxonomic and functional groups utilising a variety of processes. Species with large population sizes and fast population turnover rates, such as phytoplankton, are likely to demonstrate the potential to achieve the adaptation rates required for future climate change (e.g., [108]), making them a model species for laboratory experimental evolution. However, these experiments will likely be carried out in the absence of more complex trophic and ecological interactions. In order to clarify the effects of anthropogenic climate change on community- and ecosystem-levels, future research should be directed towards identifying key species, and establishing their interactions with coexisting species, particularly if those ecological interactions vary with season or ontogeny [194,195]. The choice of species could be associated with the needs for either ecosystem's services or functioning, such as the disproportionate role that coccolithophores play in the carbon-cycle, or societal needs, such as for food security, or possibly in an ecological context, being habitat forming or a keystone species.

Given the differential sensitivities and responses of different life-stages, future research needs to identify which life-stages are most affected by climate change and the key interactions (among species and to different climatic scenarios) within ecosystems [196]. The negative results from short-term studies on early life-stages often make it difficult to extrapolate to longer-term impacts [197–199], especially when multiple stressors interact, since the sensitivity of early life-stages may not be representative when responses are considered across all ontogeny and life-stages. The exposure of previous generations to environmental conditions will influence the response of subsequent generations (*i.e.*, carry-over effects). As such, the use of chronic long term multigenerational experiments should contribute to our understanding of both developmental and trans-generational plasticity [108,155,198]. An additional important consideration is the current local-scale variability of environmental conditions. If the adverse conditions that we expect by the end of the century are already being experienced by marine organisms, and are within the range of the current environmental variability (e.g., CO_2-enriched upwelling, Kiel Fjord, western Baltic Sea [138]), then these transient extreme conditions may result in a pre-selection for tolerance (e.g., [131]). This pre-selection might be achieved through the divergent selection of specific genes in candidate loci (e.g., [114]), and contribute to the maintenance of positive life-history traits.

Phenotypic plasticity may provide the potential for species to achieve sufficient tolerance in the short-term, such that they may actually be able to achieve adaptation to environmental change. To attain a mechanistic understanding of this process will require an interdisciplinary approach, including investigations at different levels of biological hierarchy. This is because the capacity of a species' phenotypic plasticity might be set at the cellular level, for example through changes in oxygen demand via mitochondrial activity. However, it is important to consider that these responses might be first observed through changes in abundance (or distribution), using more phenomenological approaches at the population level. Alternatively, the persistence of a species could be attributed to its dispersal ability and the availability of suitable habitat and hence potential spatial scale of gene flow. As such, research needs to be carried out at biologically-relevant scales. Therefore, a crucial first step in understanding responses at the population level will require linking the intra-individual physiology (e.g., transcriptional and cellular responses) to the fitness-related traits of the whole-organism, in order to more reliably estimate the effects of climate change on contemporary population demographics into the future.

7. Conclusions

Biotic factors such as competition and trophic interactions shape marine communities at local spatial scales and over relatively short timescales. Other extrinsic factors, such as oceanic and atmospheric environmental conditions will influence patterns of biodiversity over longer timescales, and at regional or global scales [186]. Since climate change is occurring rapidly, it is likely that biotic interactions may play a more important role, compared to abiotic factors, when it comes to evolution (*i.e.*, the Red Queen hypothesis [200]). As such, establishing the association between local environmental conditions and the genomic-physiological features of key species, that are known to be influential in communities (including their interactions with co-existing species), should elucidate how community processes will be affected, and whether evolutionary potential can be realised. However, investigating broader spatial scales will require determining the link between the genomic-physiological responses of contemporary populations and population dynamics. This could establish a deeper understanding between the physiological stress responses of marine organisms to both biotic and abiotic factors, and critical (yet often unknown) demographic processes such as effective population size.

Both adaptation and acclimatisation may enable organisms to persist in future oceans, and understanding how factors at different levels of biological hierarchy will influence these important evolutionary responses to climate change is crucial. Future research needs to investigate biological responses both spatially and temporally, by utilising spatially representative replication across different scientific disciplines and research institutes, in an effort to integrate responses and adaptive mechanisms at regional or global scales. This will help to achieve direct comparisons and a more integrative picture of the responses at the community and ecosystem levels. Only then can we establish whether the future organisational structure of marine ecosystems will resemble the communities of today, and what role acclimatisation and adaptation will play in the persistence of marine organisms.

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Author Contributions

Original concept, drafting and editing manuscript: Ben Harvey. Group leaders: molecular and cellular responses – Amit Kumar; whole-organism - Rebekah Cioffi; population-level responses – Balsam Al-Janabi; community composition and interactions – Stefanie Broszeit. Figure 1 – Ben Harvey. All other authors contributed with concept development, writing and commented on the manuscript at all stages.

Conflicts of Interest

The authors declare no conflict of interest.

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Paper II

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Genotypic variation influences tolerance to warming and acidification of early life-stage *Fucus vesiculosus* L. (Phaeophyceae) in a seasonally fluctuating environment

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Keywords: bladder wrack *Fucus vesiculosus*, fucoid germlings, reproduction, global warming, ocean acidification, german Baltic Sea, genetic diversity, chlorophyll fluorescence

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ORIGINAL PAPER

Genotypic variation influences tolerance to warming and acidification of early life-stage *Fucus vesiculosus* L. (Phaeophyceae) in a seasonally fluctuating environment

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Abstract Global change exposes brown algal *Fucus vesiculosus* populations to increasing temperature and pCO_2, which may threaten individuals, in particular the early life-stages. Genetic diversity of *F. vesiculosus* populations is low in the Baltic compared to Atlantic populations. This might jeopardise their potential for adaptation to environmental changes. Here, we report on the responses of early life-stage *F. vesiculosus* to warming and acidification in a near-natural scenario maintaining natural and seasonal variation (spring 2013–2014) of the Kiel Fjord in the Baltic Sea, Germany (54°27′N, 10°11′W). We assessed how stress sensitivity differed among sibling groups and how genetic diversity of germling populations affected their stress tolerance. Warming increased growth rates of *Fucus* germlings in spring and in early summer, but led to higher photoinhibition in spring and decreased their survival in late summer. Acidification increased germlings' growth in summer but otherwise showed much weaker effects than warming. During the colder seasons (autumn and winter), growth was slow while survival was high compared to spring and summer, all at ambient temperatures. A pronounced variation in stress response among genetically different sibling groups (full-sib families) suggests a genotypic basis for this variation and thus a potential for adaptation for *F. vesiculosus* populations to future conditions. Corroborating this, survival in response to warming in populations with higher diversity was better than the mean survival of single sibling groups. We conclude that impacts on early life-stages depend on the combination of stressors and season and that genetic variation is crucial for the tolerance to global change stress.

Introduction

Anthropogenic global change exposes marine populations, inter alia, to increases in temperatures and pCO_2 concentrations (IPCC 2013). The magnitude of these changes varies among geographic regions; in the Baltic Sea, warming and acidification are expected to increase up to 3–6 °C and to 1000 µatm, respectively, by 2100 (Graham et al. 2008; Elken et al. 2015). In many regions of the Baltic Sea, *F. vesiculosus* represents the dominant perennial large brown alga (Wahl et al. 2015a). On Baltic intertidal and shallow subtidal shores, its ecological role is important for both the biotic and the abiotic environment. As a foundation species, *F. vesiculosus* provides a three-dimensional habitat for a large number of epibionts and other associated organisms. It plays a key role in community structuring and provides shelter and food to the invertebrate community (Lotze et al. 2001; Wikström and Kautsky 2007). Rockweeds also provide various important ecosystem services including exceptionally high carbon retention (Schmidt et al. 2011) and buffering of environmental changes: the fixation of

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carbon and uptake of nitrogen provides the associated community, at least temporally, with favourable conditions regarding nutrients, oxygen and pH (Bertness and Leonard 1997; Wahl et al. 2015c). Therefore, intertidal and subtidal *F. vesiculosus* may, to some extent, buffer physical stress for many other community members, such as primary consumers (herbivores), secondary consumers (carnivores) and other associated organisms (epiphytes).

In the twentieth century, a drastic decline has led to great losses of *F. vesiculosus* biomass, as, e.g., by almost 95 % in Kiel Bay, Western Baltic Sea (Vogt and Schramm 1991), and surveys covering large parts of the Baltic Sea report marked disappearance of *F. vesiculosus* from deeper zones (Eriksson et al. 1998; Torn et al. 2006). Abiotic factors such as eutrophication and decreased irradiation have been proposed to be the most important drivers for this decline (Berger et al. 2004). However, declines of the Baltic bladderwrack are rather due to multiple factors (Wahl et al. 2011), where indirect effects may outweigh direct effects (Wahl et al. 2015a). For instance, heavier nutrient loads lead to higher phytoplankton densities and enhanced growth of epiphytes with the former reducing light penetration and the latter shading the macroalgae thallus (Rohde et al. 2008).

The increase in water temperature and pCO_2 concentrations may further impact *F. vesiculosus* populations by direct and indirect effects and possibly cause distributional shifts or range contractions. It was demonstrated that high-pCO_2 conditions increased photosynthetic rates of *F. vesiculosus* and enhanced growth in *F. vesiculosus*, *F. serratus* and other non-calcifying macroalgae (Gordillo et al. 2001; Nygård and Dring 2008; Olischläger et al. 2012, 2013). The assumed explanation for a positive CO_2 effect is the down-regulation of energy-consuming carbon-concentrating mechanisms (CCMs) under these conditions (Beardall and Giordano 2002; Wu et al. 2008). In contrast, warming causes negative effects on the physiological performance of *F. vesiculosus* populations (Graiff et al. 2015), particularly in the warm season. Thus, a poleward shift of seaweed populations has been observed since 1940 until present as a response to increased warming (Wernberg et al. 2011) and is predicted to continue for *F. vesiculosus* populations in the twenty-first and twenty-second century (Jueterbock et al. 2013). As a consequence of warming, further range retractions and local extinctions of *F. vesiculosus* populations may occur (Nicastro et al. 2013). To date, the combined effects of CO_2 and temperature increase on *F. vesiculosus* performance have not been investigated yet.

Early life-stages of seaweeds may be particularly sensitive towards global change stress and environmental fluctuations (Coelho et al. 2000). Post-settlement mortality of young *F. vesiculosus* is generally high in the field, but varies among seasons and years (Lamote and Johnson 2008). Similarly, elevated temperature impacts growth rates, survival, photosynthetic efficiency and sensitivity to copper more strongly in *F. serratus* germlings than in adults (Nielsen et al. 2014). A decreased germination success of Baltic *F. vesiculosus* at 25 °C compared to 15 °C was observed by Maczassek (2014). Since this early ontogenetic stage may constitute the bottleneck of macrophyte survival, it is crucial to assess how a combination of expected future temperature and pCO_2 will influence the performance of *F. vesiculosus* germlings. It is also unknown to which extent variations in responses to these two factors have a genetic basis in *F. vesiculosus*. Since selection is only effective if variation on traits responsive to global change factors exists, this knowledge is important to assess the adaptive potential of a species (Caruso et al. 2005).

Population resistance to global change stress is mediated by a high genetic diversity which guarantees a broad adaptive potential (Frankham et al. 2009). The loss of genetic diversity restricts the potential for adaptation (Frankham 2003, 2010; Hoffmann and Sgro 2011; Pauls et al. 2013). Genetic diversity of the eelgrass *Zostera marina* enhanced ecosystem resilience under a summer heat wave (Reusch et al. 2005; Ehlers et al. 2008) as well as community resistance to grazing by geese (Hughes and Stachowicz 2004). Also at early life-stages, genetic diversity improved recruitment of the barnacle *Balanus improvisus* (Gamfeldt et al. 2005) and enhanced the early life-stage performance in the solitary ascidian *Ciona intestinalis* (Aguirre and Marshall 2012).

Adaptive responses to climate change occur more on the population than on the species level (Harvey et al. 2014) and may be particularly critical in the Baltic Sea, where species and genetic richness is lower compared to other ecosystems (Pereyra et al. 2009). The Baltic Sea, a semi-enclosed water basin formed 8–10 thousand years ago, constitutes a geographically and ecologically marginal ecosystem with particular environmental conditions due to a gradient of decreasing salinity towards from the North Sea (32 psu) towards the north-eastern part (Bothnian Bay <4psu) (Bonsdorff 2006). This steep salinity gradient and restricted water exchange at the entrance of the Baltic Sea contribute to the lower genetic diversity of Baltic *F. vesiculosus* populations *versus* North Sea populations and to high genetic distances between them (Johannesson and André 2006; Tatarenkov et al. 2007). These remarkable differences may have additionally derived from low population connectivity due to limited egg dispersal in *F. vesiculosus* (Serrão et al. 1996). Despite its ecological importance, our understanding about adaptive processes in *F. vesiculosus* under global change conditions are still scarce and require further investigations (Johannesson et al. 2011).

In the present study, the single and combined effects of warming and acidification on *F. vesiculosus* germlings were assessed in all four seasons in genetically different sibling groups (families) and in populations of differing genetic diversity. Experimental populations of *F. vesiculosus* in different artificially generated diversity levels (low, medium and high) were treated with current and future temperature and pCO_2 conditions to explore whether diversity effects exist. We hypothesised that (1) the responses of *F. vesiculosus* to single and combined effects to temperature and pCO_2 vary with season, (2) genetically dissimilar germling groups (full-sib families of germlings) differ among each other in their response to single and combined effects of temperature and pCO_2 and (3) genetically more diverse experimental groups of *F. vesiculosus* are more resilient to stress than genetically less diverse groups.

Materials and methods

Experimental approach

To test the existence of sensitivity differences among genetically dissimilar sibling groups (full-sib families of germlings), sibling groups were produced following a controlled protocol. Since all germlings within one sibling group had the same mother and father, they were all 'sisters and brothers', i.e. full-sibs. Other sibling groups had other parents. We thus assume that genetic variability within families was substantially less than that among families. These eight genetically different families were placed in each of 12 mesocosms (termed Kiel Outdoor Benthocosms below). This 'common garden'-setting reducing between-family environmental differences to a minimum allows us to assume that between-family differences in responses has a strong genetic basis. To test hypothesis (2), we used sibling group as fixed factor.

To test hypothesis (3), the differences in responses between diversity levels, we produced three artificial diversity levels: one-family experimental populations, where all germlings came from the same parental pair (low diversity level); two-family mixtures, where equal numbers of germlings were taken from two families (medium diversity level) and four-family mixtures, where equal numbers of germlings were taken from four families (high diversity level). Hence, in the two- and four-family mixtures, germlings from any one family could account for no more than 50 and 25 % of the experimental population, respectively.

Sampling, gamete acquisition, rearing of germlings and experimental design

We experimentally studied germlings' performance in all four seasons. In the German Baltic Sea, individuals of *F. vesiculosus* reproduce in summer or autumn or even in both seasons (Maczassek 2014). *F. vesiculosus* germlings were allowed to settle on sandstone cubes with 2 cm edge length, where each sandstone represents an experimental population. To cover all four seasons of the year, two cohorts of germlings were used to establish the experimental populations. 'Cohort 1' originated from autumn 2012 spawning *F. vesiculosus* and was used in the experiments from January 2013 to September 2013, and 'cohort 2' originated from summer 2013 spawning *F. vesiculosus* and was exposed to the experiments from September 2013 to March 2014.

For producing cohort 1, a total of 53 fertile specimens (33 females, 20 males) of *F. vesiculosus* were collected in a wave-exposed area with mainly hard substrate located in the south-western Baltic Sea (Bülk, Germany, 54°27.327′N, 10°11.977′W) during the end of November 2012. Individuals collected were distanced above two metres which is the estimated maximum dispersal distance of most *F. vesiculosus* eggs (Serrão et al. 1996), thereby reducing the probability of sampling of siblings and enhancing genetic variability. Algae were immediately transported to the laboratory after collection in cooler boxes. Fertile receptacles were cut from male and female algae, rinsed with tap water, blotted dry and stored in the dark at 8–10 °C for 5 days. To generate full-sib germling groups, all receptacles of one female and all receptacles of one male were each combined in plastic dishes by random pairing. Gamete release (and the following fertilisation) was induced by the immersion of the receptacles in sand-filtered seawater (15–16 psu) and exposing them to light irradiated from an aquarium lamp (110 μmol photons m^{-2} s^{-1}) for 3 h. The term 'sibling group' is used hereafter to represent an experimental population, where all germlings were full-sibs settled on a sandstone surface. For cohort 1, eight sibling groups were generated, which were genetically different among each other, since they stemmed from different parents. Germlings were settled as follows: a 0.67-ml solution of fertilised eggs from one parental pair was pipetted homogeneously onto a 2 × 2 cm sandstone surface. Hence, single sandstones bore descendants from one single parental pair. From cohort 1, eight different sibling groups were established and replicated three times (methodological replicates of exactly the same offspring identities in each of the separate benthocosm tanks). Germlings were cultured and their presence on each sandstone was monitored weekly for 8 weeks in a room with windows allowing for natural light conditions at 8 °C with weekly water exchange (15–16 psu) until transfer to the Kiel Outdoor Benthocosms (see 'Experimental conditions: Kiel Benthocosms' section).

Cohort 2 was produced with the same method regarding sampling and gamete acquisition, but here a total of 66 fertile specimens (36 females, 30 males) were collected in June 2013. Receptacles were stored in the dark at 15 °C for 8 days prior to introducing gamete release and fertilisation.

For cohort 2, different levels of genetic diversity were generated. The term 'diversity level' is used hereafter to denote different compositions of experimental germling populations settled on the sandstone surface: the low diversity level, consisting of single sibling groups, was generated by pipetting a 0.75-ml solution of fertilised eggs from one parental pair each onto each sandstone surface. To generate the medium diversity level, a 0.375-ml solution of fertilised eggs from each of two parental pairs (pairs) was combined onto each sandstone surface. To generate the high diversity level, a 0.1875-ml solution of fertilised eggs from each of four parental pairs was combined onto each sandstone surface (quartets). In total, a 0.75-ml solution of fertilised eggs was pipetted homogeneously onto every sandstone cube. Germlings were cultured and monitored over the following 9 weeks at 15 °C until transfer to the Kiel Benthocosms. Eight low diversity level experimental populations, four medium diversity level populations and two high diversity level populations were generated, with each population being produced by different parents: i.e. parent identities were replicated. This whole set-up was replicated (methodological replicates of exactly the same offspring identities in each of the separate benthocosm tanks). For an illustration of the experimental design, see Online Resource 1. Germlings were cultured over the following 9 weeks at 15 °C until transfer to the Kiel Benthocosms.

Experimental conditions: Kiel Outdoor Benthocosms

The experiments were performed at the near-natural scenario in the Kiel Outdoor Benthocosms (KOB) under natural ambient and manipulated temperature and CO_2 conditions at 2 levels ('present' and '2110'). 'Present' conditions were the actual environmental conditions in Kiel Fjord transferred to the experimental tanks via continuous flow-through (1 tank-volume per day, i.e. 1500 l/24 h). '2110' conditions were simulated by adding 5 °C to the actual fjord temperature, as it has been predicted for the next 100 years (Elken et al. 2015). pCO_2 was increased in the hooded headspace above the tanks to 1100 µatm, according to the predictions by Schneider et al. (2015). These two delta-treatments increased the mean of the respective factors while preserving the frequency and amplitude of natural fluctuations. During all seasons, abiotic factors were logged continuously, nutrients and total alkalinity (TA) were measured twice a week and dissolved inorganic carbon (DIC) was measured monthly. The technical set-up and all measured parameters are described in detail by Wahl et al. (2015b). In addition, temperature was measured daily with a calibrated sensor (pH, Mettler Toledo GmbH, Giessen, Germany) (Online Resource 2) and the seasonal variation in solar radiation was measured in the Kiel Fjord by Rickert et al. (2015) (Online Resource 3). Light intensities (µmol photons m^{-2} s^{-1}) of the Kiel Fjord were reduced by ca. 20 % in the Kiel Benthocosm tanks, as shown by Wahl et al. (2015b). Across the 12 experimental units of KOB, the two factors warming and acidification were orthogonally crossed, creating the four treatment levels: $T+CO_2+$ (warmer and acidified), $T+CO_2-$ (warmer, non-acidified), $T-CO_2+$ (ambient temperature, acidified) and $T-CO_2-$ (ambient temperature, non-acidified) in three replicates. 'Non-acidified' refers to the ambient CO_2 conditions of Kiel Fjord with naturally fluctuating, sometimes acidified conditions, but without receiving an additional pCO_2 treatment in the KOB. All experimental populations (sibling groups of cohort 1 and 2 and diversity level of cohort 2) were introduced to each of the experimental units of KOB and were hence exposed to each of the four treatment levels with three replicates per treatment. Within the Bioacid II project and the 'Benthic Consortium', experiments of different disciplines were performed (www.bioacid.de). In each tank, a benthic community was analysed in the KOB consisting of macrophytes (adult *F. vesiculosus*) and their associated epibiotic communities as fauna, flora and consumers. These experiments were subdivided into four consecutive experiments (E1, E2, E3 and E4) each run for 11–12 weeks, covering all four seasons of the year: E1: spring (01.04.2013–21.06.2013), E2: summer (01.07.2013–20.09.2013), E3: autumn (07.10.2013–20.12.2013) and E4: winter (13.01.2014–01.04.2014).

Prior to the first experiment with cohort 1, germlings were acclimatised to the treatment conditions for 9 weeks. Temperature was increased gradually over 2 days until reaching the +5 °C treatment. When tanks were serviced in between experiments, germlings were stored in indoor mesocosms at same temperature and pCO_2 conditions as in the benthocosms for 1–3 weeks. Germlings were kept in four indoor mesocosms, one for each treatment. The temperature treatment was established by using internal heater elements (600 W, Schego Titan, Schemel & Goetz, Offenbach am Main, Germany), and values were set according to the actual fjord temperatures for the ambient treatment and +5 °C for the warmed treatment. Acidification was achieved by aeration with CO_2-enriched air (1000 µatm CO_2) directly into the water of the mesocosms. pH and temperature of the mesocosms were monitored daily. Between the core experiments E2 and E3 (cohort 2), technical problems in one of the indoor mesocosms damaged germlings of the treatment $T-CO_2+$ and led to the deletion of this treatment combination. To compensate this loss, a new experiment was initiated. Twenty-one sibling groups previously treated at ambient conditions as supplementary material (with three replicates) were distributed randomly (illustrated by a non-significant ANOSIM among the created communities, *p* value >0.05) to the four treatment levels described above resulting in five sibling groups in each benthocosm tank. The new experiment covered all four treatment levels and was used for survival and growth analysis for the following seasons. The previous experiment, consisting of the three treatment levels (T+CO_2+, T+CO_2− and T−CO_2−), was used for diversity level and sibling group difference analysis.

From summer until the end of the experiments, sibling groups had to be protected from germlings originating from fertile adult *F. vesiculosus* kept in the same tanks. Therefore, germlings were kept in PVC boxes (70 cm × 40 cm × 12 cm). These boxes were enclosed to the water content of the tank but uncovered towards the upper part and positioned above the water surface of the benthocosm tank while separating the water contents from each other. The thin PVC walls (6 mm) allowed for temperature exchange and the open upper part allowed for gas exchange. The box water was exchanged once a week by filtered water (50 µm) of the benthocosms. The filtration prevented the accidental introduction of new *F. vesiculosus* eggs (100 µm diameter), but exchanged water with the temperature and gas conditions of the benthocosm tanks. The light intensity (µmol photons m^{-2} s^{-1}) in the box was 5 % lower than in the main tank.

Survival

Germlings' survival was determined as the % of surviving germlings between the beginning (t_0) and the end (t) of each time period. All germlings on a 1-cm^2 area were counted under a binocular at 25× magnification. When a given cohort was used in two successive time periods, the number of survivors (t) of the previous experiment was taken as the start number for the following season. Survival was calculated for all time periods of the year. Summer was separated into the two time periods early summer and late summer; thus, temperatures of the warming treatment reached values above the tolerance range (>27 °C) of *F. vesiculosus* (Graiff et al. 2015). Survival % for spring, early summer, late summer, autumn, winter 2013 and for spring 2014 were calculated as:

$$\text{Survival } \% = \frac{\text{Number } t}{\text{Number } t_0} \cdot 100$$

Growth

Digital images were recorded of 10–15 individual germlings per sibling group at 40× magnification (SteREO Discovery. V8—Carl Zeiss Jena GmbH), according to Steen and Scrosati (2004). The projected side-view area of individual germlings was measured using the image analysis software Image J 1.45s (National Institutes of Health, USA). For each of the four treatments, eight sibling groups were measured for cohort 1 and five sibling groups for cohort 2 at the beginning and end of a time period of 6–9 weeks. These time periods were chosen for achieving uniform analysis over the year. In summer, growth was measured separately in early summer and late summer. Hence, time periods for growth measurements were in spring, early summer, late summer, autumn, winter 2013 and for spring 2014.

Relative growth rate (RGR) in % d^{-1} was calculated according to the exponential growth rate as

$$\text{RGR} = \left[(\text{Area } t/\text{Area } t_0)^{1/\Delta t} - 1\right] \cdot 100$$

where Area t_0 is the mean area of eight sibling groups (five sibling groups for cohort 2) at day 0, Area t is the mean area at day t and Δt the number of days between t and t_0. From each experimental population, the mean of 15 individuals in Area t and Area t_0 was calculated. Since individuals were too small for labelling, the 15 individuals were chosen randomly for each measurement. In successive experiments, Area t, the area measured at the end of the previous experiment, was set as the initial size Area t_0 of the subsequent experiment. Negative growth rates were given when bigger individuals within a sibling group died while smaller ones remained.

