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Physiology of juvenile hydroids - High food availability mitigates stress responses of *Hydractinia echinata* to increasing seawater temperatures

Yvonne Eder[a,b,*], Daniel Tschink[a], Gabriele Gerlach[a,b,c,d], Julia Strahl[a,c]

[a] Institute of Biology and Environmental Sciences, Carl von Ossietzky University Oldenburg, Carl von Ossietzky Str. 9-11, 26111 Oldenburg, Germany
[b] Institute of Chemistry and Biology of the Marine Environment, Carl von Ossietzky University Oldenburg, Carl von Ossietzky Str. 9-11, 26111 Oldenburg, Germany
[c] Helmholtz Institute for Functional Marine Biodiversity Oldenburg (HIFMB), Ammerländer Heerstraße 231, 23129 Oldenburg, Germany
[d] Centre of Excellence for Coral Reef Studies and School of Marine and Tropical Biology, James Cook University, Queensland, Australia

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ABSTRACT

Ocean warming due to climate change is predicted to profoundly affect marine ecosystems. These effects are expected to be intensified for shelf seas like the North Sea, where sea surface temperatures are predicted to increase 3 °C by 2100. Increasing seawater temperature is suggested to alter physiological performance of benthic marine invertebrates, while predicted changes in ocean dynamics and primary production might limit nutrient availability. At present, the combined effect of temperature and nutrition stress on marine biota is largely understudied. We investigated physiological responses in highly polymorphic hydroids *Hydractinia echinata* from two locations in the North Sea in response to two temperatures (18 °C = ambient, 21 °C = increased) cross factored with two food regimes (high, low). After 50 days of experimental exposure, morphological (biomass), physiological (standard metabolic rate) and biochemical performance (contents of protein and protein carbonyl) was determined in juvenile hydroids. Reduced growth and low total protein contents combined with high standard metabolic rates and high protein carbonyl contents in *H. echinata* exposed to 21 °C/low food compared to 18 °C/high food point towards an energy deficiency in the former animals. Meanwhile, high food availability seems to mitigate negative effects of elevated temperature, as energy budgets were sufficient to maintain growth and to keep oxidative damage accumulation low at 21 °C/high food. Our results suggest that