Chlorophyll a fluorescence parameters

In order to determine the photosynthetic parameters of *F. vesiculosus* germlings, the in vivo chlorophyll *a* fluorescence of photosystem II (PSII) was measured with a portable pulse-amplitude-modulated fluorometer (Pocket PAM, Gademann Instruments GmbH, Würzburg, Germany) at the end of the experiments (March 2014). Before each measurement, the germlings were carefully cleaned of epiphytes with a brush and seawater. The distance between the fibre optic (diameter 1.5 mm) and the germling was always kept constant at 1 mm with the help of a binocular and a micromanipulator. The determination of the potential maximum quantum yield (F_v/F_m) was performed according to Hanelt (1998). After 5 min of dark adaptation and a 5 s of far red light, the minimal fluorescence F_0 was recorded with a pulsed measuring light (650 nm, 0.3 µmol photons m^{-2} s^{-1}), followed by short pulses of completely saturating white light pulse (0.4–0.8 s, 1000–5000 µmol photons m^{-2} s^{-1}) to record F_m ($F_v = F_m - F_0$). The relative PSII electron transport rate (rETR) of each germling was quantified by incubations for 5 min in darkness and followed by exposures to nine increasing photon flux densities (PFD) of actinic red light (0, 25, 45, 66, 90, 125, 190, 285 and 420 µmol photons m^{-2} s^{-1}, LED 650 nm), each for 1 min. The photosynthesis vs. irradiance curves (PI curves) with rETR as a function of PFD were fitted after Walsby (1997). From each single curve, the maximum relative electron

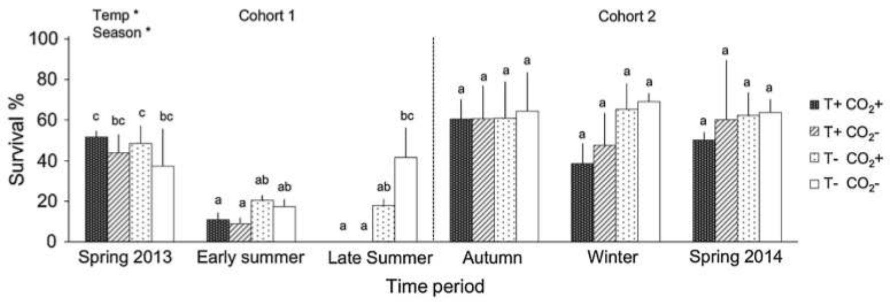

Fig. 1 Germlings' survival (%, mean +SD, $n = 3$, in 7–8 weeks) at the four treatment combinations: T+CO_2+, T+CO_2−, T−CO_2+ and T−CO_2− over a period of the year 2013 and spring 2014. Means were calculated across eight sibling groups (cohort 1) and across five sibling groups (cohort 2) within one benthocosm tank. Significant effects are indicated here (*asterisk*) after the repeated-measures ANOVA for cohort 1 (from spring 2013 through early summer and late summer) and cohort 2 (from autumn 2013 through winter and spring 2014). In cohort 2, the temperature effect was insignificant ($p = 0.0556$). Different letters above the bars indicate significant differences (p value <0.05) between the treatments after Tukey's test

transport rate (rETR$_{max}$) and the light saturation coefficient of the curves (I_k) were calculated. In addition, the non-photochemical quenching (NPQ) for each PFD was recorded and calculated (Govindjee 1995). For a quantitative description of NPQ as a function of PFD, NPQ versus PFD curves were fitted after Serôdio and Lavaud (2011). Depending on this model, NPQ$_{max}$ as the maximum NPQ value of the curve was calculated.

Variance analysis

The variance between groups of each diversity level was analysed to explore variations between groups of the low diversity level relative to the variance between experimental populations of the high diversity level. For the low diversity level, the variances between sibling groups were calculated; for the medium diversity level, the variance between pairs and for the high diversity level, variances between quartets were calculated. The cohort 2 was chosen for the variance analysis in order to compare three diversity levels. Variances were calculated as: Variance = $\frac{\sum(x-\bar{x})^2}{n}$

Statistical analysis

Growth rates (% d^{-1}) and survival (%) values were analysed in a repeated-measures ANOVA for each cohort, where temperature, CO_2 and time period were fixed factors and sibling group a random factor. Survival % values were arcsin-transformed. The factor time period defined the repeated measures. Due to enhanced mortality in the high-temperature treatments during late summer, for this period only the factor CO_2 was analysed in a mixed-model ANOVA with the fixed factor CO_2 and the random factor sibling group. Diversity level differences were analysed for cohort 2 in a mixed-model ANOVA with regard to the response variables growth rate (% d^{-1}) and survival (%): the fixed factors were treatment (three treatment levels: T+CO_2+, T+CO_2− and T−CO_2−) and diversity level (three treatment levels: low, medium and high), while sibling groups were treated as a random factor. Differences between sibling groups were tested with a three-way ANOVA with the factors temperature, CO_2 and sibling group for each time period with regard to survival (arcsin-transformed) and relative growth rate.

For the chlorophyll *a* fluorescence parameter, a mixed-model ANOVA was used to test the two fixed factors temperature and CO_2 (two levels: high and low) with the random factor sibling group. Data were tested for normality and for homogeneity of variances. The ANOSIM analysis was performed with Primer 6 (Clarke 1993), and all other analysis, including model assumptions for ANOVA, were performed using R (R Development Core Team 2014).

Results

Survival

Germlings' survival over the year ranged between 0 ± 0 % in late summer and 69.09 ± 4.18 %, $n = 3$ in winter (Fig. 1) and differed significantly among the time periods until late summer [time period, $F(2,253) = 41.08$, $p < 0.0001$; Table 1]. The high-temperature treatment reduced survival significantly to ca. 50 and 0 % in summer and late summer, respectively [temperature, $F(1,253) = 14.45$, $p < 0.001$; Table 1]. While increased temperature had no effect on survival in spring and autumn, survival decreased in winter from 69.09 ± 4.18 to 47.37 ± 16.07 %, $n = 3$ under non-acidified conditions and from 65.22 ± 12.89 to 38.65 ± 9.99 % under acidified conditions. Although

Table 1 Results of the repeated-measures ANOVA testing the effect of the fixed factors temperature, CO_2 and time period on survival (%) including the random factor sibling group for (a) cohort 1 (spring, early summer and late summer) and (b) cohort 2 (autumn, winter and spring 2014)

Source of variation	numDF	denDF	*F* value	*p* value
(a) Cohort 1				
Temperature	1	253	14.451	0.001
CO_2	1	253	0.001	0.981
Time period	2	253	41.075	<0.001
Temp × CO_2	1	253	1.209	0.273
Temp × time period	2	253	7.643	<0.001
CO_2 × time period	2	253	3.073	0.048
Temp × CO_2 × time period	2	253	1.410	0.246
(b) Cohort 2				
Temperature	1	148	3.721	0.056
CO_2	1	148	0.587	0.445
Time period	2	148	0.801	0.451
Temp × CO_2	1	148	0.239	0.625
Temp × time period	2	148	1.046	0.354
CO_2 × time period	2	148	0.726	0.486
Temp × CO_2 × time period	2	148	0.242	0.785

the effect was not significant, due to the low *p* value we consider it likely that a temperature effect existed and would be significant at higher replication [temperature, $F(1,148) = 3.72$, $p = 0.056$; Table 1]. Acidified conditions tended to increase survival in spring 2013 from 37.33 ± 18.37 to 48 ± 8.88 % at ambient and 43.71 ± 9.1 to 51.56 ± 3.09 % at high-temperature conditions, decreased survival in late summer from 41.56 ± 14.67 to 17.82 ± 3.33 % at ambient temperatures although not significantly [CO_2, $F(1,253) = 0.0006$, $p = 0.981$; Table 1], whereas there was no CO_2 effect on survival during the other time periods. The importance of time periods is reflected in the significant interaction between CO_2 and time period [CO_2 × season, $F(2,253) = 3.07$, $p = 0.048$; Table 1].

Growth

Germlings' relative growth rate ranged from −0.14 ± 0.19 % d^{-1} in winter to 4.95 ± 0.43 % d^{-1}, $n = 3$, in late summer under ambient temperature and acidified conditions (Fig. 2). In summer, the high-temperature treatment increased growth significantly from 0.52 ± 0.46 to 1.67 ± 0.33 % d^{-1} under acidified and from 0.04 ± 0.93 to 2.63 ± 1.45 % d^{-1} under non-acidified conditions [temperature, $F(1,134) = 8.19$, $p = 0.005$; Table 2]. High temperatures affected growth stronger in summer than in spring, as underscored by the significant interaction between temperature and time period [temperature × time period, $F(1,134) = 4.79$, $p = 0.03$; Table 2]. Acidification had a tendency to increase growth during late summer from 3.45 ± 1.03 to 4.95 ± 0.43 % d^{-1} at ambient temperatures, though not significantly [CO_2, $F(1,7) = 0.925$, $p = 0.3682$; Table 2]. In cohort 1, growth varied under ambient conditions between 3.33 ± 0.45 % d^{-1} in spring, 0.04 ± 0.93 % d^{-1} in summer and 3.45 ± 1.03 % d^{-1} in late summer [time period, $F(1,134) = 22.82$, $p < 0.001$; Table 2]. Variations among time periods at ambient conditions were also significant in the cohort 2, where growth was 2.91 ± 0.94 % d^{-1} in autumn but decreased considerably in winter with 0.23 ± 0.28 % d^{-1} and in spring 2014 with 0.63 ± 0.18 % d^{-1} under ambient conditions [time period, $F(2,105) = 67$, $p < 0.001$; Table 2].

Growth rates in all treatment levels from November 2013 until March 2014 did not exceed 0.63 ± 0.18 % d^{-1}

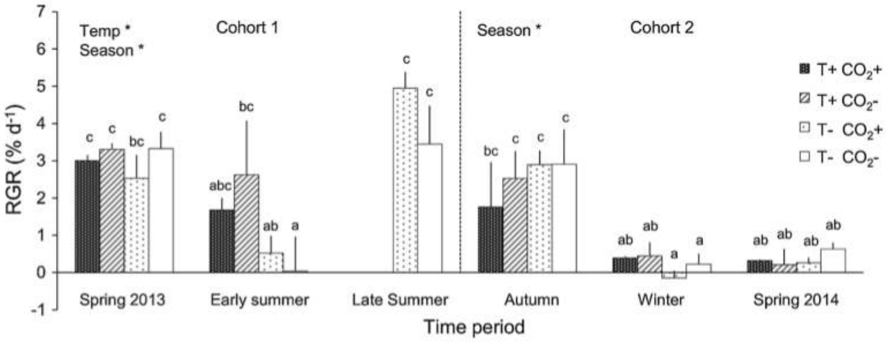

Fig. 2 Germlings' relative growth rate (% d^{-1}, mean + SD, $n = 3$, in 6–8 weeks) at the four treatments combinations: T+CO_2+, T+CO_2−, T−CO_2+ and T−CO_2− over a period of the year 2013 and spring 2014. Means were calculated across eight sibling groups (cohort 1) and across five sibling groups (cohort 2) within one benthocosm tank. Significant effects are indicated here (*asterisk*) after the repeated-measures ANOVA for cohort 1 (from spring 2013 through summer) and cohort 2 (from autumn, through winter and spring 2014). Different letters above the bars indicate significant differences (*p* value <0.05) between the treatments after Tukey's test

Table 2 Results of the repeated-measures ANOVA testing the effect of the fixed factors temperature, CO_2 and time period on the relative growth rate (% d^{-1}) including the random factor sibling group. Cohort 1 (a) include the spring and early summer and cohort 2 (c) include the autumn, winter and spring 2014. Results of the mixed-model ANOVA (b) testing the effect of the fixed factor CO_2 and on the relative growth rate (% d^{-1}) including the random factor sibling group

Source of variation	numDF	denDF	*F* value	*p* value
(a) Cohort 1 (spring–early summer)				
Temperature	1	134	8.194	0.005
CO_2	1	134	1.734	0.190
Time period	1	134	22.824	<0.001
Temp × CO_2	1	134	0.363	0.548
Temp × time period	1	134	4.788	0.03
CO_2 × time period	1	134	0.027	0.87
Temp × CO_2 × time period	1	134	1.578	0.211
(b) Cohort 1 (late summer)				
CO_2	1	7	0.925	0.368
(c) Cohort 2 (autumn–spring 2014)				
Temperature	1	105	0.520	0.472
CO_2	1	105	1.951	0.166
Time period	2	105	67.004	<0.001
Temp × CO_2	1	105	0.737	0.393
Temp × time period	2	105	2.923	0.058
CO_2 × time period	2	105	0.069	0.933
Temp × CO_2 × time period	2	105	1.009	0.368

and were lower compared to growth rates in autumn. While in October the high-temperature treatment reached 19 °C, values fell below 13 °C in November and 8 °C in January (Online Resource 2).

Chlorophyll a fluorescence parameters

The maximum quantum yield (F_v/F_m) did not differ significantly among the treatments. Mean values for F_v/F_m (±SD) for T+CO_2+, T+CO_2−, T−CO_2+ and T−CO_2− were 0.62 (±0.06), 0.65 (±0.05), 0.65 (±0.05) and 0.67 (±0.04), respectively. The maximum electron transport rate ($rETR_{max}$) of *F. vesiculosus* germlings measured in March 2014 tended to be slightly higher under ambient (38.5 µmol e^- m^{-2} s^{-1}) as compared to warmer and acidified conditions (31.9 µmol e^- m^{-2} s^{-1}) (Fig. 3a). Accordingly, the light saturation point I_k had a mean of 45.8 ± 16.2 µmol photons m^{-2} s^{-1} under ambient conditions, whereas at acidified conditions I_k reached mean values with 41.7 ± 18 µmol photons m^{-2} s^{-1}. However, all differences between all treatment levels were not significant with regard to the PI curve parameters $rETR_{max}$ and I_k [$rETR_{max}$ and I_k, $F(1,39)$, $p > 0.05$; Table 3]. High temperatures decreased NPQ_{max} significantly to 1.86 as compared to 2.81 at ambient temperatures [NPQ_{max}, $F(1,39)$, $p = 0.014$; Table 3; Fig. 3b].

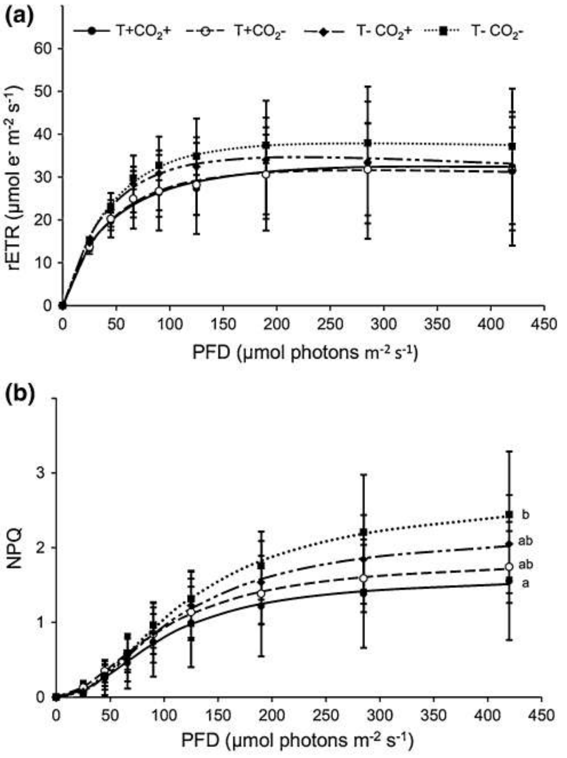

Fig. 3 Effect of the four treatments combinations: T+CO_2+, T+CO_2−, T−CO_2+ and T−CO_2− on photosynthesis irradiance curves **a** mean relative electron transport rate (rETR) and **b** non-photochemical quenching (NPQ) as a function of increasing photon flux density (PFD) of *Fucus vesiculosus* germlings measured at the end of March 2014. Data expressed as the mean ± SD ($n = 3$). Different letters indicate significant differences (p value <0.05) between the treatments after Tukey's test

Genotypic diversity level effect

In autumn, the highest diversity levels tended to feature the highest survival of all treatments, but this trend was not significant [diversity level, $F(2,11) = 0.70$, $p = 0.517$; Fig. 4a; Table 4]. In winter, lowest survival (20.07 ± 14.63 %) was observed in the lowest diversity level under high-temperature and acidified conditions, whereas highest survival was measured in the intermediate diversity level under high-temperature conditions (ca. 76.03 ± 6.03 %; Fig. 4b). Under increased temperatures, high diversity levels also showed higher survival than the low diversity level [diversity level, $F(2,11) = 4.1$, $p = 0.0467$; Fig. 4b; Table 4]. There was no diversity level effect with regard to growth in any time period under any treatment (diversity level, p value >0.05; Table 5).

Table 3 Results of mixed-model ANOVA with the two fixed factors temperature and CO_2 and the random factor sibling group on chlorophyll *a* fluorescence parameters measured at the end of the experiment (March 2014) on *F. vesiculosus* germlings

Source of variation	numDF	denDF	*F* value	*p* value
(a) Maximum relative electron transport rate ($rETR_{max}$)				
Temp	1	39	1.033	0.316
CO_2	1	39	0.364	0.549
Temp × CO_2	1	39	0.254	0.617
(b) Light saturation coefficient (I_k)				
Temp	1	39	0.020	0.888
CO_2	1	39	0.338	0.564
Temp × CO_2	1	39	0.061	0.807
(c) Non-photochemical quenching (NPQ_{max})				
Temp	1	39	6.522	0.015
CO_2	1	39	2.058	0.159
Temp × CO_2	1	39	0.452	0.506
(d) Maximum quantum yield (F_v/F_m)				
Temp	1	35	1.159	0.289
CO_2	1	35	3.977	0.054
Temp × CO_2	1	35	1.658	0.206

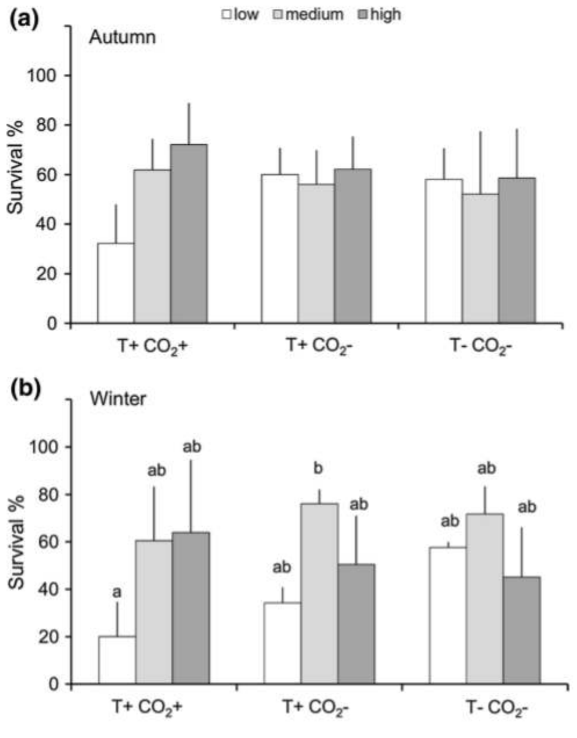

Fig. 4 Survival (%) of the different diversity levels: low (*white bars*), medium (*grey bars*) and high (*dark-grey bars*) at three treatments in **a** autumn and **b** winter. Mean values +SD are shown ($n = 3$). Different letters above the bars indicate significant differences (*p* value <0.05) between the treatments after Tukey's test

The variance in survival ($\%^2$) between groups of the low diversity level was high compared to the variance between groups of medium and high diversity levels during autumn and winter (Fig. 5a, b). The high variance of single sibling groups was present at all treatments except in winter under high-temperature and non-acidified conditions, where groups of the median diversity level showed highest variance (Fig. 5b). The response variability among experimental populations tended to decrease with increasing genetic diversity level. In 5 out of 6 treatment/season combinations, genetic diversity explained more than 60 % of response variability.

Sibling group differences

Sibling groups (1–8) showed strong differences in their sensitivities with regard to survival (%) under high-temperature and high-pCO_2 conditions. Differences between some of the sibling groups were significant in summer and winter [sibling group, in summer $F(7,16) = 2.479$, $p = 0.026$ and in winter $F(7,16) = 2.478$, $p = 0.029$; Table 5a). The single and combined effects of high-temperature and high-CO_2 treatments on survival were beneficial for some of the groups but prejudicial for other groups: high-temperature treatment enhanced survival in one sibling group but decreased survival in six sibling groups compared to ambient temperatures. Acidification led to increased survival of six sibling groups but to decreased survival in two sibling groups compared to ambient CO_2 conditions. The combined effect of high temperature and high CO_2 increased survival of three sibling groups but caused lower survival of five sibling groups compared to ambient conditions. There were no significant differences in spring and autumn [sibling group, in spring $F(7,16) = 1.169$ $p = 0.324$ and in autumn $F(7,16) = 2.478$, $p = 0.338$]. The interaction of both factors, temperature and CO_2, seemed to be mainly antagonistic (regarding germlings' mortality) on all sibling groups in spring and in summer. Sibling group variation was also high with regard to growth rates ($\%\ d^{-1}$) during all seasons. However, differences in growth rates were significant only in spring [sibling groups, $F(7,16) = 1.373$, $p = 0.014$; Table 5].

Discussion

The sensitivity of *Fucus vesiculosus* germlings to warming was higher than towards acidification, although responses to both factors are highly season dependent. Accordingly, warming had positive effects on growth in early summer but caused severe mortalities in late summer. Also the photophysiological parameter NPQ_{max} decreased under warming, while acidification did not have an effect on this

Table 4 Results of the mixed-model ANOVA testing the two fixed factors diversity level (Div.) (three levels: low, medium and high) and treatment (three levels: T+CO_2+, T+CO_2− and T−CO_2−) including the random factor sibling group on *Fucus vesiculosus* germlings regarding (a) survival (arcsin-transformed) and (b) relative growth rates (% d^{-1})

Source of variation	numDF	denDF	Autumn		Winter	
			F value	*p* value	*F* value	*p* value
Survival						
Treatment	2	106	0.737	0.481	3.125	0.048
Div	2	11	0.701	0.517	4.101	0.047
Treatment × Div	4	106	2.108	0.085	2.524	0.045
Growth rate						
Treatment	2	73	28.509	<0.001	2.135	0.126
Div	2	7	0.539	0.606	0.299	0.750
Treatment × div	4	73	0.498	0.738	1.928	0.115

Table 5 Results of three-way ANOVA for each season showing differences between sibling groups testing the factors: temperature, CO_2 and sibling group (sib) in (a) survival (arcsin-transformed) and (b) relative growth rate (% d^{-1})

Source of variation	Df	*F* value	*p* value	*F* value	*p* value
(a) Survival					
		Spring		Summer	
Temp	1	0.025	0.876	12.507	<0.001
CO_2	1	1.167	0.284	1.879	0.175
Sib	7	1.184	0.324	2.479	0.026
Temp × CO_2	1	0.010	0.921	0.233	0.631
Temp × sib	7	0.441	0.873	1.816	0.099
CO_2 × sib	7	0.320	0.942	0.754	0.628
Temp × CO_2 × sib	7	0.951	0.474	1.660	0.135
Residuals	64				
		Autumn		Winter	
Treatment	2	3.600	0.035	6.311	0.004
Sib	7	1.169	0.338	2.478	0.029
Treatment × sib	14	0.849	0.615	1.496	0.150
Residuals	48				
(b) Growth rate					
		Spring		Summer	
Temp	1	3.886	0.054	5.875	0.019
CO_2	1	12.193	<0.001	0.246	0.622
Sib	6	2.960	0.014	0.466	0.830
Temp × CO_2	1	3.306	0.075	0.950	0.334
Temp × sib	6	1.247	0.297	1.084	0.385
CO_2 × sib	6	2.377	0.041	0.580	0.745
Temp × CO_2 × sib	6	1.550	0.179	0.992	0.441
Residuals	50				
		Autumn		Winter	
Treatment	2	10.953	<0.001	7.631	0.001
Sib	7	1.373	0.239	1.640	0.147
Treatment × sib	14	1.916	0.049	1.863	0.056
Residuals	46				

parameter. The variability in the responses of the sibling groups to warming and acidification was high, supporting the hypothesis that the tolerance against environmental stress is increased in populations of higher genetic diversity. Corroborating this, survival of germlings increased under warming in the high diversity level compared to the

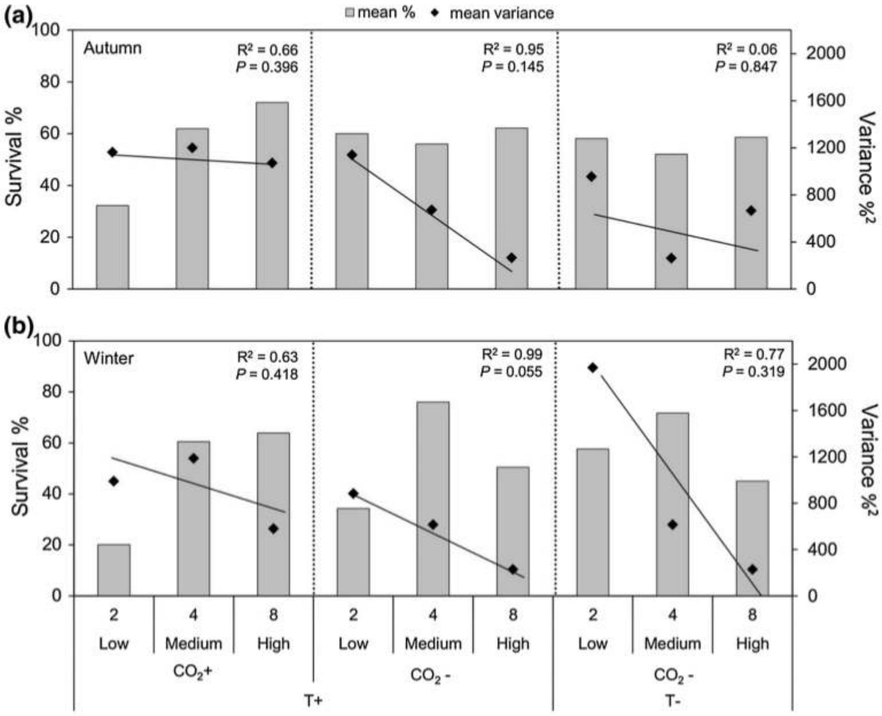

Fig. 5 Variances (%^{2}) between the groups of each of the three diversity levels (low: single sibling groups, medium: pairs, high: quartets) with 2, 4 and 8 parents each diversity level, respectively, for survival (%). *Bars* show means ($n = 3$) for each combination of diversity and treatment level in **a** autumn and **b** winter. Trend lines for the variances within each treatment level are shown as well as the determination coefficient R^2

low diversity level, supporting the hypothesis that the tolerance against environmental stress is increased in populations of higher genetic diversity. Macroalgal germlings, growing under the environmental gradients of the Baltic shallow subtidal, are regularly subjected to strong fluctuations of temperature and pCO_2 (Saderne et al. 2013). This, over time, may have led to a preadaptation to global changes (e.g. Pansch et al. 2014). However, in the near-natural environment of the KOB, so far unknown sensitivities were revealed regarding the interactive effects of temperature, acidification and season. Responses in physiological performance of *F. vesiculosus* germlings to warming were positive until July as reflected in enhanced growth rates and are consistent to those of adult *F. vesiculosus* in the northern Baltic proper (Lehvo et al. 2001). Enhanced growth rates under warm conditions may be explained by stimulated metabolic processes according the Q10-rule (Nygård and Dring 2008; Nielsen et al. 2014). During a natural heat wave in August, however, temperatures reached >27 °C (Online Resource 2) exceeding the thermal tolerance range of juveniles of this species (Li and Brawley 2004; Maczassek 2014) as reflected in a severe mortality. These results indicate that future ocean warming effects on survival are strongest during summer, differing among seasons. As a consequence of warming, local extinctions and range shifts of *F. vesiculosus* were predicted for the next century by Jueterbock et al. (2013) and were observed for other seaweed species (Wernberg et al. 2011).

Warming-induced mortality was also observed in winter, when twice as many germlings died under warming and acidification compared to ambient conditions. This seems surprising, considering that the increase in temperatures ranged from ambient 3–7 °C to only experimental 8–12 °C (December to January), which, at least for adults, is towards the optimum temperature range of *F. vesiculosus* (Graiff et al. 2015). Warming during winter may enhance all metabolic processes (e.g. respiration), while photosynthesis is still limited due to low-light conditions (Rohde et al. 2008). In winter the daily dose of irradiation is probably below the light compensation point of the *F. vesiculosus* germlings (Wahl et al. 2011) (Online Resource 3). Consequently, the resulting light limitation may have biased towards heterotrophic processes. Therefore, it is assumed that the accelerated metabolism under warming has provoked an overexploitation of storage products and hence a lethal energy debt. The interactive effect of temperature and light might be relevant for early life-stages of seaweeds which often occur in the shaded understory vegetation.

Acidification affected growth and survival of the germlings less strongly than warming, but high pCO_2 increased survival in spring and growth in late summer. The reason for this is either an enhanced carboxylation at high-pCO_2 conditions as previously reported for other algae

(Olischläger et al. 2012, 2013; Koch et al. 2013; Saderne et al. 2013) or down-regulation of energy-consuming carbon-concentrating mechanisms (CCMs) under these conditions (Beardall and Giordano 2002; Wu et al. 2008).

Under ambient conditions, germlings seem to require less light than adults possibly reflecting an adaptation to their natural shaded habitat, i.e. the understory of adults. The photosynthetic performance, expressed as relative electron transport rate and light saturation point, of *F. vesiculosus* germlings under ambient conditions was only half compared to adults (A. Graiff, unpublished results). The photosynthetic apparatus of juveniles may not be fully developed and hence be more sensitive to environmental stress. The maximum quantum yield (F_v/F_m) of the germlings under warming at the end of the winter experiment (March 2014) was not reduced, but they showed a significantly decreased non-photochemical quenching (NPQ_{max}) compared to ambient temperatures (Fig. 3b). Lower NPQ_{max} and reduced $rETR_{max}$ under the high-temperature treatment can be explained by the presence of temperature sensitive enzymes of photophosphorylation and the stability of PSII (e.g. D1 protein), as reviewed by Allakhverdiev et al. (2008). Non-photochemical quenching is a proxy of xanthophyll pigment cycling, which protects photosystems from overexcitation (Lavaud et al. 2002a, b; Ruban et al. 2007). Therefore, data on non-photochemical quenching regulation better reflect the actual physiological state of algae under stress than other chlorophyll *a* fluorescence parameters, indicating the decreased capacity of *F. vesiculosus* germlings for stress resistance under warmed conditions.

Chlorophyll *a* fluorescence parameters from *F. vesiculosus* germlings were not significantly influenced by acidification neither under ambient nor under elevated temperatures. In contrast, stimulating effects of increasing external DIC on the photosynthesis and relative electron transport rates of adult brown algae have been reported, possibly due to increased activity of RuBisCO (Forster and Dring 1992; Nygård and Dring 2008; Johnson et al. 2012). In particular, the kelps *Laminaria digitata* and *Saccharina latissima* as well as Baltic *F. vesiculosus* responded to moderately increased DIC with elevated rates of photosynthesis and carbon acquisition (Schmid et al. 1996; Klenell et al. 2004; Nygård and Dring 2008). It is assumed that photorespiration is reduced under elevated pCO_2 and less energy is required for recharging the internal carbon storage after periods of high photosynthetic activity. However, these beneficial effects of increased pCO_2 on photosynthetic performance on *F. vesiculosus* germlings may have been masked by the interaction with other fluctuating abiotic factors (e.g. nutrients, irradiances) as was previously reported for other non-calcifying algae (Sarker et al. 2013).

F. vesiculosus germlings in populations with higher genetic diversity tended to survive better under warmer conditions than those in the low diversity level. These findings support that populations with higher genetic diversity are more resilient towards environmental stress than those of lower genetic diversity. Ongoing genetic analysis will reveal the causes for this observed pattern; hence, for the moment all interpretation is speculative. Possibly, neighbour effects exist that are more beneficial for germlings if their neighbours are more different among each other than full-sibs would be. There are several possible ways how diverse neighbouring germlings could have influenced each other positively, in analogy to processes and complementarity found in species-rich communities and modelled in ecosystem functioning concepts (Hooper et al. 2005). *F. vesiculosus* genotypes vary in their capacity of antifouling defence strength and in the release of defence metabolites (Honkanen and Jormalainen 2005). Higher genetic diversity level may present well-defended genotypes that protect more weakly defended neighbours [associational defence sensu Wahl and Hay (1995)]. Hence, antifouling capacity is indeed crucial for seaweed fitness in general (da Gama et al. 2014) and may be particularly important for early ontogenetic stages (Wahl et al. 2011). Facilitation and niche differentiation under warming have been observed in other species, as among different genotypes of the eelgrass *Zostera marina* (Reusch et al. 2005), where complementarity dominated over selection processes, as well as improved settlement success in the early life-stage of the barnacle *Balanus improvisus* (Gamfeldt et al. 2005). The higher resistance conferred by intraspecific variability for *F. vesiculosus* to environmental stress underpins the increasing plea for the inclusion of intraspecific variability in ecosystem functioning concepts (Reusch and Hughes 2006). In fact, there is a growing recognition of including the within-species variation when using an upscaling approach: the genotypic variability determines the width of species niches and has therefore implications on an interspecific level and on the community structure (Violle et al. 2012).

In conclusion, our study highlights the importance of evaluating the interactive impacts of multiple stressors (Folt et al. 1999) in the context of variable environmental conditions (seasons), natural fluctuation (Wahl et al. 2015c) and variable intraspecific diversity (Reusch and Hughes 2006; Reusch and Wood 2007; Pauls et al. 2013). Our results also show the strong pressure on early life-stages of *F. vesiculosus* under climate change conditions, mainly caused by warming during summer. Sufficient genetic diversity in the population, however, provides a strong stress-driven selection which might quickly enhance tolerance to global change. Although the observed antagonistic interaction between temperature and pCO_2 may mitigate high impacts of global change variables, further genetic analyses are required for understanding the exact significance. The past massive retreat of this brown alga in the Baltic Sea indicates that additional abiotic or biotic stressors such as increased grazing, overfishing or fouling in combination with the inherently low genetic variation of *F. vesiculosus* are responsible for this *quasi* collapse. Filling these knowledge gaps will help to better predict the fate of *F. vesiculosus* in the Baltic Sea.

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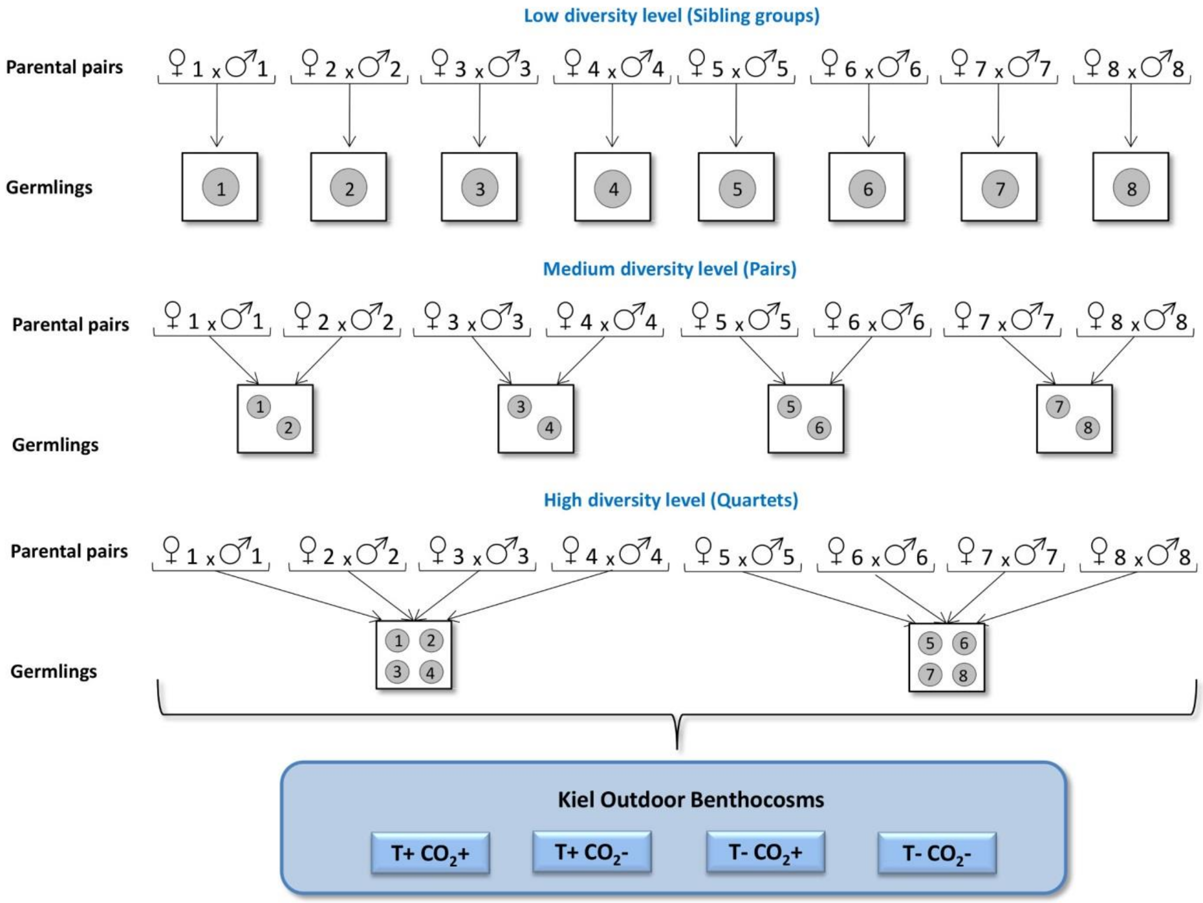
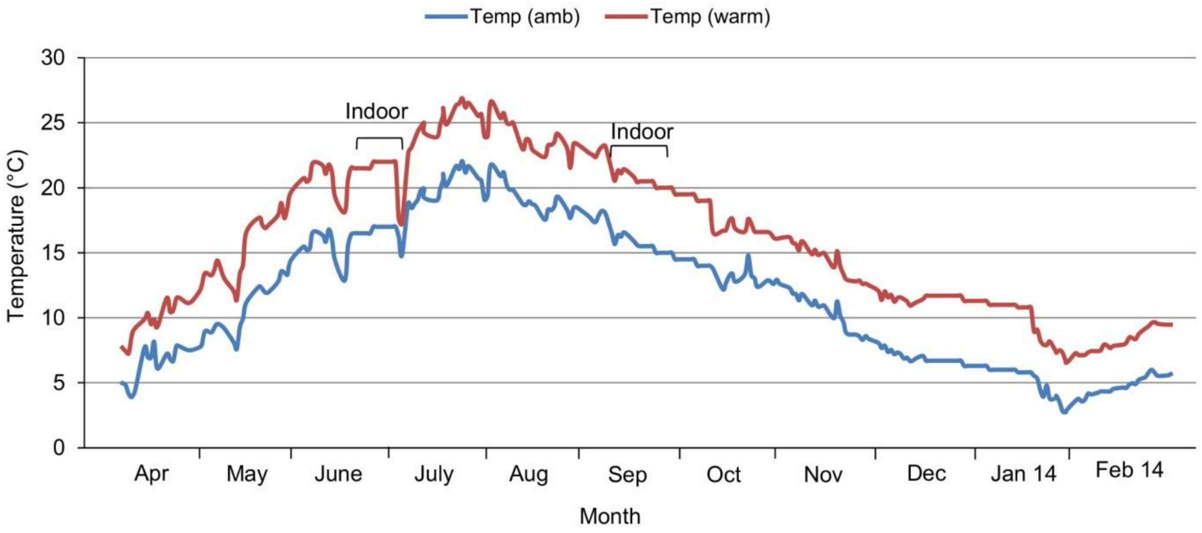
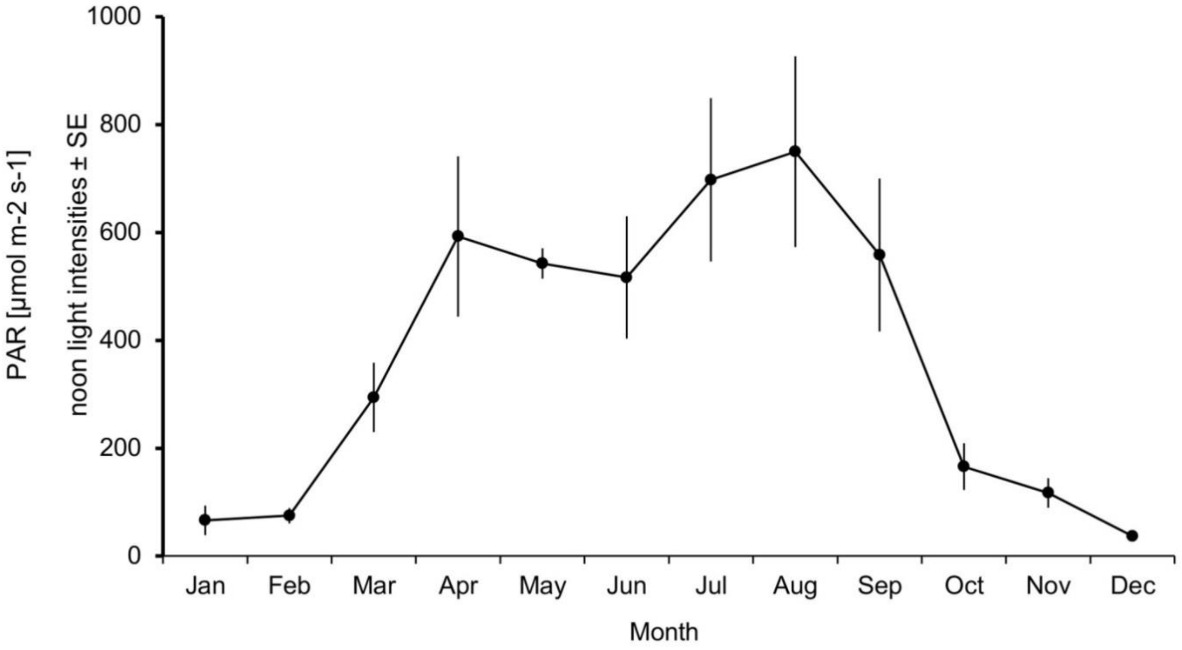
Supplementary Information



Online Resource 1 Scheme of the experimental design showing three diversity level of *Fucus vesiculosus* germlings in cohort 2. For each experimental population, the parental source is shown. Fathers (♂) and mothers (♀) are numbered and their combination of parental pairs is indicated. Numbers in circles represent the sibling groups and boxes represent sandstone surfaces (2 cm^2), where germlings were settled. For cohort 1, only the low diversity level was established and introduced into the Kiel Outdoor Benthocosms (KOB). All experimental populations were introduced into each of the 12 experimental subunits of the KOB. Across the 12 subunits of KOB, the two factors warming and acidification were orthogonally crossed, creating the four treatment levels: T+ CO_2+ (warmer and acidified), T+ CO_2- (warmer, non-acidified), T- CO_2+ (ambient temperature, acidified), T- CO_2- (ambient temperature, non-acidified) in three replicates.



Online Resource 2 Seasonal variation of temperatures from April 2013 to February 2014 in the ambient and warm treatment (mean, n=3) at ambient CO_2 concentrations in the outdoor Kiel Benthocosms. Temperatures were recorded every day with a calibrated sensor (pH, Mettler Toledo GmbH, Giessen, Germany). Values measured in the indoor mesocosms used for maintenance of germlings between the benthocosms experiments are indicated as “indoor”.



Online Resource 3 Seasonal variation of light intensities measured in the outer Kiel fjord (Bülk, Germany) during the year 2013. Measurements took place in 0.5 m water depth (depending on the water level) with a light logger (HOBO, Onset Computer Corporation). Mean light intensities were calculated of the first seven days in each month (n=3). Error bars indicate ±SE. This figure was modified after Rickert et al. (2015).

Paper III

Manuscript submitted to Plos One

Buffering and amplifying interactions among OAW (ocean acidification & warming) and nutrient enrichment on early life-stage *Fucus vesiculosus* L. (Phaeophyceae) and their carry over effects to hypoxia impact

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Statement of authorship: B. Al-Janabi and M. Wahl designed the study; B. Al-Janabi and V. Winde collected the data; B. Al-Janabi, V. Winde and M. Lenz analysed the data; B. Al-Janabi wrote the manuscript and all authors commented and corrected the manuscript.

Keywords: bladder wrack, *F. vesiculosus* germlings, global climate change, German Baltic Sea, genetic diversity, eutrophication, hypoxia, local upwelling

Abstract

In many marine coastal regions, global change causes ocean acidification and warming (OAW). Additionally, at a more local scale the spreading of hypoxic conditions is promoted by eutrophication and warming. In the semi-enclosed brackish Baltic Sea, occasional upwelling in late summer and autumn may expose even shallow-water communities including the macroalga *Fucus vesiculosus* to particularly acidified, nutrient-rich and oxygen-poor water bodies. During summer 2014 (July – September) sibling groups of early life-stage *F. vesiculosus* were exposed to OAW in the presence and absence of enhanced nutrient levels and, subsequently to a single upwelling event in a near-natural scenario which included all environmental fluctuations in the Kiel Fjord, southwestern Baltic Sea, Germany (54°27 ´N, 10°11 ´W). We strove to elucidate the single and combined impacts of these potential stressors, and how stress sensitivity varies among genetically different sibling groups. Enhanced by a circumstantial natural heat wave, warming and acidification increased mortalities and reduced growth in *F. vesiculosus* germlings. This impact, however, was mitigated by enhanced nutrient conditions. Survival under OAW conditions strongly varied among sibling groups hinting at a substantial adaptive potential of the natural *Fucus* populations in the Western Baltic. A three-day experimental upwelling caused severe mortality of *Fucus* germlings, which was substantially more severe in those sibling groups which previously had been exposed to OAW. Our results show that global (OAW), regional (nutrient enrichment) and local pressures (upwelling), both alone and co-occurring may have synergistic and antagonistic effects on survival and/or growth of *Fucus* germlings. This result emphasizes the need to consider combined stress effects.

Introduction

Global climate change will expose marine populations to increased eutrophication and upwelling events at a regional scale and to ocean acidification and warming (OAW) at a more global scale [1]. For the Baltic Sea, current models predict an increase in pCO_2 from currently almost 400 µatm to 1000 µatm, an increase in sea surface temperature by up to 5 °C and considerable enhancements of eutrophication and hypoxia during the next 100 years [2]. The combined effects of these global change factors will likely affect Baltic ecosystems [3]. Anthropogenic activities, such as agriculture, lead to eutrophic conditions in the largest part of the Baltic Sea, including the southwestern part with Kiel Bight [4]. Eutrophication may further intensify due to increased precipitation and river runoff [5, 6]. Hypoxic conditions (< 2 mg O_2 L^{-1}) are predicted to increase in the Baltic Sea during this century as modelled by Meier and Andersson [7]. In Kiel Bay, seasonal oxygen depletion has been known for decades [8], but is likely to intensify further.

Macrophytes play a key role in ecosystem services by the retention of excessive nutrients [9, 10], acting as 'nutrient filters' [11] and providing the benthic ecosystem with oxygen [12]. A combination of global, regional and local stressors endangers macroalgae worldwide [13, 14]. Among these, the bladder wrack *Fucus vesiculosus* is a dominant perennial macroalga in the intertidal and shallow subtidal of the Western, Central and Eastern Baltic Sea [15]. As a foundation species, *F. vesiculosus* provides habitat and food for a large variety of invertebrate assemblages [16, 17]. A drastic decline in *F. vesiculosus* during the last five decades was observed, e.g. by almost 95 % in Kiel Bay, Western Baltic Sea [18, 19]. The shoaling of *F. vesiculosus* was mainly assigned to the severe indirect effects of eutrophication [18, 20-22]. These are *i.a.* the increased turbidity [20], competition with ephemeral algae [23] and increased palatability [24]. Also, enhanced sedimentation reduces the attachment and survival of *F. vesiculosus* zygotes [25, 26]. Regarding direct effects, elevated nutrient concentrations positively affect photosynthesis and growth in adult *F. vesiculosus* [27].

As a consequence of eutrophication in the Baltic Sea, sedimentation and bacterial re-mineralization are enhanced, leading to seasonal hypoxia and hypercapnia in sub-surface waters [28]. Further spread of (periodic) hypoxic areas in the Baltic Sea is predicted until the end of the 21st century due to the interplay between eutrophication and lower oxygen solubility under warming [7]. Hypoxia induced mortality of benthic organisms was reviewed by Gray et al. [29] and recognized at a Baltic [30] and a global scale [31]. Also, higher frequencies of local upwelling in the last decades have been documented in the Baltic Sea [32].

In addition to hypoxia, warming was observed to impact growth, survival and photosynthetic efficiency in *F. vesiculosus*, while acidification showed weaker effects in the early [33] and adult life-stage [34]. As a consequence of warming, poleward range shifts of seaweed populations have been observed worldwide [14, 35, 36]. Also in the Baltic Sea, northward range shifts of *F. vesiculosus* populations have been observed and are predicted to continue during the 21st and 22nd century [37]. In contrast, ocean acidification was reported to increase growth in non-calcifying macroalgae [27, 38]. Assumed reasons are enhanced availability of CO_2 to saturate the carbon demand during photosynthesis or the saved energy when carbon concentrating mechanisms are downregulated [39]. However, physiological responses of the macroalga *Macrocystis pyrifera* to acidified conditions showed that increased CO_2 conditions did not affect growth or photosynthesis [40]. Early life-stage macroalgae may be particularly threatened by global change [41]: Warming lowers the germination success of *F. vesiculosus* at 25 °C [42] and reduces survival in *F. serratus* germlings more severely than in adults [43]. Enhanced sedimentation caused by eutrophication is more detrimental for young [23, 44] than for adult *Fucus* [15, 45]. Despite recognized differences in stress sensitivity among life-stages of a species, past research has mainly focussed on adult forms [46, 47].

Adaptation of marine populations to global change is favoured by genetic diversity [48, 49]. Conversely, low intraspecific genetic diversity and high phenotypic plasticity and gene flow restrict adaptation to environmental stress [50-53]. The increased tolerance of the eelgrass *Zostera marina* with higher genetic diversity to a summer heat wave [54] showed that genetic diversity may buffer warming stress at the population level [55].

Baltic *F. vesiculosus* populations show reduced genetic variation compared to Atlantic populations probably due to isolation and bottlenecks as well as the eroded genetic variation due to selection [56]. It has been argued that environmental stress, e.g. osmotic stress in the brackish Baltic, lower genetic diversity and limited dispersal capacity of *F. vesiculosus* gametes [57] may favour local extinctions [58]. However, this study is the first one to test genetic variation in Baltic *F. vesiculosus* with regard to the sensitivity towards OAW, nutrient enrichment and subsequent hypoxia.

The aim of the present study was to investigate (1) how OAW, (2) nutrient enrichment and (3) upwelling events affect the survival and growth of *F. vesiculosus* germlings, (4) how OAW interacts with simultaneous exposure to high nutrient concentrations, (5) how these treatments modify hypoxia sensitivity and (6) whether sibling groups vary in their tolerance towards these environmental parameters. Our experimental concept, hence, consisted in the exposure of genetically different sibling groups of *F. vesiculosus* germlings to increased temperature, pCO_2 and nutrient conditions and to a final upwelling event while maintaining the natural variations.

Material and Methods

Collection, gamete acquisition, experimental design

A total of 64 fertile *F. vesiculosus* were collected in a wave exposed area with mixed hard substrate and sand bottom in the southwestern Baltic Sea (Bülk, Germany, 54°27.327 ´N, 10°11.977 ´W) in mid-June 2014. To avoid the collection of siblings and ensuring for genetic variability, individuals sampled were distanced by at least 2 meters, which is the estimated maximum dispersal distance of most *F. vesiculosus* gametes [57]. After collection, algae were transported to the lab in cooler boxes. Fertile receptacles were cut from these dioecious algae and gender was determined (46 females, 18 males) under the microscope at 100 x magnification (Olympus BH-2). Receptacles were rinsed with tap water, blotted dry and stored in the dark for 6 days at 14 °C. Before gamete release, all receptacles from one female and one male individual (i.e. one parental pair) were put in a small dish. Gamete release followed by egg fertilisation was induced by immerging receptacles into sand-filtered seawater (15-16 psu) and exposing them to light (110 µmol photons m^{-2} s^{-1}) for 3 hours. In this way, gametes were obtained from16 parental pairs. No specific permits were required for this study, the location is not privately-owned or protected and the study did not involve an endangered or protected species.

One mL of homogeneously suspended fertilised eggs was pipetted onto the upper surface of each of 2 x 2 cm sandstone cubes. Each cube with its settled germlings represented one experimental population. 16 different populations, each composed of germlings stemming from one parental pair, were thus produced. Culture and monitoring of germlings took place in a room with windows approximating natural light conditions during 3 weeks with weekly water exchange (15-16 psu) at 15 °C until introducing them to the Kiel Outdoor Benthocosms (KOB). This facility maintains the natural *in situ* fluctuations and simultaneously allows manipulating environmental variables (e.g. temperature, pCO_2) on top of these fluctuations, i.e. "delta treatments". Target temperatures were obtained and maintained by computer controlled heaters and coolers, while acidification was achieved by increasing the atmospheric pCO_2 within the enclosed head space above the tanks by injecting pre-mixed gas to maintain an atmospheric pCO_2 of 1100 µatm. Details of the experimental set-up of the KOB and the logged conditions in the tanks are given in Wahl et al. [59].

OAW x nutrient and upwelling treatment

The OAW x nutrient experiment took place during 2 summer months from mid-July until mid-September 2014. One PVC box (70 cm x 40 cm x 12 cm) was placed within each of the 12 benthocosm main tanks, each of them containing all 16 experimental populations of germlings. Since the upper rim of the lid-less boxes was a few centimetres above the water surface, the water body within the PVC boxes was separated from the water body of the main tank but open to the atmosphere. Thus, the boxes experienced the same treatments as the main tank regarding OAW but were insulated from gene flow from the adult *Fucus* population in the main tank. Twice a week the water of the PVC box was exchanged by water from the main tank which had been filtered through a 50 µm mesh to prevent the introduction of *F. vesiculosus* eggs (100 µm diameter). As the single-factor and combined effects of temperature and pCO_2 on *F. vesiculosus* germlings have been investigated previously [33], we combined warming and acidification into a single factor (OAW) in the present run. The two fully crossed factors OAW and nutrients were applied at two levels each (ambient and future). Ambient and predicted future levels of OAW and nutrients were simulated by adding the expected shift to the natural fluctuations of the ambient fjord conditions as delta treatments [59].

The "ambient" condition represents the natural fjord conditions transported into the main tanks of the KOB by a continuous flow-through (1 tank-volume per day, i.e. 1500 L/ 24 h) of Kiel Fjord water pumped from 1 m depth. "Future" conditions were simulated by adding 5 °C to the actual temperature of the Kiel Fjord and by increasing the pCO_2 concentration in the hooded headspace of the tanks to 1100 µatm according to the predictions for the year 2110 for the Baltic Sea [2]. The bi-weekly nutrient enrichment (2 x ambient) was achieved by doubling the "ambient" concentration which was taken as the seven years (2006-2013) mean for each specific date of nutrient addition (Table 1, Figure in S1 Fig). $NaNO_2$ (Merck, Germany), $NaNO_3$ (Carl Roth, Germany) and $H_2NaO_4P.H_2O$ (ACROS organics, Germany) were dissolved in fjord water 10 minutes before addition to the nutrient treatments. The ratio P : N of the Kiel Fjord is approximately 1 : 1.5 and does not match the Redfield Ratio, probably due to the nutrient input of the nearby located river Schwentine. Additionally, high organism activity during summer months decreases the overall nutrient availability in shallow water. This ambient P : N ratio was not altered when NO_3, NO_2 and PO_4 concentrations (µmol L^{-1}) were doubled under "future" conditions. Our analysis on CN ratio in *Fucus* tissue (see result section below) showed no differences under "future" conditions, indicating that carbon did not become a limiting factor under nutrient enrichment.

The four treatment combinations, each replicated three times, thus were: OAW- N- (ambient OAW & ambient nutrients), OAW- N+ (ambient OAW & high nutrients), OAW+ N- (future OAW & ambient nutrients) and OAW+ N+ (future OAW & high nutrients). These treatment combinations were regularly distributed among the 12 experimental units.

The upwelling experiment was performed immediately after the end of the OAW x nutrient treatment phase, i.e. when all the treatments in the tanks were set back to "ambient". During three days, hypoxic fjord water from 15 m depth (O_2 = 2.75 ± 0.41 mg L^{-1}, T = 16.52 ± 0.33 °C, pH = 7.40, Sal = 22.8) was pumped as flow-through into the KOB and, via a bypass into the germlings boxes continuously during 3 days. This experiment was meant to assess the effect of the compound factor "upwelling" on sibling groups pre-conditioned by the foregoing treatment combinations (OAW x nutrient enrichment). Upwelled water in this region and this season usually is characterized by lower oxygen, higher salinity and higher nutrients than surface water.

Table 1. Nutrient concentration in ambient and future conditions

	July		August		September	
	Ambient	Future	Ambient	Future	Ambient	Future
PO_4	0.46	0.93	0.59	1.19	1.06	2.11
NO_3	0.53	1.05	0.77	1.54	1.27	2.54
NO_2	0.18	0.36	0.20	0.40	0.22	0.44

"Ambient" nutrient concentrations of PO_4, NO_2, NO_3 (µmol L^{-1}) for the respective summer months and "future" nutrient concentrations (µmol L^{-1}).

Measurement of abiotic factors

pH and temperature of the main tank of the KOB were measured daily with a calibrated hand-held sensor (pH, Mettler Toledo GmbH, Giessen, Germany) while oxygen was measured with a Multi WTW Oxy 3515 (oxygen, Wissenschaftlich Technische Werkstätten, Weilheim, Germany). Salinity was measured with a portable conductivity meter (WTW Cond 3110 + Tetra Con 325, Wissenschaftlich Technische Werkstätten, Weilheim, Germany). Additionally, temperature, pH, O_2

and salinity were continuously logged at 10 min time intervals (GHL Advanced Technology, Kaiserslautern, Germany). During the hypoxia experiment, O_2 and temperature within the PVC boxes were logged every 10 min using the Multi WTW Oxy 3515. Samples for nutrient concentrations were taken from the KOB before each water exchange in the PVC boxes as initial concentration. For this, water samples were immediately filtrated through a 0.45 µm Minisart syringe filter (Sartorius) in 10 mL tubes, stored at -20 °C and measured with a QuAAtro nutrient analyzer [60] (SEAL Analytical; Figure in S1 Fig.). More details about the measurement of the abiotic variables in the KOB are described by Wahl et al. [59].

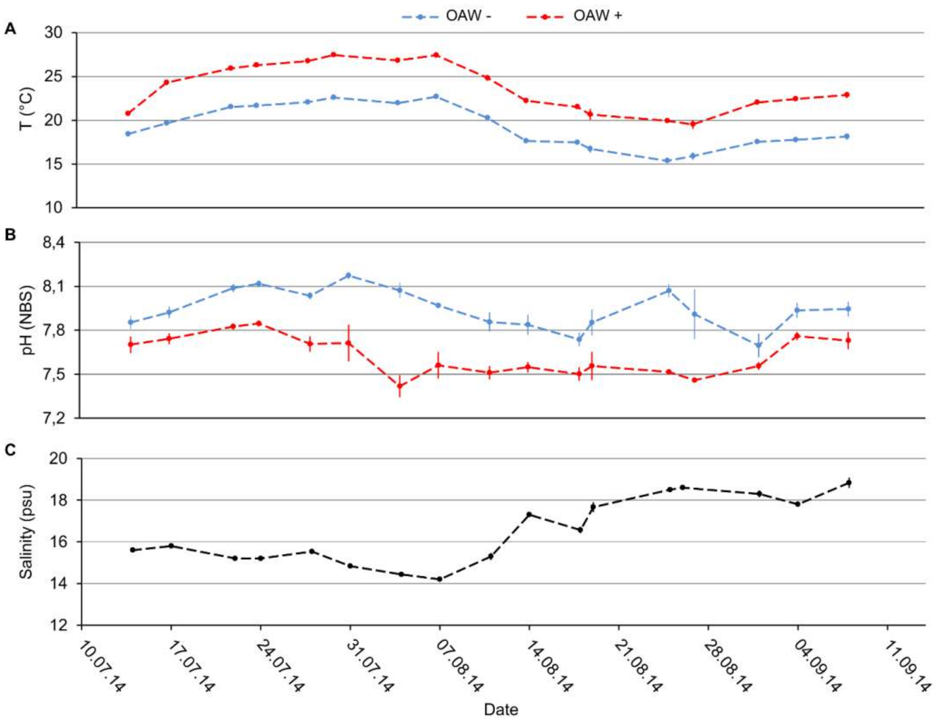

Fig 1. Temperature, pH and salinity during the OAW x nutrient experiment. (**A**) Temperature (°C), (**B**) pH (NBS, National Bureau of Standards) at the two treatment combinations ambient (OAW-), warmed and acidified (OAW+) and (**C**) salinity (psu) at the ambient treatment (OAW-). Data are the mean ±SD of measurements in three replicates of each treatment combination.

Abiotic conditions

During the OAW x nutrient experiment, the bi-weekly measured oxygen concentrations (mean = 8.91 ± 0.38 mg L^{-1}) varied in the low OAW treatment with a minimum of 8.01 ± 0.23 mg L^{-1} and a maximum of 9.44 ± 0.16 mg L^{-1} (mean ± SD) and within the high OAW treatment (mean = 7.76 ± 0.56 mg L^{-1}) with a minimum of 6.75 ± 0.56 mg L^{-1} and a maximum of 8.72 ± 0.21 mg L^{-1}. Day-night fluctuations measured in two hours intervals showed that O_2 concentrations varied between a minimum of 7.64 mg L^{-1} at 4:30 am and a maximum of 9.97 mg L^{-1} at 16:30 pm under ambient conditions.

Ambient temperatures (factor level ‘ambient’) (mean = 19.26 ± 2.38 °C) varied between bi-weekly measurements with a minimum of 15.37 ± 0.19 °C and a maximum of 22.7 ± 0.08 °C. Elevated temperatures (factor level ‘future’) (mean = 23.64 ± 2.68 °C) varied between a minimum of 19.53 ± 0.52 °C and a maximum of 27.47 ± 0.26 °C, which occurred during a natural summer heat wave (mean ± SD) (Fig 1). Temperatures during day and night fluctuated between a minimum of 16.1 °C at 10:30 pm and a maximum of 17.7 °C at 8:30 am under ambient conditions.

Response variables

Growth

For growth measurements, digital images were taken of 10-15 randomly chosen individual germlings per population at 40 x magnification (SteREO Discovery. V8 – Carl Zeiss Jena GmbH) similar to Steen and Scrosati [61]. Measured individuals were chosen randomly, since germlings were too small for labelling. The projected side-view of the single germlings was measured with the image analysis software Image J 1.45s (National Institutes of Health, USA) and the mean of germlings’ area of the perpendicular projection was calculated for each population. Germlings’ area was measured at the beginning (area t_0) and after 8 weeks (area t) at the end of the OAW x nutrient experiment. Relative growth rate (RGR) in % d^{-1} was calculated as exponential growth:

$$RGR = \left[\left(\frac{Area\ t}{Area\ t_0}\right)^{1/\Delta t} - 1\right] .100$$

Where Δt is the time period between t_0 and t in days.

Survival

Germling number was counted under a binocular at 25x magnification between the start (number t_0) and after 8 weeks (number t) of the OAW x nutrient experiment. Survival of germlings was expressed as the percent of surviving germlings and calculated as:

$$Survival\ \% = \frac{Number\ t}{Number\ t_0}\ .\ 100$$

For determining survival during the final upwelling experiment, the germling number at the end of the preceding OAW x nutrient experiment was set as t_0 and number t was the germling number after the upwelling treatment.

Log-effect ratio

Log effect ratios were performed to show the direction and the strength of the sibling groups' phenotypical responses to the different OAW and nutrients enhancements relative to the respective ambient conditions. These differences among sibling groups' responses were determined in order to assess whether higher genetic diversity also increases the variance in responses. Sibling groups' sensitivity to high OAW and N were calculated separately as the log effect ratios of growth or survival under future relative to actual conditions, as:

$$log\,effect\,ratio\ OAW = \log\left(\frac{Growth\ OAW+}{Growth\ OAW-}\right)$$

at ambient and high nutrient conditions and:

$$log\,effect\,ratio\ N = \log\left(\frac{Growth\ N+}{Growth\ N-}\right)$$

at ambient and high OAW. The same procedure was used for calculating the log effect ratios for survival. Negative growth rates observed in 3 populations were attributable to the mortality of the bigger sized individuals and a decrease in mean area t compared to t_0. To avoid negative values for log effect ratio analysis, growth was measured as ratios of area t and area t_0 as:

$$Growth = \frac{Area\ t}{Area\ t_0}$$

CN ratios

Germlings of five sibling groups (1, 3, 7, 12 and 14) of the two treatment combinations OAW- N- and OAW- N+ were pooled for CN analysis. For the analysis of carbon and nitrogen content, freeze-dried algal material was ground to powder and three subsamples of 2 mg from each treatment was packed and loaded into tin cartridges (6×6×12 mm). Then, the packages were combusted at 950 °C and the absolute C and N contents in % dry weight (% DW) were automatically quantified in an elemental analyser (Elementar Vario EL III, Germany) using acetanilide as standard according to Verardo et al. [62].

Statistical Analysis

Growth (RGR, % d^{-1}) and survival (%) were analysed using a split-plot ANOVA with the fixed factors 'OAW' (with two levels: OAW- and OAW+) and 'Nutrients' (with two levels: N- and N+) as well as the random factors 'Mesocosm' (with 12 levels) and 'Sibling Group' (with 16 levels). 'Mesocosm' was nested in the 'OAW' x 'Nutrients' interaction. This model allowed us to analyse the influence of the fixed factors. Furthermore, we were able to identify possible random-by-fixed factor interactions between 'Sibling Group', 'OAW' and 'Nutrients'. Survival data were arcsine transformed prior to the analysis to overcome data truncation. Differences in carbon and nitrogen contents (% DW) as well as in CN ratios between five single sibling groups were identified in a further split-plot ANOVA with the fixed factor 'Nutrients' (two levels: see above), which was combined with OAW- only, and the random factors 'Mesocosm' (12 levels) and 'Sibling Group' (16 levels), while 'Mesocosm' was nested in 'Nutrients'. Split-plot ANOVAs were performed by using Satterthwaite's method for denominator synthesis [63], which calculates appropriate error terms for the F-ratios of the respective effects. Normality of errors and homogeneity of variances were verified by using residual plots (Q-Q Plot and Standardized Residuals Plot, respectively) in STATISTICA and R. The significance level of all analyses was $\alpha = 0.05$. Post-hoc tests were performed using Tukey's HSD. All statistical analyses were conducted using the software STATISTICA v. 12 [64] and R.

Results

OAW x nutrient experiment (mid-July – mid-September 2014)

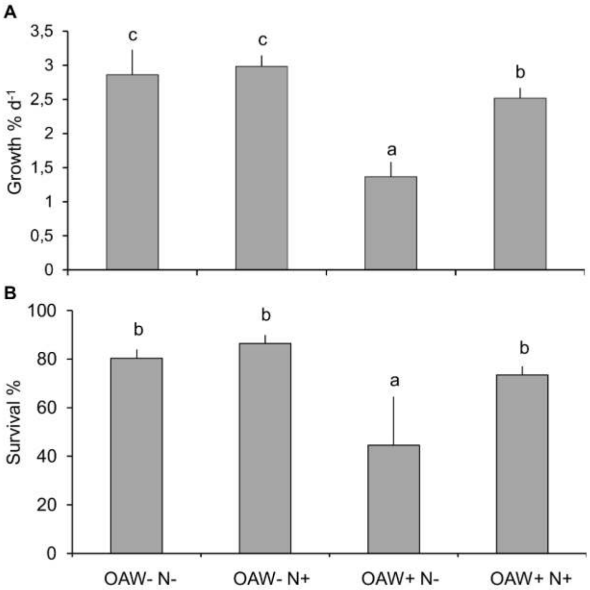

Fig 2. Growth and survival of *F. vesiculosus* germlings during the OAW x nutrient experiment. (A) Growth (% d^{-1}) and (B) survival (%) (mean +SD, n=3, 8-9 weeks) during summer 2014 at the four treatment levels OAW-N-, OAW-N+, OAW+N-, OAW+N+. Means were calculated from 16 sibling groups for each treatment 'OAW' and 'Nutrient' treatment combination. Different letters above the bars indicate significant differences (p-value < 0.05) between the treatments after Tukey's HSD.

Growth and survival

Under ambient temperature, pCO_2 and nutrient conditions, *F. vesiculosus* germlings' relative growth rate (RGR) and survival was 2.86 ± 0.37 % d^{-1} (Fig 2A) and 80.34 ± 3.70 % (Fig 2B), respectively. Under ambient temperature and pCO_2 conditions, the addition of nutrients (i.e. OAW-N+) did not change RGR and survival (Fig 2). In contrast, high OAW conditions under ambient nutrient conditions (OAW+N-) reduced RGR and survival by about 50 %; when RGR was 1.37 ± 0.21 % d^{-1} and survival was 44.48 ± 20.03 % (mean, ± SD, n=3, Table 2). The addition of nutrients almost entirely compensated the negative impact of OAW as reflected in a RGR of 2.52 ± 0.15 % d^{-1} and survival of 73.49 ± 3.49 %. The significant interaction between the factors OAW and nutrients regarding growth rate reflects this compensation effect of the nutrient treatment (Split-plot ANOVA, $p < 0.05$, Table 2A).

Table 2. OAW and nutrients effects on growth and survival.

Results from split-plot ANOVA with the fixed factors ‘OAW’ and ‘Nutrients’ and the random factors ‘Mesocosm’ and ‘Sibling group’. Effects are shown for (A) growth rates (% d^{-1}) and (B) survival during the OAW x nutrient experiment and (C) survival during the upwelling experiment. Df: degrees of freedom, SS: sums of squares and MS: mean squares. ‘Den. Syn. Error df’ and ‘Den. Syn. Error MS’ describe the denominator synthesis of degrees of freedom and mean squares, respectively. (Datasets of area and growth values can be found in the PANGAEA dataset).

Source of variation	*df*	*SS*	*MS*	*Den. Syn. Error df*	*Den. Syn. Error MS*	*F-value*	*p-value*
(A) Growth rates OAW x nutrient experiment							
OAW	1	47.659	47.659	6.156	1.188	40.111	< 0.001
Nutrients	1	20.468	20.468	8.469	1.428	14.335	< 0.05
OAW x Nutrients	1	13.473	13.473	8	1.339	10.055	0.013
Mesocosm (OAWxNutrients)	8	10.720	1.340	135	0.389	3.443	0.001
Sibling	15	27.120	1.808	5.276	0.325	5.558	0.030
Sibling x OAW	15	3.561	0.237	135	0.389	0.610	0.863
Sibling x Nutrient	15	7.156	0.477	135	0.389	1.226	0.260
Error	135	52.542	0.389				
(B) Survival OAW x nutrient experiment							
OAW	1	4,069	4,069	14.366	0.867	4.694	0.048
Nutrients	1	2,903	2,903	7.061	0.513	5.656	0.049
OAW x Nutrients	1	1,272	1,272	8	0.535	2.376	0.162
Mesocosm (OAWxNutrients)	8	4,281	0,535	135	0.163	3.284	0.002
Sibling	15	11,786	0,786	12.535	0.473	1.662	0.186
Sibling x OAW	15	7,420	0,495	135	0.163	3.036	< 0.001
Sibling x Nutrient	15	2,117	0,141	135	0.163	0.866	0.603
Error	135	21,998	0,163				
(C) Survival Upwelling experiment							
OAW	1	4.907	4.907	8.398	0.550	8.914	0.017
Nutrients	1	0.322	0.322	5.284	0.423	0.761	0.421
OAW x Nutrients	1	2.913	2.913	8	0.516	5.647	0.045
Mesocosm (OAWxNutrients)	8	4.127	0.516	135	0.164	3.150	0.003
Sibling	15	10.711	0.714	3.504	0.105	6.793	0.052
Sibling x OAW	15	2.976	0.198	135	0.164	1.212	0.270
Sibling x Nutrient	15	1.057	0.070	135	0.164	0.430	0.968
Error	135	22.106	0.164				

Sibling group differences in growth

RGR of the 16 sibling groups differed significantly (Split-plot ANOVA, factor: sibling group, p-value < 0.05, Table 2A). Nutrient enrichment at ambient temperature and CO_2 enhanced growth significantly only of the sibling groups 3, 4 and 7 (Fig 3B). In contrast, nutrient enrichment under high OAW conditions enhanced growth in most sibling groups, notably in the groups 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15 and 16. High OAW at ambient nutrient conditions decreased growth in most sibling groups, i.e. 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 15 and 16. In contrast, under nutrient enrichment the negative effect of high OAW was mitigated so that growth was significantly decreased only in the 6 sibling groups 2, 4, 7, 8, 10 and 12 (Fig.3A). The factor 'sibling group' did not interact significantly with 'OAW' or with 'nutrients' (Split-plot ANOVA, sibling group x OAW, sibling group x nutrients, p-value > 0.05, Table 2A).

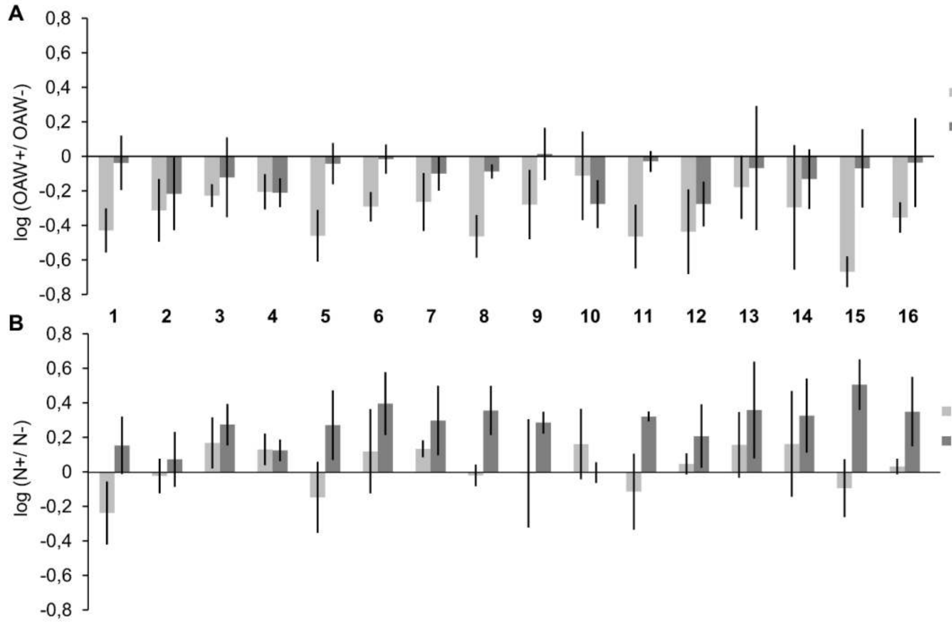

Fig 3. Log effect ratios of growth in 16 sibling groups during the OAW x nutrient experiment. Log effect ratios (mean ± 95 % confidence intervals, n=3) of (A) OAW effects were calculated as log (growth OAW+/ growth OAW-) under ambient and high nutrient conditions (N-, N+) and (B) log effect ratios for nutrient effects calculated as log (growth N+/ growth N-) at ambient and high OAW (OAW-, OAW+).

Sibling group differences in survival

OAW+ at ambient nutrient concentrations decreased survival significantly in 5 out of 16 sibling groups, notably 3, 7, 12, 14 and 15. The different survival responses to increased OAW of sibling groups are reflected in the significant interaction of the factors 'OAW' x 'Sibling group' (Split-plot

ANOVA, p-value < 0.001, Table 2B). Nutrient enrichment tended to mitigate the negative effect of OAW and this buffering influence was significant in the 4 sibling groups 7, 12, 14 and 15 (Fig 4A). Nutrient enrichment under ambient temperatures and CO_2 did not affect sibling groups' survival. In contrast, nutrient enrichment under warming and acidification generally tended to improve survival and significantly enhance survival in the groups 3, 7, 12 and 15 (Fig 4B, Table 2B).

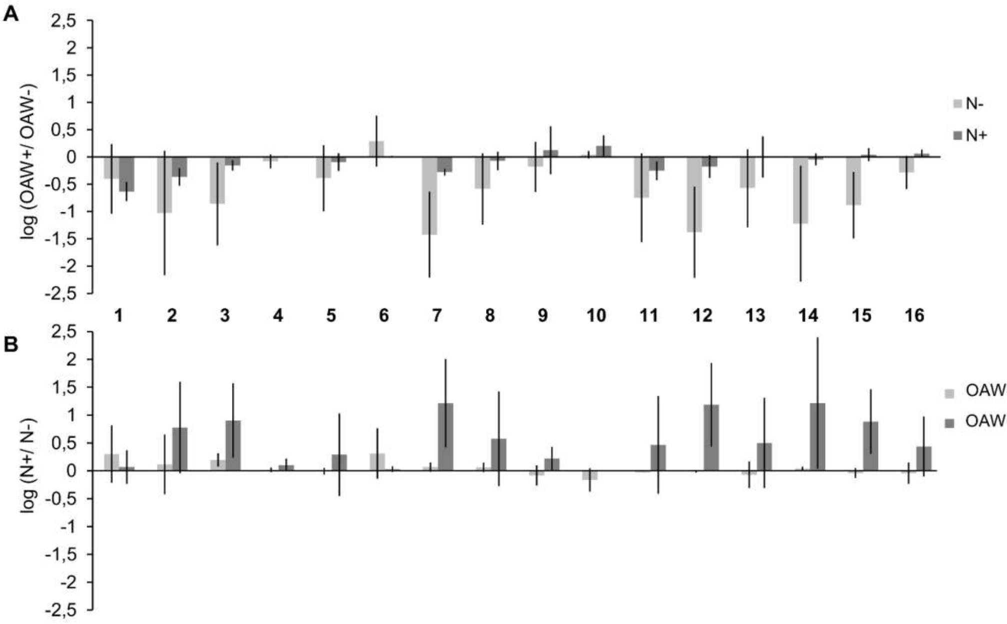

Fig 4. Log effect ratios of survival in 16 sibling groups during the OAW x nutrient experiment. Log effect ratios (mean ± 95 % confidence intervals, n=3) of (A) OAW effects were calculated as log (survival OAW+/ survival OAW-) under ambient and high nutrient conditions (N-, N+); (B) log effect ratios for nutrient effects were calculated as log (survival N+/ survival N-) at ambient and high OAW (OAW+, OAW-).

CN ratio

Germling carbon and nitrogen content (% DW) as well as the CN ratio did not differ significantly among the nutrient treatments (Split-plot ANOVA, p-value > 0.05, Table 3). The CN ratio was 10.56 ± 1.13 under ambient nutrient conditions and 7.18 ± 3.06 (mean ± SD) under high nutrient conditions.

Table 3. Nutrient effect on carbon and nitrogen content (% DW) and on the CN ratio.

Split-plot ANOVA with the fixed factor 'Nutrient' and the random factors 'Mesocosm' and 'Sibling group'. Effects of the nutrient treatment were analysed for (A) the carbon content (% DW), (B) the nitrogen content (% DW) and (C) the CN ratio. Df: degrees of freedom, SS: sums of squares and MS: mean squares. 'Den. Syn. Error df' and 'Den. Syn. Error MS' describe the denominator synthesis of degrees of freedom and mean squares, respectively.

Source of variation	*df*	*SS*	*MS*	*Den. Syn. Error df*	*Den. Syn. Error MS*	*F-value*	*p-value*
(A) Carbon content							
Nutrients	1	36.563	36.563	1.998	17.839	2.05	0.289
Mesocosm (Nutrients)	4	79.878	19.969	16	15.482	1.290	0.315
Sibling	4	145.584	36.396	4	13.352	2.726	0.177
Sibling x Nutrients	4	53.407	13.352	16	15.482	0.862	0.507
Error	16	247.718	15.482				
(B) Nitrogen content							
Nutrients	1	6.676	6.676	3.669	38.545	0.173	0.700
Mesocosm (Nutrients)	4	159.792	39.948	16	5.462	7.314	0.002
Sibling	4	16.647	4.162	4	4.059	1.025	0.491
Sibling x Nutrients	4	16.235	4.059	16	5.462	0.743	0.576
Error	16	87.387	5.462				
(C) CN ratio							
Nutrients	1	< 0.001	< 0.001	4.488	333.810	< 0.001	1
Mesocosm (Nutrients)	4	1226.652	306.663	16	42.265	7.256	0.002
Sibling	4	297.260	74.315	4	69.412	1.071	0.474
Sibling x Nutrients	4	277.649	69.412	16	42.265	1.642	0.212
Error	16	676.237	42.265				

Upwelling experiment (Mid-September 2014)

O_2, salinity and temperature conditions

The mean (± SD) of O_2 concentration during the experimental upwelling in the PVC boxes was 2.71 ± 0.37 mg L^{-1} (range 1.44 mg L^{-1} to 5.61 mg L^{-1}). Mean temperature during the hypoxia experiment was 16.35 ± 0.29 °C (range 15.8 °C to 17.5 °C). pH was 7.40 and salinity was 22.8 psu in the deep water measured once on the day before the start of the hypoxia treatment.

Survival

In all sibling groups with different treatment histories, the three days of hypoxia reduced germling survival significantly. Survival was highest for germlings pre-treated at ambient conditions (OAW-N-) and lowest for germlings previously treated at high OAW and ambient nutrients (OAW+N-) (Fig 5). Thus, high OAW significantly doubled the sensitivity to subsequent hypoxia as compared to a nonwarmed, non-acidified regime (Split-plot ANOVA, OAW, p-value < 0.05, Fig 5, Table 2C). Nutrient addition during the preceding experiment enhanced sensitivity to hypoxia in germlings stemming from a regime of ambient temperature and CO_2 conditions but not for those from a OAW+ regime as reflected in the significant interaction between 'OAW' and 'Nutrients' (Split-plot ANOVA, OAW x Nutrients, p-value < 0.05, Fig 5, Table 2C).

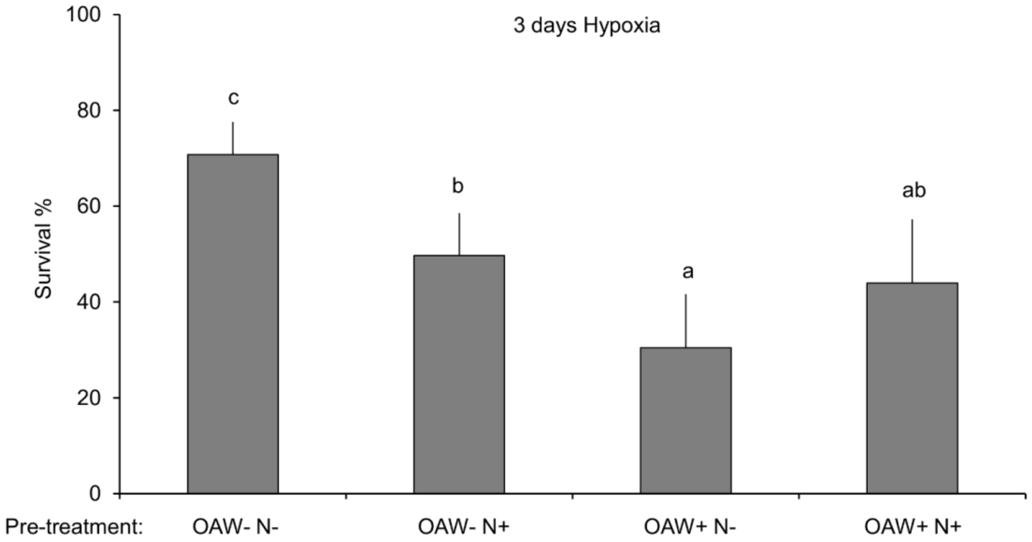

Fig 5. Survival of *F. vesiculosus* germlings after 3 days of hypoxic upwelling. Survival (%, mean +SD, n=3) in 16 sibling groups of *F. vesiculosus* germlings previously treated under the four treatment combinations of the OAW x nutrient experiment: OAW-N-, OAW-N+, OAW+ N-, OAW+N+. Different letters above the bars indicate significant differences (p-value < 0.05) between the treatments after Tukey's HSD.

Discussion

The simulated OAW as expected for 2110 strongly reduced survival and growth of *F. vesiculosus* germlings. The analysis of the single factors warming and acidification in a previous study at the KOB showed that warming was the main driver of mortality of *F. vesiculosus* germlings, while acidification played a minor role [33]. At temperatures > 27 °C, reduction in growth and photosynthetic efficiency were observed in adult *F. vesiculosus*, finally resulting in necrosis [34]. Reduced growth of germlings under heat stress (> 25 °C) was also observed in this work. This shows that the upper limits of thermotolerance of *F. vesiculosus* performance are similar in early and adult life-stages.

However, in our multi-factorial design, the negative effects of warming on germling survival and growth rates were strongly mitigated by high nutrient concentrations. Earlier studies on Baltic adult *F.*

vesiculosus have shown that nutrient enrichment increases nutrient uptake [65] and enhances photosynthetic efficiency [27]. Similar responses were also observed in other algal species. *Ulva rigida* cultured under nutrient enrichment reacted with higher nitrogen uptake, higher nitrate reductase activity and higher growth rates. Moreover, the nitrogen reductase activity was enhanced under future (1000 µatm) compared to ambient pCO_2 (400 µatm) conditions [66]. Similarly, high pCO_2 enhanced nitrogen assimilation in the brown alga *Hizikia fusiforme* [67] and may have decreased the relative investment in the nitrogen-intensive protein biosynthesis [67]. Consequently, nitrogen may have been freed for other processes, such as growth and nitrogen storage [68]. Possibly, the *F. vesiculosus* germlings in our experiments also took up more nitrogen under nutrient enrichment when additional CO_2 was provided under acidified (and warmed) conditions, resulting in increased growth and survival. Our experimental design did not allow for disentangling the different possible mechanisms of mitigating effects of nutrients on either warming or acidification. Photosynthesis is regarded as one of the most heat sensitive metabolic activities in the plant cell [69, 70], with at least three major heat-stress sensitive sites in the photosynthetic machinery: the photosystems (mainly photosystem II with its oxygen-evolving complex), the ATP generating and the carbon assimilation processes [71]. Moreover, respiration rates are increased under warming [72]. As our response variables growth and survival represent responses integrating over many metabolic processes, several compensatory effects caused by high nutrient levels appear possible. In conclusion, nutrient enrichment compensated to some degree the severe negative effects of future heat stress on *F. vesiculosus* germlings, which may be further mitigated by higher carbon availability under acidified conditions. However, such direct beneficial effects of nutrient enrichment may be overridden by indirect detrimental effects of eutrophication (such as increases in water turbidity, sedimentation, grazing and abundance of epibiotic filamentous algae) at field conditions, as reviewed by Berger et al. [45]. During the OAW x nutrient experiment, the epibiota under ambient and enriched nutrient conditions have not been determined. Regular filtration of the water content of our experimental boxes kept the fouling load relatively low under both, ambient and high nutrient conditions, assuming that epibiota had no strong effects on the *F. vesiculosus* germlings.

Increased nitrogen uptake is accompanied by higher CO_2 uptake (even at ambient pCO_2), hence a constant CN ratio is maintained [66]. Our findings show that the CN ratio in *F. vesiculosus* germlings was lower in the high nutrient treatment. Although this difference was not significant, it suggests higher uptake rates of nitrogen under nutrient enrichment. Since the nitrogen concentration in Baltic *F. vesiculosus* thalli is lowest in summer [73], nutrient enrichment effects may be most conspicuous in this season. This may have contributed to the observed stress-mitigating effect of nutrient enrichment in *F. vesiculosus* germlings. The buffering of stress impact by additional resources was also observed in juvenile blue mussel *Mytilus edulis*, when high food conditions enhanced the tolerance to ocean acidification [74].

The different sibling groups showed high variations in survival under warming and acidification, indicating the enhanced potential for adaptation in genetically diverse populations [48]. The crucial role in genetic variation for recovery from disturbances has also been reported in estuarine macrophytes [75] allowing for adaptation under global change stress [76].

The three day hypoxia experiment in the KOB simulating an upwelling event induced substantial germling mortality. During a local upwelling event, deep water with low oxygen concentration, low temperatures an increased pCO_2 and high salinity is shoaling [77, 78]. In our upwelling treatment, temperature did not decrease considerably (16.41 ± 0.33 °C) compared to previous values of 19.26 ± 2.38 °C. Likewise, salinity (22.8 psu) did increase only slightly relative to the previous condition (Fig 1C) and the elevated CO_2 (as associated with hypoxic upwelling) has minor effects on germling survival [33] (Fig 1C). This leaves the low oxygen concentration (2.75 ± 0.41 mg L^{-1}) during the upwelling event as the most likely driver of germling mortality. Mortality during the three days of upwelling was considerably higher than during the two months of the preceding experiment, illustrating the high susceptibility of *F. vesiculosus* germlings to hypoxia. This susceptibility is probably due to reduced respiration rates under dark conditions, as it was also observed under hypoxic conditions in *Cladophora vagabunda* and *Gracilaria tikvahiae* [79]. Reduced respiration rates are accompanied by decreased provision of ATP and biosynthetic precursors leading to higher stress sensitivity [80] and to a reduced metabolism [79]. Susceptibility to hypoxia impacts was highest on germlings which previously experienced warmed and acidified conditions. Thus, the impact of hypoxic upwelling events in the future may be amplified by synchronous OAW. The assumed increased respiration under warming [72] may have further increased the O_2 debt, which could not be balanced under hypoxic conditions. Consequently, *F. vesiculosus* germlings grown under high compared to ambient temperatures were less tolerant to hypoxia. Thus, germlings grown under high nutrient levels experienced higher mortality under hypoxia compared to those grown at low nutrient levels. Zou et al. [68] demonstrated that under high-nitrogen conditions, respiration was enhanced by high CO_2 compared to ambient CO_2 conditions in the macroalga *H. fusiforme*. Consequently, in algae growing under nutrient enrichment increased respiration might be necessary to support higher maintenance demands (e.g. due to increased RUBISCO contents) and greater uptake of extra nitrogen [68]. This nutrient-driven higher metabolism may have rendered these germlings more susceptible to hypoxia.

In summary, the responses to hypoxia depended on the preceding OAW x nutrient treatments we applied. This suggests that there are different protective mechanisms in *F. vesiculosus* germlings that vary with the type of stressor. Future expansions of hypoxic areas in the Baltic Sea [7] will have severe effects on *F. vesiculosus* recruitments, as observed in this experiment, as well as on the benthic community in general [81]. We demonstrated that the net impact of global change including warming, acidification, eutrophication and hypoxia may depend on the interaction among these global and regional factors. This finding underscores the importance for analysing the combined effects of multiple stressors and their interconnectivity for accurate predictions of future scenarios [3]. Moreover, the indirect effects of global change may be more significant than the direct effects [13]. Scaling up multiple stressors is crucial for predicting the fate of *F. vesiculosus* populations [82].

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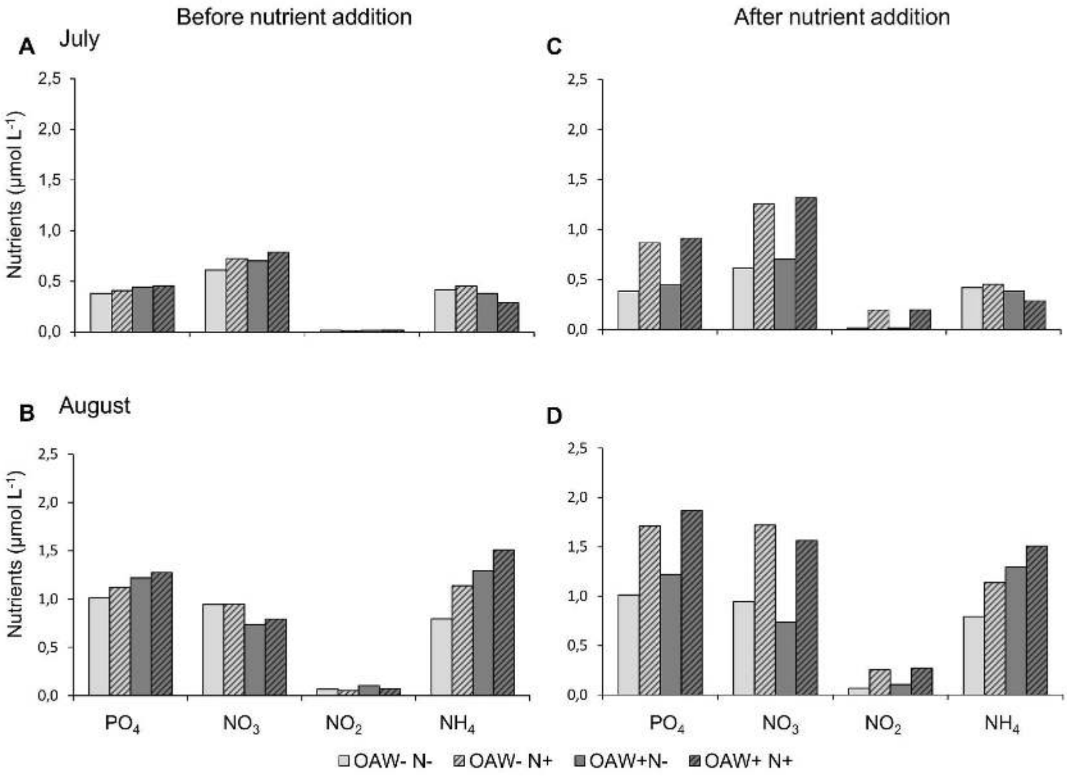
Supplementary Information



S1 Fig. Nutrient concentrations in the experimental boxes

Nutrient concentrations within the experimental germling boxes before (A, B) and after (C, D) the bi-weekly addition of the nutrients (PO_4, NO_3, NO_2) as well as initial NH_4 conditions in µmol L^{-1} in July and August. Initial nutrient concentrations were measured six times per month in the main KOB tank before the water addition to the boxes. Nutrient concentrations after additions were determined by adding the sum of the initial and additional nutrient concentration.

Paper IV

Manuscript will be submitted to Ecology Letters

Genotypic correlation in response to global change parameters in seaweed germlings indicates potential for modulation of adaptation success

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Keywords: Seaweed *Fucus vesiculosus*, global climate change, ocean warming, ocean acidification, eutrophication, local upwelling, hypoxia, multifactorial change, genetic diversity

Abstract

Populations responses to a changing climate will be driven by multiple factors interacting in their effects. We explored the potential at the genetic level of correlation patterns of sensitivities towards single drivers to modulate the sum response to multiple changes. In a near-natural scenario, genetically different families (germlings) of the seaweed *Fucus vesiculosus* were exposed to warming and acidification. Combined warming and acidification (OAW) was crossed with nutrient enrichment, followed by a final hypoxic upwelling. Families tolerant to acidification also tended to tolerate warming, indicating a positive genetic correlation of the two sensitivities. Seaweed may adapt to these two conditions simultaneously and faster than estimated so far. OAW tolerance correlates positively with nutrient enrichment but anticorrelates with tolerance towards hypoxic upwelling. Genetic selection for tolerance to OAW would enhance a population's sensitivity to hypoxia. Concluding, genotypic correlations should be incorporated into evaluations of the adaptive potential towards multifactorial climate change.

Introduction

Marine primary producers contribute about 50% of the world's carbon fixation (Field *et al.* 1998). Whether Climate Change will increase or decrease marine net primary production is has been under discussion (Hein & Sand-Jensen 1997; Gattuso & Buddemeier 2000; Schippers *et al.* 2004; Gao *et al.* 2012). This is mainly because interactions of multiple stressors are complex in their effects (Wahl *et al.* 2015b) and the speed of adaptation of photosynthesizing species to the forecasted changes (Reusch & Wood 2007; Reusch & Boyd 2013) is difficult to predict.

Similar to research on terrestrial plants (Way *et al.* 2015), few studies in marine research have focused on factorial treatments of CO_2 x temperature and fewer studies have attempted to additionally include genetic variation to assess adaptive potentials (but see: Reusch & Boyd 2013; Schlüter et al. 2014). Seaweeds are major contributors to coastal primary production (Mann 1973). Some authors report that most seaweed species are not saturated at current inorganic carbon concentrations (DIC) in seawater and thus are expected to increase their photosynthetic and growth rates under elevated CO_2 (Koch *et al.* 2013). Other authors state that many seaweeds use carbon concentrating mechanisms (CCMs) and thus are saturated under current DIC (Beardall *et al.* 1998). However, fundamental linkages between elevated CO_2 and temperature on photorespiration, enzyme systems and carbohydrate production exist in seaweeds (Sukenik *et al.* 1987; Surif & Raven 1989; Koch *et al.* 2013), but net interactive effects of elevated CO_2 and temperature are largely unknown (Fernandez *et al.* 2015).

In addition to temperature and CO_2 increase, many coastal ecosystems worldwide are challenged by increasing eutrophication and hypoxia in the course of Global Change (Rabalais *et al.* 2009). In many parts of the Baltic Sea, eutrophication occurs as a result of increasing nutrient input from land to sea due to a combination of intensifying agriculture and higher precipitation (HELCOM 2007 - 2011). A more stable stratification due to warming and more intense organic sedimentation after algal blooms (e.g. Ærtebjerg *et al.* 2003) will increase hypoxic areas within this century (< 2 mg O_2 L^{-1}, Meier *et al.* 2011). This increase of hypoxic areas affects local biota mainly through reduction of respiration rates leading to energy debts (Gray *et al.* 2002). Furthermore, upwelling events sporadically transport such stressful conditions also to shallow habitats by shoaling acidified, nutrient rich, hypoxic and cooler waters (Melzner *et al.* 2013). Hence, there is a high need of expanding our knowledge towards multiple drivers in a natural context to better understand and predict climate change impacts on adaptive processes in marine populations (Jutterström *et al.* 2014; Riebesell & Gattuso 2015).

The seaweed *Fucus vesiculosus* is a foundation species of the intertidal and shallow subtidal habitat in the North Atlantic including the Baltic Sea. It is highly sensitive to ocean warming. When a threshold of 27°C is exceeded, growth rates and survival of adult *F. vesiculosus* are reduced (Graiff *et al.* 2015). Survival of early life-stages in the closely related *Fucus serratus* is also reduced, but growth rates of the surviving germlings are increased with temperatures rising from 6 to 22 °C (Nielsen *et al.* 2014). Generally, the early life-stages of marine organisms are presumed more sensitive to global climate change compared to adults (Pineda *et al.* 2012) and hence, early development stages may constitute the critical stage and therefore the bottleneck for the persistence of seaweed species (Coelho *et al.* 2000). However, despite the emerging recognition that global change is multifactorial and the responses to it are multivariate, to date most research of coastal ecosystems was limited to single factors and single (mostly adult) life-stages (e.g. Wahl *et al.* 2015c).

In a recent study, considerable variation among genetically dissimilar families of *F. vesiculosus* germlings in response to a warming x acidification treatment was found (Al-Janabi *et al.* 2016). This variation is a prerequisite for positive selection. Whether the variation among genotypes of sensitivities towards different environmental factors correlates or not will determine selection under multiple drivers. Genetic correlations in responses to ocean warming and acidification, eutrophication and hypoxia are explored in the present study. Genetic correlations among traits may increase or decrease the rate of adaptive evolution depending on whether they are positively or negatively correlated with respect to the fitness landscape (Sunday *et al.* 2014: Box 2, Figure II). When sensitivities towards different drivers are genetically positively or negatively related, selection under one driver, such as ocean warming, may modulate the sensitivity towards other drivers such as acidification or hypoxia. For example, gastrulation success in the sea urchin *Centrostephanus rodgersii* showed a significant genetic correlation between the responses to ocean warming and acidification, indicating a potential for correlated responses to selection imposed by the two stressors

(Foo *et al.* 2012). The analysis of phenotypic correlations of sensitivities towards environmental changes is a convenient estimate of genetic correlations (Roff 1996).

In the present study, we recorded the phenotypic responses growth and survival of genetically dissimilar families of *F. vesiculosus* germlings exposed to ocean warming, acidification, nutrient enrichment and hypoxia (in varying combination). We hypothesized that the responses in *F. vesiculosus* families correlate positively or negatively towards (i) ocean warming and acidification (ii) combined ocean warming and acidification (OAW) and hypoxia and (iii) OAW and nutrient enrichment.

We found positive genetic correlation between sensitivities towards acidification and warming, and between combined OAW and nutrient enrichment. In contrast, we found a negative correlation between the sensitivities towards OAW and hypoxia.

Material and Methods:

Experimental design and course of the experiments

For all experiments, germlings from full-sib families were produced following a controlled protocol and reared on sandstones, so that all germlings on one sandstone had the same mother and father. Different families had different parents. We thus assume that genetic variability within families was substantially less than that among families. Sandstones with germlings were then distributed among the different treatment combinations. Each family was placed into each of the 12 mesocosms (described as Kiel Outdoor Benthocosms below), with n=3 replication of the same treatment and family. This ‘common garden’-setting reducing between-family environmental differences to a minimum allows us to assume that between-family differences in responses have a strong genetic basis. In spring and summer 2013, eight and seven full-sib families, respectively, were exposed to a temperature x CO_2 (Temp x CO_2) treatment, whereas in summer 2014, 16 families were exposed to a combined treatment of warming and acidification, “OAW” crossed with a nutrient treatment (OAW x N). These latter 16 families were subsequently exposed to an upwelling experiment.

Collection and gamete acquisition

To create eight different families of germlings for the experiments in spring and summer 2013, a total of 53 fertile *F. vesiculosus* were sampled in the southwestern Baltic Sea (Bülk, Germany, 54°27.327 ´N, 10°11.977 ´W) at the end of November 2012. To ensure for genetic variation among prospective parents, the distance between individuals sampled was at least 2 meters, which is the estimated maximum dispersal distance of *F. vesiculosus* gametes (Lifvergren 1996; Serrão *et al.* 1997).

Collected algae were transported to the lab in cooler boxes. From each alga, all fertile receptacles were cut and their gender was determined under the microscope at 100x magnification (Olympus BH-2). Parental pairs were formed by combining the receptacles of a male and a female adult *Fucus* individual. Receptacles were rinsed in tap water, blotted dry and placed in the darkness at 8-10 °C. After 5 days, the receptacles were immersed in sand-filtered seawater (15-16 psu) and exposed to light emitted by an aquarium lamp (110 µmol photons m^{-1} s^{-1}) for 3 hours to allow for gamete release and for egg fertilisation. A homogeneous zygote suspension was created by vigorous stirring and 0.67 ml solution was pipetted onto the upper surface of a sandstone cube with 2 cm edge length. *F. vesiculosus* germlings were cultured in sand-filtered seawater and monitored during 8 weeks in a room with natural light conditions with weekly water exchange (15-16 psu) at 8 °C until being transferred to the Kiel Outdoor Benthocosms (KOB). The KOB facility is described in detail by Wahl *et al.* (2015a).

For the experiments in summer 2014, 16 families were established following the same procedure regarding collection and gamete acquisition but with the following exceptions: here, a total of 64 fertile *F. vesiculosus* (46 females, 18 males) were collected in mid-June 2014, receptacles were stored in the dark for 6 days at 14 °C, 1 ml of a homogeneous suspension of fertilised eggs was pipetted onto the sandstone surface for each family, and all germlings were cultured during 3 weeks at 15 °C until introducing them to the Kiel Outdoor Benthocosms (KOB).

Temperature, CO_2, nutrient enrichment and local upwelling treatments

All experiments were performed at a near-natural scenario in the Kiel Outdoor Benthocosms (Wahl *et al.* 2015a). In spring and summer 2013, *F. vesiculosus* germlings were exposed to all combinations of the two treatment factors temperature and CO_2 at the two levels "present" and "future". The "present" conditions corresponded to the actual in situ conditions of Kiel Fjord at 1 m depth transferred to the experimental tanks of the KOB in real time by a continuous flow-through (1 tank-volume per day, i.e. 1500L/ 24 h). "Future" conditions according to the predictions of the year 2110 in the Baltic Sea (Graham *et al.* 2008; Elken *et al.* 2015) were achieved by dynamically adding 5 °C to the actual ambient temperature of the Kiel Fjord and by increasing the CO_2 concentration in the hooded headspace of the tanks to 1100 µatm CO_2.

The continuous flow-through assured that the natural fluctuations (Fig S1) of all environmental variables were transported into the experimental tanks in all treatment combinations. In the 12 experimental units of the KOB, warming and acidification were orthogonally crossed producing four treatment combinations replicated thrice: ambient temperature and non-acidified; warmer and non-acidified, ambient temperature and acidified; warmer and acidified. During the natural reproduction periods, sandstones were protected from newly settling germlings by placing them into PVC boxes (70 cm x 40 cm x 12 cm) suspended within the tanks. This set-up ensured the maintenance of the treatments by temperature exchange through the thin PVC walls (6 mm) and for CO_2 exchange with the KOB headspace at their water surface. Water with the temperature and CO_2 conditions of the KOB tanks was partially replaced biweekly into the PVC box after filtration through 50 µm mesh to prevent the accidental introduction of new *F. vesiculosus* eggs (about 100 µm in diameter). In mid-June, when tanks of KOB were serviced, *F. vesiculosus* germlings were stored in indoor mesocosms during 14 days while maintaining the respective temperature and CO_2 conditions. Germlings were kept in four mesocosms, one for each of the treatment combinations. Temperatures were controlled by using internal heater elements (600 W, Schego Titan, Schemel & Goetz, Offenbach am Main, Germany) and values were set according to the delta treatment. The highCO_2 conditions were achieved by aeration with CO_2 enriched air (1000 µatm CO_2) directly into the water of the mesocosms. Daily measurements of temperature and pH were performed. *F. vesiculosus* germlings were acclimatized at the KOB during 8 weeks before the experiment started. Growth and survival were measured for spring (02.04.2013 – 30.05.2013) and for summer (31.05.2013 – 08.08.2013).

In summer 2014 (mid-June until mid-September 2014) the factor nutrient was crossed with the OAW treatment. Nutrient enrichment, i.e. the increase of the concentrations of NO_2, NO_3, and PO_4, was achieved by doubling the mean concentration of each nutrient measured during the last seven years (2016 – 2013) (Table 1). We kept the natural P:N ratio of the Kiel Fjord, which is rather N-limited and off the Redfield ratio. The chemicals $NaNO_2$ (Merck, Germany), $NaNO_3$ (Carl Roth, Germany) and $H_2NaO_4P.H_2O$ (ACROS organics, Germany) were dissolved in fjord water 10 minutes before adding them with syringes to the PVC boxes. The treatments were as follows: the warming and acidification were combined to a single factor "ocean warming and acidification" (OAW) in the two levels "present" (ambient temperature and non-acidified) and "future" (warmer and acidified) (Fig. S2) and crossed orthogonally with the factor nutrient with the two levels: ambient (actual nutrient concentration) and nutrient enrichment. The resulting four treatments were: OAW- N- (ambient temperature and CO_2 and low nutrients), OAW- N+ (ambient temperature and CO_2 and high nutrients), OAW+ N- (future temperature and CO_2 and low nutrients) and OAW + N+ (future temperature and CO_2 and high nutrients).

	July		August		September	
	Present	Future	Present	Future	Present	Future
NO_2	0.53	1.05	0.77	1.54	1.27	2.54
NO_3	0.18	0.36	0.20	0.40	0.22	0.44
PO_4	0.46	0.93	0.59	1.19	1.06	2.11

Table 1 Nutrient concentrations in µmol l^{-1} in the present (mean of the last 7 years of the respective months) and future nutrient conditions (doubled amounts of the present nutrient concentrations) for NO_2, NO_3 PO_4.

In mid-September, subsequent to 2 months of the OAW x Nutrient experiment, the 16 families of all experimental units experienced an upwelling event during three days by a continuously flow-through of water pumped from 15 m water depth from Kiel Fjord, which was hypoxic during that time (O_2 = 2.71 ± 0.37 mg l^{-1}, T = 16.35 ± 0.29 °C, pH = 7.403, Sal = 22.8). Acidified conditions, occurring also during upwelling events, were not observed to decrease *F. vesiculosus* survival in previous experiments (Al-Janabi *et al.* 2016). Compared to the previous conditions in the KOB, temperature and salinity were not strongly affected by the upwelling treatment (Fig. S2). Hypoxia with a mean of (± SD) 2.75 ± 0.41 mg O_2 L^{-1} differed from the previous oxygen concentrations under ambient conditions (OAW -) 8.913 ± 0.38 mg O_2 L^{-1} (Fig. S3).

Temperature and CO_2 were measured daily with a calibrated sensor (pH, Mettler Toledo GmbH, Giessen, Germany) and salinity was measured with a conductivity meter (WTW Cond 3110 + Tetra Con 325, Wissenschaftlich Technische Werkstätten, Weilheim, Germany) (Fig. S1). During the hypoxia treatment, oxygen and temperature were logged every 10 minutes with a Multi WTW Oxy 3515 (Fig. S3), while pH and salinity from 15 m depth were measured on the day before the experiment as described above.

Survival

Survival of *F. vesiculosus* germlings was determined as the % of surviving germlings between the start (t_0) and the end (t) of respective experiments: Temp x CO_2 spring and summer experiments 2013; OAW x N experiment 2014; and upwelling experiment 2014. The number of germlings for each experimental population was determined under the binocular at 25 x magnification. Survival % was determined as: $\mathrm{Survival} = \frac{\mathrm{Number\ t}}{\mathrm{Number\ t_0}}.100$

Growth

Germlings' growth was determined by recording digital images of 10-15 germlings per experimental population at 40 x magnification (SteREO Discovery. V8 – Carl Zeiss Jena GmbH) according to the method used by Steen and Scrosati (2004). The side-view of the single germlings was measured by means of image analysis using Image J 1.45s (National Institutes of Health, USA). For each experimental population, the mean area was calculated. The germling areas were measured at the beginning (Area t_0) and at the end (Area t) of the experiments (Temp x CO_2 experiments in spring and summer 2013; OAW x N experiment summer 2014 and upwelling experiment 2014). Individuals from each experimental population were chosen randomly for measurements since germlings were too small to be labelled. Growth was calculated as the ratio between Area t and Area t_0 as: $\mathrm{Growth} = \frac{\mathrm{Areat}}{\mathrm{Areat_0}}$

Correlation of sensitivities

Following the conceptual model of Vinebrooke *et al.* (2004), the correlation of sensitivities was explored by ranking the families according to their mean response (n = 3) to the factors (temperature, CO_2, OAW, Hypoxia) and by correlation of these factor-specific sensitivity ranks. Rank 1 always represented the highest sensitivity, i.e. strongest response, to the given factor. Significances (p-value) and correlation coefficients (*R*) were calculated by using Spearman Rank correlation (R Development Core Team, 2014). If the correlation was positive and significant we interpret this as a positive genetic correlation of the response towards these two factors. If the correlation was negative and significant we interpret this as a negative genetic correlation of sensitivities towards these two factors. Responses to the single treatments by each family were calculated as described below.

Calculations of the sensitivities to temperature and CO_2

$\text{Warming sensitivity} = \frac{\text{Survival T+}}{\text{Survival T−}}$ at non-acidified conditions and

$\text{Acidification sensitivity} = \frac{\text{Survival CO}_2\text{+}}{\text{Survival CO}_2\text{−}}$ at ambient temperatures.

$\text{Warming sensitivity} = \frac{\text{Growth T+}}{\text{Growth T−}}$ at non-acidified conditions and

$\text{Acidification sensitivity} = \frac{\text{Growth CO}_2\text{+}}{\text{Growth CO}_2\text{−}}$ at ambient temperatures.

Eight families were used in spring and seven families in summer due to die-off of one family.

Calculations of the sensitivities to OAW and Hypoxia

$$\text{OAW sensitivity} = \frac{\text{Survival OAW+}}{\text{Survival OAW−}}$$

Only those germlings were analysed on the hypoxia sensitivity which had experienced ambient conditions previously:

$$\text{Hypoxia sensitivity} = \frac{\text{Survival Hypoxia}}{\text{Survival OAW−}}$$

Sensitivities of 16 families were calculated.

Calculations of the sensitivities to OAW and nutrient enrichment

$\text{OAW sensitivity} = \frac{\text{Growth OAW+}}{\text{Growth OAW−}}$ at ambient nutrient conditions.

$\text{Nutrient enrichment sensitivity} = \frac{\text{Growth N+}}{\text{Growth N−}}$ at ambient temperature and CO_2 conditions.

Sensitivities of 16 families were calculated.

Results

Abiotic factors

During the warming x acidification experiment in spring and summer 2013, mean temperature (± SD) was 14.34 ± 5.23 °C under ambient conditions, and 18.89 ± 5.73 °C under warmed conditions. The mean difference between the ambient and the warm treatment was 4.54 ± 0.85 °C. The mean pH (± SD) was 8.30 ± 0.32 under ambient conditions and 8.09 ± 0.33 under acidified conditions, with a mean difference of 0.22 ± 0.08. Mean salinity (± SD) under ambient conditions was 14.5 ± 1.27 psu (Fig. S1).

During the OAW x nutrient experiment (summer 2014), the mean temperature (± SD) under ambient conditions was 19.26 ± 2.38 °C and under warmed condition 23.64 ± 2.68 °C. The mean difference between the warmed and ambient conditions was 4.38 ± 0.61 °C. Mean pH (± SD) under ambient conditions was 7.95 ± 0.13 and under acidified conditions 7.63 ± 0.13. The mean difference in pH between the ambient and the acidified treatment was 0.32 ± 0.14. The mean salinity (± SD) under ambient conditions was 16.45 ± 1.54 psu (Fig. S2). During the local upwelling event, mean temperature (± SD) was 16.35 ± 0.29 °C and the mean O_2 concentration (± SD) was 2.71 ± 0.37 mg O_2 L^{-1} (Fig. S3).

Correlation of sensitivities to warming and acidification

Sensitivities to warming and acidification, resp., showed a significantly positive correlation in spring with regard to survival (%) (Fig. 1,a) and in spring and summer with regard to growth (% d^{-1}) (Fig. 1 b,c) (Spearmans' rank correlation, survival in spring, growth in spring and summer, respectively: R = 0.952, 0.929, 0.821; N = 8, 8, 7; p = 0.001, 0.002, 0.034, respectively). The correlation of families' sensitivities in summer with regard to survival was not significant but showed a positive trend (R = 0.143, N = 7, p = 0.783) (Fig. 1 d).

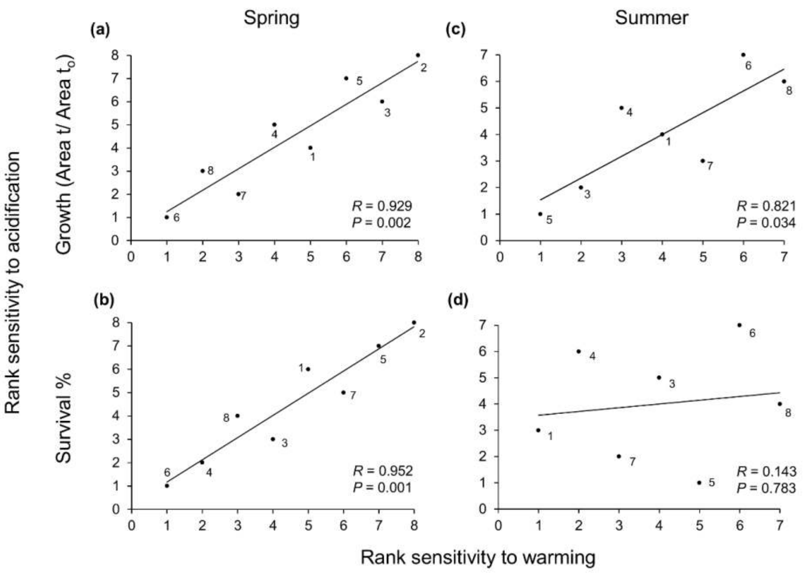

Fig. 1 Positive correlation of rank sensitivity per family representing sensitivities towards warming and acidification (a, b**:** growth and survival in spring 2013 and c, d: growth and survival in summer 2013, respectively). For each family, sensitivities towards each treatment factor were calculated in relation to the responses under ambient conditions (means for each family from n = 3). Ranks of families' sensitivities to warming were correlated to the rank of their sensitivity to acidification, where rank 1 stands for the group with the highest sensitivity and rank 8 for the lowest sensitivity to the given factor. In spring, the correlation was calculated for 8 families, in summer for 7 families. P-value *P* and correlation coefficient *R* from Spearman's Rank correlations are indicated. Numbers in the graph indicate family identity.

Correlation of sensitivities to warming, acidification and hypoxia

A significantly negative correlation of the survival responses to OAW and hypoxia was observed in the 16 families (Spearman's rank correlation, R = - 0.8088, p-value < 0.001, Fig. 2).

Correlation of sensitivities to nutrient enrichment and the combined factor warming and acidification

A positive correlation was observed regarding the sensitivities towards OAW and towards nutrient enrichment in growth in the 16 families (Spearman's rank correlation, R = 0.776, p-value < 0.001, Fig. 3). There was no correlation of the sensitivities towards OAW and towards nutrients regarding survival (Spearman's rank correlation, R = - 0.097, p-value > 0.05).

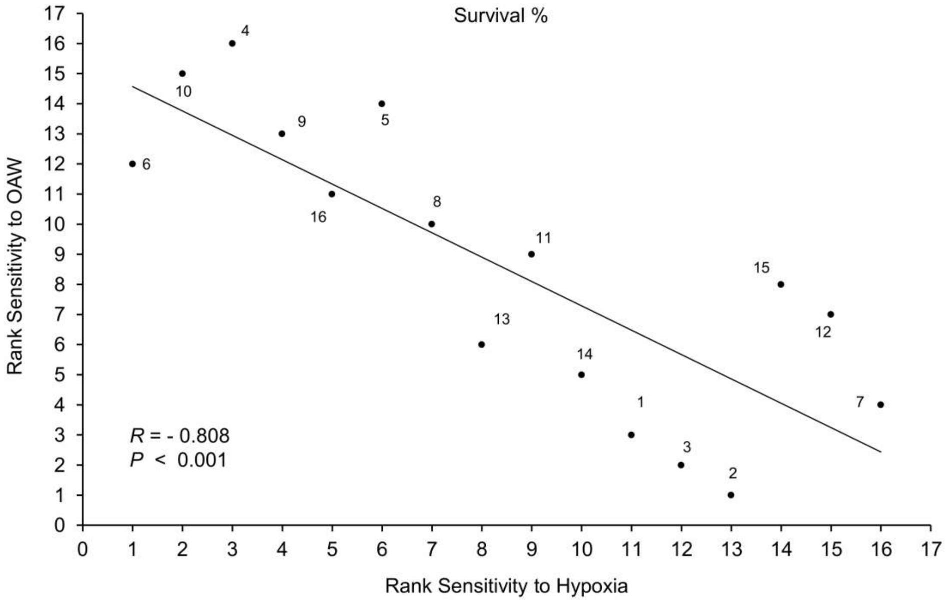

Fig. 2 Negative correlation of rank sensitivities per family representing sensitivities towards OAW and hypoxia in 16 families with regard to survival. For each family, the sensitivities towards each treatment factor were calculated in relation to the responses under ambient conditions (means for each family from n = 3). Families were ranked according to their sensitivities and ranks of both sensitivities were correlated. Rank 1 indicates highest sensitivity, rank 16 the lowest sensitivity to the given factor. P-value *P* and correlation coefficient *R* from Spearman's Rank correlation are indicated. Numbers in the graph indicate family identity.

Discussion

Estimates of species' adaptation speed to global change are a major issue in evolutionary biology and ecology (Hoffmann & Sgro 2011). Depending on the capacity of species to keep step with environmental changes, evolutionary rescue, migration or extinction will follow (Bell & Gonzalez 2009, 2011). Recent studies over many generations on species with short generation times have found that adaptation speed to new environments can be surprisingly fast, so that evolutionary rescue under climate change appears possible (Bell 2013; Lohbeck *et al.* 2013; Schlüter *et al.* 2014) at least when generation time is short. However, for species with several years of generation times, examples of fast adaptation exist also (Munday *et al.* 2013) but still selection experiments under global change conditions are rare (Reusch & Wood 2007). Only very few experimental studies provide insight into adaptation potentials of longer-lived species to multifactorial climate change. This lack of knowledge contrasts with the high ecological importance of many long-lived species in the marine realm, since typically in coastal ecosystems one or a few longer-lived species are habitat-formers (Bruno & Bertness 2001). Moreover, evolutionary rescue models are considered more appropriate for these species than for microorganisms, since macroorganisms face a greater risk of extinction (Dulvy et al. 2003).

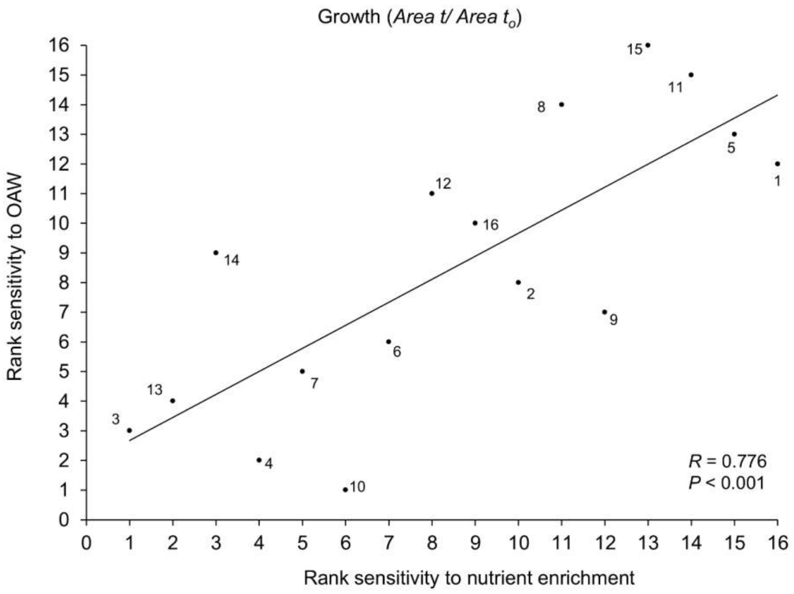

Fig. 3 Positive correlation of rank sensitivities per family representing sensitivities towards combined OAW and nutrient enrichment in summer 2014 for 16 families with regard to growth. For each family, the sensitivities towards each treatment factor was calculated in relation to the performance under ambient conditions (means for each family from n = 3). Rank 1 indicates highest sensitivity, rank 16 the lowest sensitivity to the given factor. P-value *P* and correlation coefficient *R* resulting from Spearman's Rank correlation are indicated. Numbers in the graph indicate family identities.

In the present study, we show that genetic correlations among sensitivities towards various climate change factors exist in the habitat-forming seaweed *Fucus vesiculosus.* Families more tolerant to warming were also capable to better benefit from acidification which enhanced growth and survival in spring, and growth in summer. This is, to our knowledge, the first study demonstrating for a marine photosynthesizing organism a genetic correlation of responses to the main features of climate change, ocean warming and acidification. Correlations in climate change sensitive traits have the potential to strongly affect the adaptation speed to climate change (Sunday *et al.* 2014). Sufficient genetic variation among experimental groups or organisms tested for this correlation, however, is a prerequisite to detect them. For photosynthesizing organisms, genetically based variation in responses to warming and acidification has been found in several studies, both in the terrestrial and the marine realm. Following also a family-based approach, Cantin *et al.* (1997) found significant genotypic differences for height, biomass, and water-use efficiency in jack pine seedlings. These seedlings significantly increased total biomass under OAW relative to ambient temperature and CO_2 conditions. Interestingly, seedling families kept their ranks in growth relative to other families, from the ambient to the combined warmed and acidified conditions.

Genetic correlations between sensitivities towards two environmental factors are often considered to be based on genetic pleiotropy (when one gene influences two or more unrelated phenotypic traits), or linkage disequilibrium (the non-random association of alleles of different genes at different loci) (Conner 2002; Frankham 2010). However, we find neither of the two likely. This is because first, we did not follow a Quantitative trait locus (QTL) approach on single traits, since our response variables growth and survival most likely integrated over many traits and genes, and second, we do not know which and how many traits are influenced by temperature and CO_2. Thus, we can only speculate about the causes for this correlation of responses. One possibility is a functional connection of the two factors, e.g. any temperature-driven enzyme kinetics towards better utilization of $CO_{2,}$ which is present in some families but not in others. A positive genetic correlation was also found between the responses towards OAW and nutrient enrichment. Again here, a functional linkage is conceivable, since higher carbon fixation resulting in stronger growth may require more nutrients. A negative genetic correlation between the responses towards OAW and hypoxia may point in the same direction: stronger growth under OAW may require more energy and thus higher respiration rates with higher O_2 demands which, under hypoxic conditions, is no longer possible. Since there is strong temporal and spatial habitat heterogeneity in $pCO_{2,}$ temperature, nutrient concentrations and O_2 conditions in the Baltic Sea, there is the possibility that past selection events at the habitat scale have produced these correlations of responses. Selective covariance can arise when natural selection directly causes correlated change in a suite of traits (Armbruster & Schwaegerle 1996). Thus, among-population or among-family covariance can also arise among traits that are not genetically correlated by pleiotropy or linkage.

Our results show that the ongoing direction of selection of *F. vesiculosus* will most likely move towards genotypes resistant to warming that better exploit high CO_2 and nutrient enrichment. We consider it possible that these genetic correlations can also be found in other photosynthesizing organisms. However, in areas where upwelling exists and/or where transient hypoxic conditions occur, those genotypes which are adapted to acidification and warming will be particularly threatened, thus further aggravating the decline of *F. vesiculosus* in the Baltic Sea. Our results corroborate the need of investigating responses to multiple stressors for revealing genetic correlations of sensitivities that would be hidden when stressors are analysed separately (Jutterström *et al.* 2014). Most studies also disregard natural variabilities: these are (i) fluctuations in the treatment factors during the experiments, which can have profound effects on experimental results (Small *et al.* 2015; Wahl *et al.* 2015c) and (ii) sufficient standing genetic variation in between experimental groups or organisms tested.

Understanding genotypic correlation of responses towards multiple environmental factors in the context of natural variability will help to better predict global change impact on marine populations.

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Supporting Information

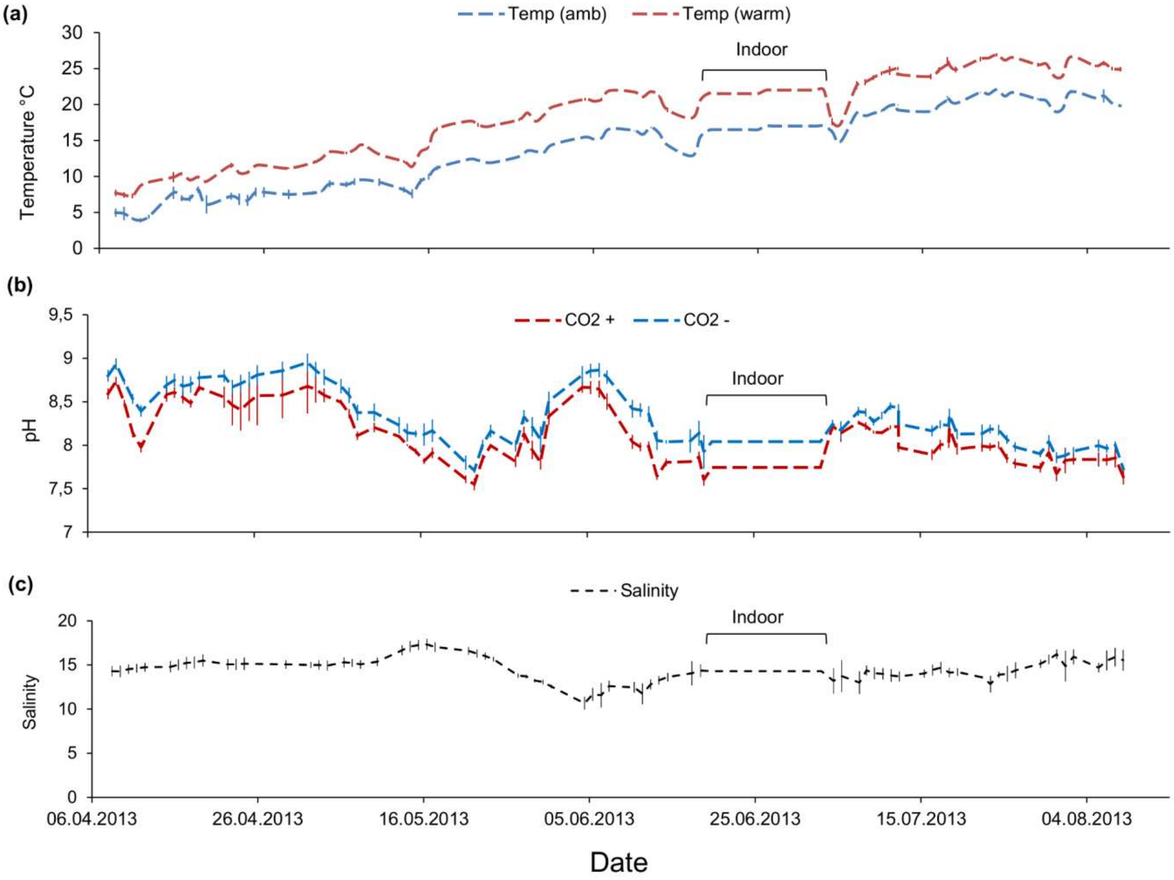

Fig. S1 Temperature (**a**, °C), pH (**b**, NBS), Salinity (**c**, psu) (mean, ±SD, n = 3) during the Temperature x CO_2 experiment in the Kiel Outdoor Benthocosms in spring and summer 2013.

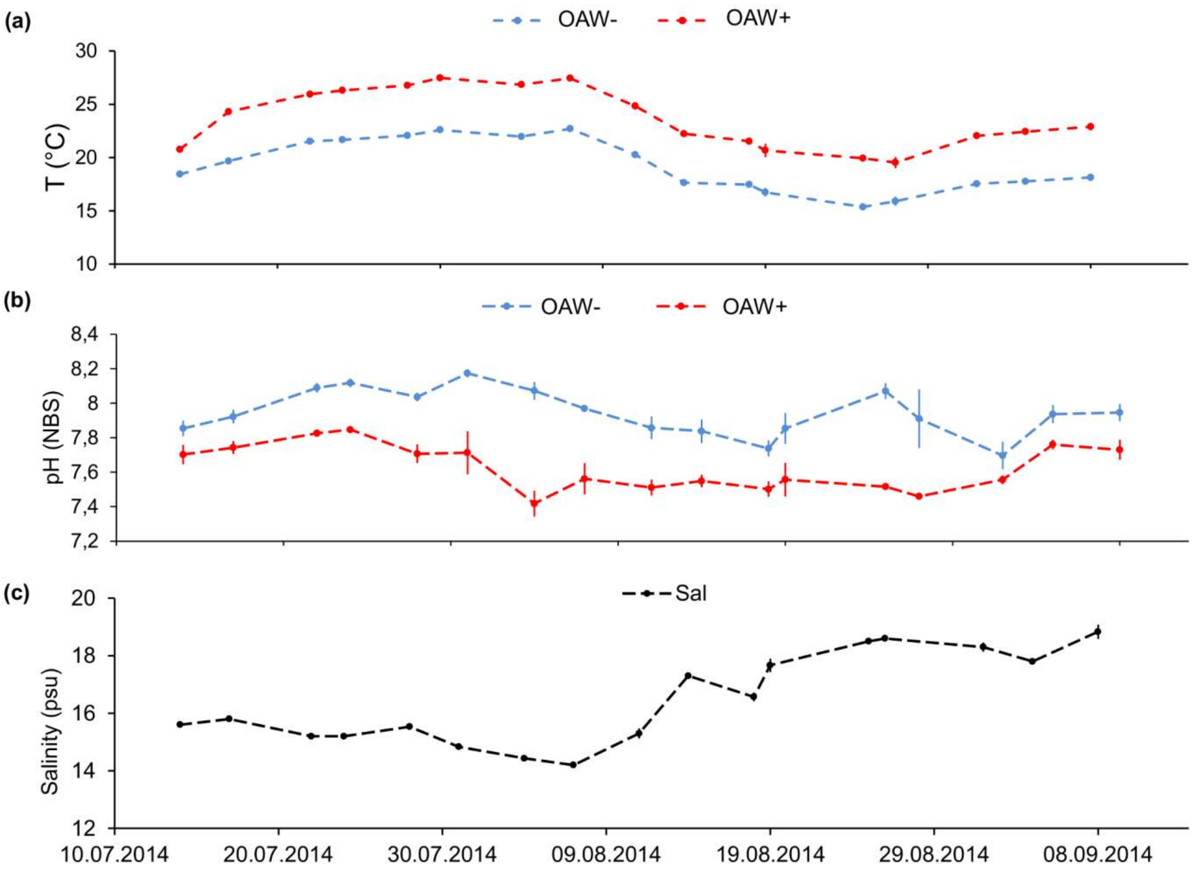

Fig. S2 Temperature (**a**, °C), pH (**b**, NBS) and salinity (**c**, at OAW-, psu) (mean, ±SD, n = 3) during the OAW x nutrient experiment in the Kiel Outdoor Benthocosms at the two treatments ambient (OAW -) and under warming and acidification (OAW+).

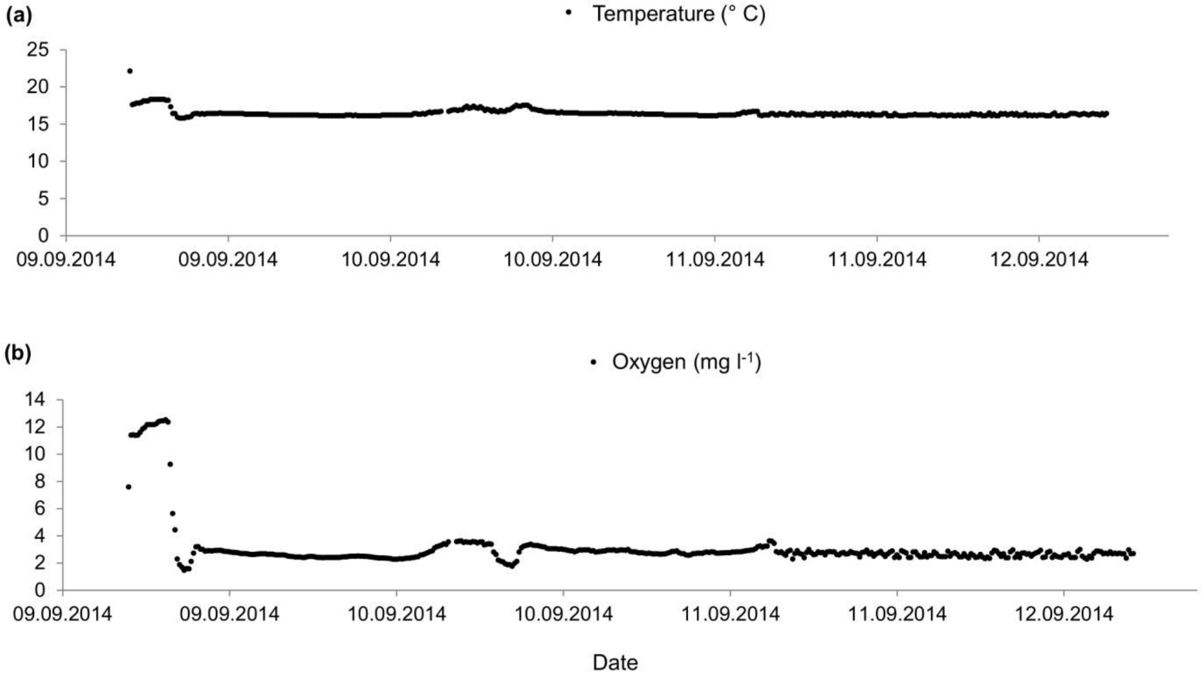

Fig. S3 Oxygen (**a**, mg l^{-1}) and T (**b**, °C) values during the three days of the upwelling event (September 2014) in the Kiel Outdoor Benthocosms.

5 General Discussion

This doctoral thesis demonstrates that the combined effect of global and local factors modulated by natural fluctuations determines the phenotypic performance of *F. vesiculosus* germlings. Furthermore, the results indicate that intraspecific genetic diversity confers the potential to adapt to environmental change. This chapter summarizes the key findings that have resulted from the four papers of this thesis. I discuss potential implications of my results for macrophyte communities under multifactorial environmental change and natural fluctuations considering the sensitivity of different life-stages. Also, consequences for *F. vesiculosus* under adverse environmental conditions, the upscaling approach in global change research and future research perspectives are discussed.

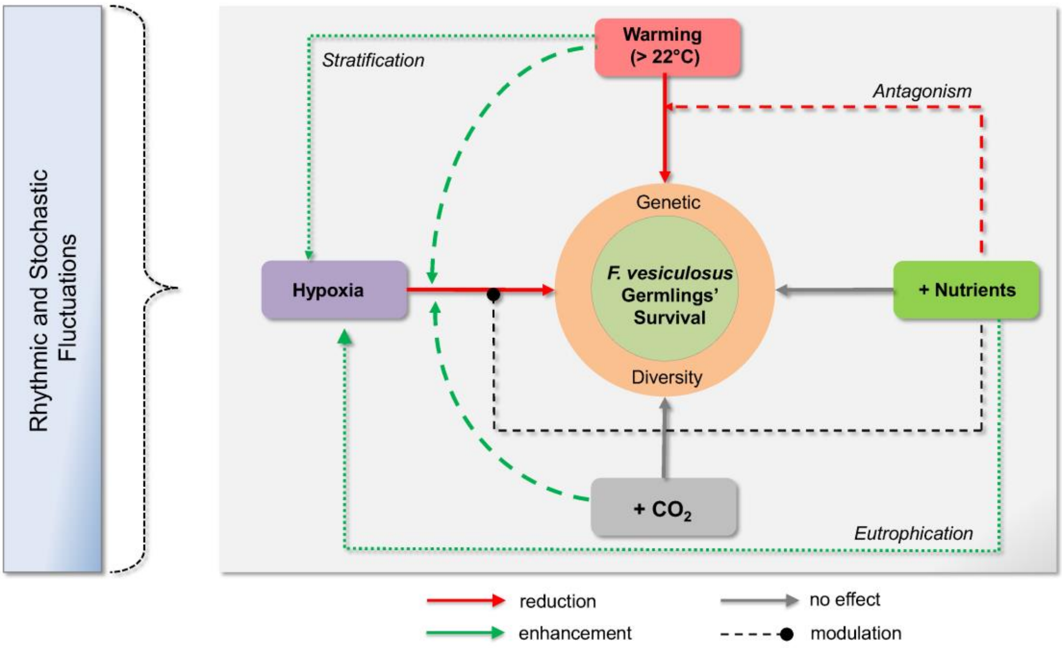

Figure 1 Single and combined impact of the environmental conditions warming (> 22°C), hypoxia, increased nutrient and CO_2 concentrations on *F. vesiculosus* germlings' survival during summer. Continuous arrows show direct effects; dashed arrows show interactive effects. Dotted arrows show biophysical interactions between abiotic factors. Warming and high CO_2 conditions enhance hypoxia sensitivity due to a negative correlation pattern: genotypes of *F. vesiculosus* selected under warming and acidification are most sensitive towards hypoxia. Similar patterns of interactions between the factors were observed regarding growth rates of *F. vesiculosus* germlings. The multifactorial effect is further modulated by rhythmic and stochastic fluctuations.

5.1 Responses of *Fucus vesiculosus* germlings to multifactorial environmental change

Environmental conditions may enhance or buffer each other via biological and physical interactions (Fig. 1) (Jutterström et al. 2014). Eutrophication enhances pelagic primary production, leading to increased sedimentation of organic material followed by hypoxic conditions and an accelerated bacterial re-mineralisation in deeper waters (Ærtebjerg et al. 2003). Warming of the upper sea surface enhances the thermal stratification of the water column especially in summer, impeding water mixing of deeper waters. In deeper water layers, CO_2 and nutrient concentrations increase while temperature and O_2 concentrations decrease (Neumann 2010).

The application of multiple factors in this study demonstrated that interactions determined the phenotypic responses of *F. vesiculosus* germlings, in agreement to Wahl et al. (2011). *F. vesiculosus* sibling groups were simultaneously exposed to the factors temperature, CO_2 and nutrients in various combinations. Additive, synergistic or antagonistic types of interactions were observed (Paper II and III), in agreement to Crain et al. (2008). Upon simulating the conditions of the end of the 21st century, phenotypic performance of *F. vesiculosus* germlings was found to be rather determined by warming than by acidification or nutrient enrichment. Under global change scenario, warming is generally an important determinant of ecological responses (Walther et al. 2002). The thermotolerance range of adult *F. vesiculosus* has been described between 5 and 20 °C; temperatures exceeding this range have been found to negatively impact the photosynthetic efficiency, growth and survival (Graiff et al. 2015a). At the Kiel Outdoor Benthocosms (KOBs), temperatures transiently reached values higher than 27 °C in summer and caused high mortality of fucoid germlings (Paper II and III). High temperatures cause damage to the enzymes involved in photosystem II and the carboxylation assimilation process (Berry and Bjorkman 1980). Corroborating this, warming decreased the non-photochemical quenching, i.e. the potential to protect against photoinhibition (Paper II). Acidification showed weak effects on the performance of *F. vesiculosus* germlings during experiments conducted at the KOBs. In contrast, constant conditions of acidification in indoor experiments in my other studies that are not shown in this thesis caused stronger effects. The growth rate of *F. vesiculosus* germlings was enhanced by 30% under the conditions predicted for the year 2110 (1120 µatm pCO_2) compared to growth under present conditions (ca. 400 µatm pCO_2) (Al-Janabi et al., unpublished data). Another indoor experiment on very young *F. vesiculosus* germlings, only three days of age, demonstrated that the rhizoid length increased by almost 50 % under the CO_2 conditions predicted for the year 2110 compared to growth under present CO_2 conditions (C. Starke, pers. comm.). Although current DIC concentrations of the seawater seem to be saturating for *F. vesiculosus*, the carbon fixation enzyme RuBisCO is not saturated by CO_2 (Koch et al. 2013). The carbon carboxylation pathway is inhibited because RuBisCO is also involved in the photorespiration pathway by binding to O_2 (Bowes and Ogren 1972). In the Indoor experiments, higher environmental pCO_2 may have enhanced the carboxylation pathway. Similarly, increased photosynthetic rates under acidified conditions were observed in adult *F. vesiculosus* (Nygård and Dring 2008). Acidification causes different mechanisms on a physiological level: high CO2 conditions are beneficial when growth is enhanced by a fertilising effect (Wu et al. 2008). However, acidification may be prejudicial when physiological stress is caused by changes in the pH. The weak carbon fertilisation under acidified conditions at the KOBs compared to the indoor experiments may be due to a possible mitigation by a fluctuating nature of the pH regime. Corroborating this, the combined effect of warming and acidification (OAW) on *F. vesiculosus* survival and growth rate at the KOBs (Paper III) approximated the single warming effect (Paper II).

Subsequently, the interactive effect between global change factors and a local factor of the Baltic Sea was demonstrated. For this purpose, the two factors “combined ocean acidification and warming” (OAW) and “nutrient enrichment” were orthogonally crossed at the KOBs (Paper III). An antagonistic interaction was observed when nutrient enrichment strongly mitigated the severe mortality and growth reduction of *F. vesiculosus* germlings’ caused by OAW in summer. Interactions between nutrient enrichment and other environmental factors are probably based on physiological processes of seaweeds. Higher nitrogen assimilation, protein synthesis and growth rates have been observed under acidified conditions, as shown in a review of plant physiology (Stitt and Krapp 1999). Acidified conditions may have enhanced the beneficial effects of nutrient enrichment in *F. vesiculosus* germlings. The positive effect of nutrient enrichment may have important implications on the development of *F. vesiculosus* under future warming, considering that currently large parts of the Baltic Sea are eutrophic (HELCOM 2007 - 2011). In contrast to positive direct effects, nutrient enrichment causes indirect negative effects on *F. vesiculosus* performance. Under eutrophic conditions, growth of microalgae is enhanced, leading to decreased light penetration and a decreased depth penetration of *F. vesiculosus* (Rohde et al. 2008). Hence, the effect of single factors depends on the interplay among biotic and abiotic factors in a natural context (Wahl et al. 2011).

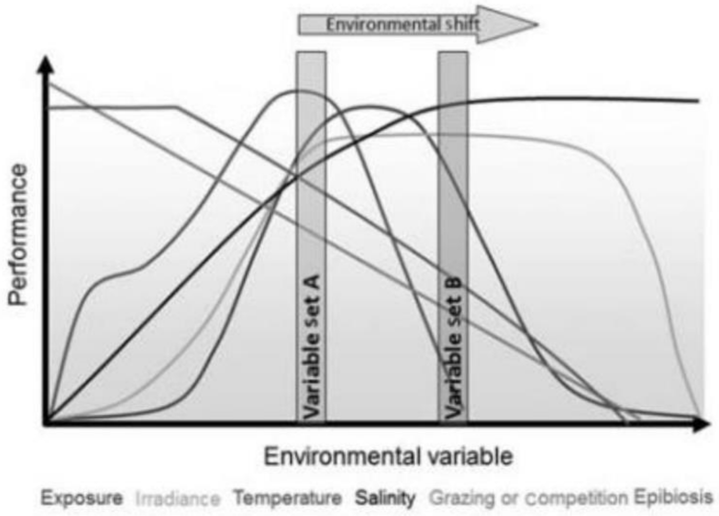

Figure 2 Hypothetical effects of multiple environmental factors on species’ performance. Two different variable sets are given depending on an environmental shift after changing the habitat or season. The magnitude and duration of different single factors and the interactions between them change. Reprinted from Wahl et al. (2011, Fig. 2.3) with permission from Elsevier.

Besides simultaneous exposure to abiotic factors, sequential actions of different factors determined the performance of *F. vesiculosus* germlings (Paper III). All *F. vesiculosus* germlings of the different treatment of the OAW x nutrient experiment were subsequently exposed to a hypoxic upwelling event lasting three days. Hypoxia induced mortality was higher in germlings that had previously experienced warmed and acidified conditions compared to germlings that had previously experienced ambient conditions (Paper III). The previous exposure to warming may have damaged the photosynthetic pathway leading to a higher uptake of storage materials in *F. vesiculosus* germlings. The reduced respiration rate during the hypoxic upwelling likely caused a decreased provision of ATP and biosynthetic precursors (Plaxton and Podestá 2006) and a reduced metabolism (Peckol and Rivers 1995). Possibly, germlings previously harmed by warming were not able to compensate the reduced metabolism under hypoxia. Moreover, previously experienced nutrient enrichment with or without combined warming reduced or enhanced the sensitivity to the subsequent hypoxic upwelling, respectively. These observations corroborate that the sequential exposure to environmental factors determines the sensitivity in marine organisms (Crain et al. 2008). As shown in Paper III, *F. vesiculosus* germlings were generally very sensitive to the three day upwelling event. However, the effect of local upwelling varies among regions. At the southern Iberian and northern African shores, upwelling brings deeper water to the surface and lead to a constant cooling of shallow waters (Marcello et al. 2011). The cooling at these upwelling areas is beneficial for the persistence of *Fucus guiryi*, considering that populations in the same regions outside the upwelling areas became extinct due to global warming (Lourenço et al., pers. comm.). Interactions among abiotic factors either amplify or buffer with single and combined impacts that vary among different life-stages.

5.2 Responses to global change factors in different life-stages of *Fucus vesiculosus*

Different developmental stages of *F. vesiculosus* are exposed to different environmental stressors: adult *F. vesiculosus* are exposed to *i.a.* epibiosis, warming, grazing and hyposalinity, as reviewed by Wahl et al. (2011). The early life-stages (zygotes, germlings and juveniles) of *F. vesiculosus* are additionally threatened by other stressors, such as sedimentation and space competition (Berger et al. 2004). Sperm motility, fertilisation, settlement and germination success as well as survival of germlings and juveniles are impacted by different environmental factors, as reviewed by Coelho et al. (2000). The cumulative impacts at all the different life-stages will determine the fate of *Fucus* populations.

The sensitivity of the early life-stage *F. vesiculosus* demonstrated in this thesis can be compared with the sensitivity of the adult life-stage documented in literature. The thermotolerance range is apparently similar in both, the early and the adult life-stage of *F. vesiculosus*. Warming within the tolerance range (< 20 °C) enhanced growth in summer in germlings (Paper II) and adult *F. vesiculosus* (A. Graiff, pers. comm.). Temperatures above 26 °C in late summer exceeded the thermotolerance range and induced high mortality in both, germlings (Paper II) and adults *F. vesiculosus* (Graiff et al. 2015b). The sensitivity of developmental stages seems to be species-specific. In the giant kelp *Macrocystis* *pyrifera*, the developmental stages such as the germination, fertilisation, germling recruitment and growth differed in their sensitivity towards warming and acidification (Harley et al. 2012). In *F. vesiculosus*, the interactive effect of nutrients, temperature and CO_2 differed between life-stages. In germlings, heat stress was strongly mitigated by nutrient enrichment (Paper III), while adults did not benefit from nutrient enrichment under warming (A. Graiff, pers. comm.). High nutrient conditions may be particularly important for the development of young *F. vesiculosus*, when the internal storage material is most likely limited. Other differences among life-stages were observed regarding their sensitivity to hypoxia. Hypoxic upwelling reduced the survival of *F. vesiculosus* germlings by 30 % after ambient conditions and by 70 % after the ocean warming and acidification (OAW) treatment (Paper III). In adult *F. vesiculosus*, tolerance towards hypoxia is likely higher than in germlings due to a relatively strong photosynthetic activity and a higher compensation of the oxygen depletion (A. Graiff, pers. comm.). Accordingly, the photosynthetic efficiency was unaffected by low oxygen concentrations in the adult-life stage of the red macrophyte *Lomentaria articulata* (Kübler et al. 1999). Under ambient conditions, the photosynthetic efficiency, of the fucoid germlings (Paper II) was only half compared to adult *F. vesiculosus* (A. Graiff, pers. comm.). This difference between the early and adult life-stage indicates an adaptation of fucoid germlings to the low light conditions at the understory of adult algae.

Sensitivity differences between life-stages regarding biotic pressures have also been reported in macroalgae. Selective grazing on different life-stages has been documented in *Enteromorpha spp.*, when different herbivore species selected either germlings or adults (Lotze and Worm 2000). In fact, the top-down control decreases the germination of settled spores and germling growth by 90 % in *Enteromorpha spp.* (Lotze et al. 1999). Grazing on juveniles should not be overlooked in conservation biology. In general, impacts on the early life-time may prevent successful recruitments, enhance the sensitivity on successive life-history stages (Wahl et al. 2015a) and limit the population size of seaweeds (Russell et al. 2012). Environmental impacts on different developmental stages of *F. vesiculosus* are modulated by natural fluctuations.

5.3 Modulation of abiotic factors by rhythmic and stochastic fluctuations

5.3.1 Short-term fluctuations

The extreme spatio-temporal fluctuations of abiotic factors determine the ultimate effect on biological assemblages (Fig. 1) (Benedetti-Cecchi 2003). The attributes of temporal fluctuations of an environmental parameter are intensity (amplitude), duration (time period) and frequency (number of changes in a certain time). A stress factor acts in alternating phases of enhancement and relaxation.

Values above the mean are stressful, while values below the mean allows for the recovery of stress (Wahl et al. 2015a).

At the KOBs, the abiotic factors temperature, pCO_2 and nutrient concentration were shifted to predicted conditions of the year 2110, while maintaining the natural fluctuations of the Kiel fjord. Day-night fluctuations (rhythmic) regarding the abiotic factors pH, salinity, temperature and oxygen showed important short-term variations (Wahl et al. 2015c). Solar radiation is an important driver of the variations between day and night. During day-time, primary producers assimilate CO_2 during photosynthesis, reduce environmental CO_2 and increase the pH. During night-time, photosynthesis of primary producers is reduced and environmental pH is decreased due to respiration (Koch et al. 2013). Day-to-day (stochastic) variations can be caused by climatic changes (e.g. rainfall, storms) or by hydrodynamic forces (e.g. currents) (Wahl et al. 2015a). For instance, local upwelling of hypercapnic and hypoxic waters temporally increase CO_2 in shallow waters to even higher concentrations than those predicted for the end of the 21st century (Paper III) (Saderne et al. 2013). Generally, variations of abiotic factors, e.g. light intensity and temperature, is highest in shallow waters (~ 1 m) and decreases with depth (Wahl et al. 2010). Fluctuations have important implications for *F. vesiculosus* as being mostly distributed at 1 – 3 m depth in the Baltic Sea (Torn et al. 2006). The consideration of *in situ* fluctuations allowed a realistic assessment of multifactorial environmental impact on *F. vesiculosus* germlings observed at the KOBs (Paper II, III and IV). Besides short-term fluctuations, global factors are modulated by mid-term (rhythmic) fluctuations during different seasons (Wahl et al. 2015a).

5.3.2 Mid-term fluctuations

The effects of warming and acidification were analysed at the KOBs during all seasons of the year. Warming showed weak effects on the performance of *F. vesiculosus* germlings in spring, but enhanced growth in early summer (Paper II). The beneficial effects of warming in early summer occurred within the thermotolerance range due to an enhanced metabolism (Graiff et al. 2015a). Similar temperature conditions occur in autumn, however warming may be less beneficial when nutrients are more limited in comparison to nutrient conditions in early summer. In contrast, warming during late summer (> 26 °C) strongly reduced growth rate and survival of *F. vesiculosus* germlings (Paper II and III). Warming induced mortality may seriously impact recruitments and reduce population sizes of *F. vesiculosus*. Additionally, growth of filamentous algae is enhanced during summer and may limit zygote settlement and germlings' survival (Berger et al. 2003). In contrast to warming, acidification only enhanced germling growth during late summer when combined with warming, though not significantly (Paper II).

In autumn, macroalgae are exposed to a different set of abiotic conditions. Local upwelling exposes macrophyte communities to an environmental change in late August and September (Saderne et al. 2013). The conditions of warming, acidification and nutrient enrichment during summer determined the sensitivity towards the subsequent hypoxic upwelling in autumn. This observation reflects the extraordinary role of seasonal variation on stress sensitivity (Paper III). A positive correlation of sensitivities towards warming and acidification during spring and summer (Paper IV) indicate a strong selection towards *F. vesiculosus* genotypes which are tolerant towards these conditions (Sunday et al. 2014). Summer selected genotypes are most sensitive towards hypoxic upwelling in autumn, because of the anticorrelation between the genotypic sensitivity towards hypoxia and combined OAW (Paper IV).

In winter, a different pattern of environmental impacts on *F. vesiculosus* germlings was observed than during the other seasons (Paper II). Warming and acidification decreased germlings' survival by almost half when compared to the survival under ambient temperature conditions. This warming effect was unexpected considering that an increase from ambient temperatures (up to 7°C) to warm conditions (up to 12 °C) approximated germlings' optimal temperature conditions (Graiff et al. 2015a). The negative effect was likely due to an increased metabolic rate under warming, while the photosynthetic rate was reduced under the light limitation in winter. This imbalance possibly caused a higher consumption of the storage products followed by a lethal energy debt. Likewise, adult *F. vesiculosus* showed lower growth rates under warmed conditions when compared to ambient temperature conditions in winter (A. Graiff, pers. comm.). These observations show that *F. vesiculosus* performance is influenced by the interaction between light and temperature in winter. The relevance of seasonal patterns for fucoid development has also been documented in other geographic regions. At the northwestern American coast (San Juan Island, USA), growth of *Fucus distichus* is reduced during spring and summer, when low tides during day-time enhance emersion stress caused by factors such as warming (Dethier and Williams 2009). Low tides occur at night during autumn and winter which reduces the adverse emersion conditions. This enhances the growth rate when compared to rates observed in spring and summer. In the same region, the survivorship of *Fucus gardneri* embryos of the autumn cohort is higher compared to the survivorship of summer cohorts. In autumn, environmental conditions are more suitable for early life-stages regarding temperature and humidity at low tide compared to the summer conditions (Wright et al. 2004).

5.4. Consequences of environmental changes for Baltic *Fucus vesiculosus*

F. vesiculosus populations have different strategies to withstand adverse environmental conditions. Phenological shifts may adjust life-history stages to favourable conditions in a seasonal environment. Phenotypic plasticity and fast adaptation allow for short or long-term solutions, while range shifts by poleward migrations may prevent populations from local extinction (Harley et al. 2012). The following discussion shows that global change effects on *F. vesiculosus* vary among the different levels of biological organisation, i.e. the molecular, cellular, whole-organism, population and ecosystem level (Paper I).

5.4.1 Phenological shifts

Phenological change can occur when changes of the reproductive and developmental timing expose organisms to more favourable environmental conditions (Walther et al. 2002). The photoperiod is an important environmental cue for the reproductive maturity (Bartsch et al. 2008). Additional environmental factors (e.g. water motion, temperature and lunar phase) modulate receptacle maturation, gamete release and the development of early life-stages in fucoid algae (Pearson and Serrão 2006).

Future warming and acidification may be an important cue for the reproductive maturity of *F. vesiculosus*. During the KOB experiments in autumn, the proportion of fertile receptacles of adult *F. vesiculosus* was higher under warmed and acidified conditions compared to ambient conditions (Graiff 2015). Under future warming, algae of the summer reproducing population may achieve sexual maturity in autumn, moving forward in time leading to a phenological shift. The early-life stages of *F. vesiculosus* are exposed to different environmental conditions when originating from the autumn-reproducing population than from the summer-reproducing population (Maczassek 2014). During autumn, the amount of deposited organic matter is higher than during other seasons. As a consequence, settlement of fucoid zygotes recruitment is reduced (Berger et al. 2003). The development of the autumn-reproducing cohorts may be protected from local upwelling events when reproduction occurs after September. As a disadvantage, the early life-stage of the autumn reproducing cohorts do not experience directed selection towards a warming tolerance during summer and may be less tolerant to heat stress during subsequent years. Early life-stages originating from the summer-reproducing populations are threatened by higher growth of filamentous algae during summer in the Baltic Sea, decreasing zygote settlement and germling survival (Berger et al. 2003). Furthermore, germlings' survival is strongly reduced under future warming during late summer (Paper II) when temperatures exceed the thermotolerance range of *F. vesiculosus* (Graiff et al. 2015a). Germlings selected under warming and acidification during summer are particularly sensitive to hypoxic upwelling in autumn (Paper IV).

Overall, autumn and summer-reproducing populations of *F. vesiculosus* do not differ in recruitment success, as reported by Berger et al. (2001). However, the developmental stages of the two reproductive populations are exposed to different environmental conditions during the different seasons. Phenological shifts may have important implications on *F. vesiculosus* under future global change.

5.4.2 Phenotypic plasticity

Phenotypic plasticity is a reversible short-term response to modify the tolerance range under a changing environment (Somero 2010). Fast adjustment of the phenotype to adverse environmental conditions, e.g. heat stress, occurs while maintaining the same genotype (Reusch and Wood 2007).

When the same phenotype is maintained during adverse environmental changes, phenotypic buffering, a type of phenotypic plasticity, occurs (Reusch 2014). Phenotypic plasticity is enabled on a molecular level by either epigenetic processes, i.e. gene regulation without changes in the DNA sequence, or by physiological changes (Paper I). Sibling groups of *F. vesiculosus* showed different phenotypic responses to warming, acidification, nutrient enrichment and hypoxia (Paper II and III). Genotypes within each sibling groups are genetically similar. Hence, different phenotypic responses of the same sibling group under different environments indicate plastic responses of *F. vesiculosus*. The adjustment to the environment is limited by the slope of the reaction; changes beyond the tolerance range cannot be endured (de Jong 2005). Plastic responses had already been documented in *F. vesiculosus*: Herbivore grazing induce the regulation of different genes responsible for defence, stress response, lipid and carbohydrate metabolism and photosynthesis (Flöthe et al. 2014). In *Fucus serratus*, transcriptomic upregulation of heat shock proteins and changes of the photosynthetic performance allow for heat stress tolerance to some extend (Jueterbock et al. 2014). Phenotypic plasticity may constitute the fastest solution for *F. vesiculosus* populations to sudden environmental changes. Moreover, the capacity for plastic responses itself can be inherited allowing for fast adjustment to global changes in future generations (Chevin et al. 2013).

5.4.3 Adaptation

Adaptive evolution, i.e. the genetic change due to natural selection that improves the fitness of a population in its environment, provides a long-term solution to global change (Frankham et al. 2010). Genetic diversity provides the substrate for adaptation, allowing for evolutionary rescue under adverse environmental conditions (Bell 2012). A relatively high population size is beneficial for adaptation when genetic loss (genetic erosion) is compensated by a higher chance of new mutations compared to small populations (Bell and Gonzalez 2009). The two most important criteria for evolutionary rescue are population size and genetic diversity (Bell 2012). Presumably, Baltic *F. vesiculosus* have a decreased genetic diversity compared to e.g. Atlantic populations. Lower genetic diversity of Baltic *F. vesiculosus* populations are assigned to bottleneck effects at the Baltic Sea entrance, genetic isolation along the salinity gradient in the Baltic Sea and the limited gamete dispersal (Johannesson and André 2006). Also, relatively strong genetic differences between Atlantic and Baltic *F. vesiculosus* populations have been documented (Johannesson and André 2006). In a population located close to the Baltic Sea entrance (outer Kiel fjord, Germany), the genetic diversity was relatively high (Allelic richness = 8.33), as analysed by means of nine microsatellite markers in 42 adult *F. vesiculosus* individuals (Al-Janabi et al., unpublished data). Accordingly, the genetic diversity of this population is higher compared to the *F. vesiculosus* populations of Kalmar sound (Sweden), located towards the inner parts of the Baltic Sea (Tatarenkov et al. 2007). The potential to adapt to environmental change may be higher for *F. vesiculosus* populations located closer to the entry of the Baltic Sea than for other *F. vesiculosus* populations located towards the inner parts of the Baltic Sea.

Phenotypic variations among genetically different sibling groups (Paper II, III and IV) showed that genetic diversity determined phenotypic diversity. High phenotypic differences were observed for only eight sibling groups (Paper II), indicating a high potential of adaptation in presence of a low number of *F. vesiculosus* genotypes, in accordance to Frankham (2010). Genetic diversity increases the variation of resource use, resistance and resilience to stress and therefore the fitness on the population level (Paper I) (Reusch and Hughes 2006). Genetic diversity has implications on a higher level by enhancing the productivity and the recovery from disturbance and replaces the role of biodiversity to some extent (Reusch et al. 2005). According to the strong genotypic variations in *F. vesiculosus* germlings in response to environmental stress (Paper I, II and III), adult *F. vesiculosus* also show genotypic variation with regard to fouling tolerance and resistance (Honkanen and Jormalainen 2005). Also, genotypic variation in resistance to grazing pressure in adult *F. vesiculosus* indicated that the potential for adaptation to environmental stress is conferred by genetic diversity (Haavisto et al. 2010). At the KOBs, highest differences among sibling groups of *F. vesiculosus* germlings were observed under increased environmental stress: differences among sibling groups were strongest in summer and in winter (Paper II). Warming induced mortality in *F. vesiculosus* germlings was lower in the higher levels of genetic diversity in winter, while diversity level effects were absent regarding the weak warming effects in autumn (Paper II). These observations indicate that tolerant genotypes may be selected under adverse environmental conditions. Local adaptation by natural selection may cause genetic impoverishment and decrease the potential to adapt to new environments (Pauls et al. 2013).

Evolutionary adaptation to multifactorial global change is influenced by different patterns of sensitivity correlations (Sunday et al. 2014). The sensitivity of different genotypes towards multiple stressors may correlate, anticorrelate or be unrelated (Vinebrook et al. 2004). In *F. vesiculosus*, the sensitivity of different genotypes towards warming, acidification and nutrient enrichment correlated, indicating a direction of selection towards genotypes that are favoured under these conditions (Paper IV). The observed positive correlation may be a consequence of pleiotropy, when adaptation towards single genes determines several traits. In plants for instance, adaptation of physiological traits often favours both growth and stress resistance (Chapin et al. 1993). The sensitivity of *F. vesiculosus* genotypes towards OAW and hypoxia anticorrelated, indicating that genotypes selected under warming and acidification are most sensitive towards hypoxia (Paper IV). The negative correlation pattern may be due to physiological trade-offs after energy allocation between different traits (Blows and Hoffmann 2005). These results demonstrate that the correlation pattern of sensitivities towards multiple factors likely determine adaptive processes under multifactorial environmental impact.

5.4.4 Range shifts

Range shifts under global warming, i.e. the expansion towards poleward regions, is an important strategy for *F. vesiculosus* populations unable to withstand thermal stress (Jueterbock et al. 2013). Predictive species distribution modelling showed future poleward shift of *F. vesiculosus* as well as the potential loss of unique genetic lineages at the southern edges of distribution until the end of the 21st century (Assis et al. 2014). At the experiments of the KOBs, conditions beyond the upper thermotolerance range in late summer caused severe mortality of germlings (Paper II and III). If *F. vesiculosus* populations are unable to adapt or to acclimatize to environmental stress, range shift may be required for the persistence of the species. In the Baltic Sea, a northwards shift would expose *F. vesiculosus* to different environmental threats, such as lower salinity concentrations.

5.4.5 Possible consequences for macrophyte communities in the Baltic Sea

Multifactorial environmental change will likely impact Baltic *F. vesiculosus* populations including other organisms associated to the macrophyte community. A shift from summer- to autumn-reproducing population may occur as a consequence of warming, as described above (Section 5.4.1). As reported in the literature, the fouling pressure by epiphytes and epizoans on *F. vesiculosus* in spring and summer is higher compared to the fouling pressure in autumn (Rickert et al. 2015). Consequently, the development of *F. vesiculosus* zygotes and germlings may be favoured in autumn and a shift towards the autumn-reproducing population may be further enhanced.

Analysing the seasonal variation of warming and acidification on the early (Paper II) and adult (Graiff et al. 2015b) life-stages show that *F. vesiculosus* will be threatened most strongly in summer, when temperatures exceed the thermotolerance range. The known effects of global change factors on different organisms during the different seasons allow for speculating future changes in Baltic macrophyte communities.

Under ocean warming, also biotic impacts on *F. vesiculosus* occur due to an increase of macroepiphyte growth in summer (Werner et al. 2015). The enhanced fouling load of macroepiphytes leads to light limitation by shading and to higher nutrient depletion (Kautsky et al. 1986). These indirect effects may accelerate the decline of western Baltic *F. vesiculosus* populations, which may have severe consequences for the associated community. The abundance and biomass of macrophyte grazers, which mainly consists of bivalves, gastropods and crustaceans, declines under warmed and acidified conditions as predicted for the end of the 21st century (Werner et al. 2015). This invertebrate community may be additionally threatened by the future decline of bladder wrack when the provision of food and shelter becomes limited.

The community structure of *F. vesiculosus* may also change as a consequence of the predicted range shift towards the northern Baltic Sea at the end of the 21st century (Jueterbock et al. 2013). The geographic shift requires the adaptation towards low salinity conditions in the northern Baltic Sea, as the decrease in salinity will be also enhanced by an increase of precipitation and riverine input, as predicted by Neumann (2010). The macrophyte community under future global change may differ from the present community in the western Baltic Sea. Moreover, *F. vesiculosus* competes with the endemic sister species *F. radicans* in the northern Baltic Sea. The associated community of both host species is to some extent specific (Schagerström et al. 2014), which suggests that invertebrates associated only to *F. radicans* may be endangered if a replacement by *F. vesiculosus* occurs.

It is conceivable, that the population dynamics will be influenced by future environmental change. It is likely that northern *F. vesiculosus* populations are restricted in their reproduction as sperm motility and fertilisation is impeded under low salinity conditions (Serrão et al. 1996). Consequently, the number of breeding individuals in northern *F. vesiculosus* populations may decrease as also the genetic diversity (Frankham 2010). Therefore, northern populations may be restricted in their potential to adapt to environmental change.

Overall, a complex interplay between abiotic and biotic factors influences the physiological and evolutionary responses of macrophyte communities. The better understanding of this interplay under global change requires an upscaling approach.

5.5 Implications of the upscaling approach and future research perspectives

In my doctoral thesis, an upscaling approach was performed regarding (1) multiple factors (2) short-term fluctuations (3) mid-term (seasonal) variations (4) sensitivity analysis of developmental stages (5) different levels of genetic diversity and (6) of biological organisation. The gained results support the increasing demand for using upscaling approaches in global change research (Russell et al. 2012; Wahl et al. 2015a). The performance of *F. vesiculosus* germlings was considerably determined by the simultaneous and sequential action of multiple global and local factors, in accordance to Russell and Connell (2012). A better understanding of global climate change impacts on marine organisms requires the comparison of various spatial scales, such as different geographic regions (Helmuth et al. 2010). On the Adelaide coasts (South Australia), human urbanization since the 1970s considerably affected the marine ecosystem due to an enhanced volume of discharge. The high release of nutrients enhanced growth of turf forming algae leading to a replacement of kelp, such as the common kelp *Ecklonia radiata*. Also, large amounts of sediment impede the settlement of kelp spores and decrease recruitment (Connell et al. 2014). These effects caused a decline of kelp forests down to 70 % (Connell et al. 2008). The anthropogenic stress on the Adelaide coast is comparable with the conditions in the Baltic Sea, considering that eutrophication impedes the settlement of Baltic fucoid germlings (Eriksson and Johansson 2003). A better restoration policy for the improvement of the water quality for the recovery of subtidal seaweed has been demanded for both regions, South Australian coast (Gorman and Connell 2009) and the Baltic Sea (Berger et al. 2003).

The maintenance of natural fluctuations at the KOBs was important for a realistic assessment of stress sensitivity under future global change conditions. However, temporal fluctuations have been neglected in most ocean acidification studies, thus most studies still use constant treatment conditions (Small et al. 2015). Since effects of the important global change factors temperature and CO_2 varied between different seasons, seasonal fluctuations should be included in global change research (Wahl et al. 2015a). Furthermore, the different sensitivity of *F. vesiculosus* developmental stages to multifactorial environmental change has been observed by comparing the results of this study with literature on adult macroalgae. So far, studies on the sensitivity of different life-stages of macroalgae to anthropogenic stress are still limited (Coelho et al. 2000). The integration of all life-stages to models of algal community structure (Schiel and Foster 2006) would lead to a better detection of critical developmental stages, allowing for target-oriented conservation strategies (Russell et al. 2012).

As a further step, data of the macrophyte community originating from different working groups within the benthic consortium (BIOACID II project) will be integrated in a BIOACID III phase. The relative importance of direct and indirect effects of different parameters during the KOB experiments will be analysed by a Structural Equation Modelling (SEM). Complex interactions between the components of the macrophyte community can be disentangled. Possible effects of abiotic and biotic factors, e.g. biofouling and grazing on *F. vesiculosus* germlings, can be quantified.

The analysis of intraspecific genetic diversity in Baltic *F. vesiculosus* populations indicate that genetic diversity allows for natural selection under global change stress. Global monitoring of genetic diversity has been suggested for a better conservation of marine populations under global climate change (Frankham 2010). Therefore, population viability analyses (PVA) include population properties, such as genetic diversity, to improve preservation strategies (Frankham et al. 2014). Genotypic correlations of sensitivities towards multiple global change factors indicate the direction of selection; nonetheless correlation analyses have been largely neglected. Sensitivity correlation patterns may even uncover unknown causes of the decline of Baltic *F. vesiculosus* populations. Furthermore, the literature research on different levels of biological organisation (molecular, cellular, whole-organism, population level) revealed that global change effects differ between each single level (Harvey et al. 2014). Conservation strategies with the incorporation of different levels of biological organisation would help to assess the fate of seaweed communities under a changing environment (Harley et al. 2012).

The reduction of global climate change requires international arrangements (e.g. reducing greenhouse gases) and rather constitutes a big challenge in the near future. In contrast, the reduction of local stressors (e.g. eutrophication and pollution) may be a more feasible short-term solution to improve the environment. A better understanding of local conditions in the Baltic Sea may enable the successful transplantation of fucoid germlings for the reintroduction and a compensation of the past decline of *F. vesiculosus*. Generally, higher technical development and resources usages, e.g. burning of fossil fuels, should come along with conservation strategies for better environmental protection. All anthropogenic impact on the environment has, although not visible at the first glance, irreversible consequences on benthic communities. The scientific and non-scientific society requires a better consciousness of conservation of the marine environment.

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8 *Curriculum Vitae*

Balsam Al-Janabi

Address: Boninstreet 47, 24114 Kiel, Germany

Birth Date: 01.02.1983

Place of Birth: Baghdad, Iraq

Nationality: German

Academic Experience

PhD student at the Benthic Ecology Department, GEOMAR, Helmholtz Centre for Ocean Research Kiel. “Interaction between intraspecific genetic diversity and environmental stress in early life-stage macroalgae”. Since October 2012

2 semesters of the Master of Science: “Biological Oceanography”. GEOMAR. October 2011 – September 2012

Diploma in Biology. Final mark: 7.48/10. Thesis title: “Marine environment consulting with focus on the biodiversity of marine benthic ecology”. University of Valencia, Valencia, Spain. September 2006 – July 2011

Final secondary-school examination. Final mark: 6.58/10. National University for distance (UNED), Madrid, Spain. June 20060

High School Graduation. Final German mark: 1.6/6, Principal subjects: Biology, German. “Abendgymnasium Köln“,Cologne, Germany. December 2005

Professional Experience

Supervision of the experiment: Ocean acidification effect in the Baltic Sea on mortality and hatch in fish larvae: *Gadus morhua* and *Pleuronectes platessa*. Supervisor: Dr. Catriona Clemmesen. GEOMAR, Helmholtz Centre for Ocean Research, Kiel, Germany. April 2012

Collaborator Student: Research in Marine Parasitology. Fish dissection, genetic analyses and maintainance of experiments“. Supervisor: Juan Antonio Balbuena. Cavanilles, Institute of Marine Zoology, University of Valencia, Valencia, Spain. September 2009 – July 2011

University integrated internship (9.5/10), Marine sediment sampling tasks. Samples triage and identification of molluscs. OCEANSNELL. Marine environment Consulting, Valencia, Spain. October 2010 – January 2011

Oral Presentations:

Al-Janabi, B. et al. 2015. Correlated and anti-correlated sensitivities to global change factors will determine the fate of *Fucus vesiculosus*. Highlight talk: Consortium “Benthic Assemblages”, Bioacid II Final Meeting, Kiel, Germany.

Al-Janabi, B. et al. 2015. The interaction between intraspecific genetic diversity and global environmental change in early life-stage *Fucus vesiculosus*. Aquatic Biodiversity and Ecosystems conference, Liverpool, England.

Al-Janabi, B. et al. 2015. Tolerance to climate change of early life-stage *Fucus vesiculosus* varies among sibling groups. European Phycology Congress, London, England.

Al-Janabi, B. et al. 2015. Interaction between intraspecific genetic diversity and environmental stress in early life-stage macroalgae. Aquatic Sciences Meeting, Granada, Spain.

Al-Janabi, B. et al. 2014. Effects of climate change on benthic communities in the Baltic Sea – Kiel Benthocosms. Akkeshi Marine Station, University of Hokkaido, Hokkaido, Japan.

Al-Janabi, B. et al. 2014. Response of genetic diversity levels of early life-stage *Fucus vesiculosus* on two climate change parameters. Scientific Conference of the Phycology Section of the German Botanical Society, Stralsund, Germany.

Supervising Activity

Claudia Starke from the University of Zittau/ Görlitz, Germany “Der Effekt von ansteigender CO_2- Konzentration auf die Auskeimung verschiedener Geschwisterscharen von *Fucus vesiculosus*” (co-supervisor, Bachelor, 2016).

Laura Käse from the University of Bremerhaven, Germany “Der Einfluss von Klimawandelstressoren auf Wachstum und Photosyntheseeffizienz bei jungen Makroalgen” (co-supervisor, Bachelor, 2013).

Skills

Computer skills: Vector NTI Advanced 11, NTSYS 2.2, Image J, POPULUS 5.4, SPSS 15.0, R, PRIMER, GeneMarker, GENETIX

Languages: German (Proficient user); English (Proficient user); Spanisch (Proficient user); Arabic: (Advanced user)

Licences: Driving licence: Classes AM, B; Boat licence: Crafts on sea-going ships; Diving licence: “Open Water Diver”

9 Eidesstattliche Erklärung

Hiermit bestätige ich, dass die vorliegende Arbeit von mir selbstständig angefertigt wurde. Die Arbeit wurde keiner anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt. Dies ist mein einziges und bisher erstes Promotionsverfahren. Ich habe keine als die angegebenen Hilfsmittel und Quellen verwendet und die Arbeit unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft erstellt.

Teile dieser Arbeit sind bereits veröffentlicht, wurden zur Veröffentlichung in wissenschaftlichen Fachzeitschriften eingereicht oder sind in Vorbereitung eingereicht zu werden:

Harvey B, Al-Janabi B, Broszeit S, Cioffi R, Kumar A, Aranguren-Gassis M, Bailey A, Green L, Gsottbauer C, Hall E, Lechler M, Mancuso F, Pereira C, Ricevuto E, Schram J, Stapp L, Stenberg S, Rosa L (2014) Evolution of Marine Organisms under Climate Change at Different Levels of Biological Organisation. Water 6: 3545-3574, DOI: 10.3390/w6113545

B. Harvey hat das Gesamtkonzept des Multi-Autoren-Artikels entwickelt. B. Al-Janabi hatte die Leitung des Autorenteam des Kapitels „population-level responses" inne, zu welchem außerdem E. Hall und J.B. Schramm betrugen.

Al-Janabi B, Kruse I, Graiff A, Karsten U, Wahl M (2016) Genotypic variation influences tolerance to warming and acidification of early life-stage *Fucus vesiculosus* L. (Phaeophyceae) in a seasonally fluctuating environment. Marine Biology 163: 1-15 doi 10.1007/s00227-015-2804-8:

Autorenbeiträge: *B. Al-Janabi, I. Kruse und M. Wahl haben die Studie entworfen; B. Al-Janabi hat die Daten erhoben; B. Al-Janabi und A. Graiff haben die Daten analysiert; B. Al-Janabi hat das Manuskript geschrieben; Alle Koautoren haben zur Entstehung des Manuskripts mitgewirkt.*

Al-Janabi B, Kruse I, Graiff A, Winde V, Lenz M, Wahl M (unter Begutachtung in Plos One) Buffering and amplifying interactions among OAW (ocean acidification & warming) and nutrient enrichment on early life-stage *Fucus vesiculosus* L. (Phaeophyceae) and their carry over effects to hypoxia impact*:*

Autorenbeiträge: *B. Al-Janabi und M. Wahl haben die Studie entworfen; B. Al-Janabi und V. Winde haben die Daten erhoben; B. Al-Janabi, M. Lenz und V. Winde haben die Daten analysiert; B. Al-Janabi hat das Manuskript geschrieben; Alle Koautoren haben zur Entstehung des Manuskripts mitgewirkt.*

Al-Janabi B, Wahl M, Karsten U, Graiff A, Kruse I (in Vorbereitung zur Einreichung in Ecology Letters) Genotypic correlation in response to global change parameters in seaweed germlings indicates potential for modulation of adaptation success:

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Kiel, im Februar 2016 Balsam Al-Janabi

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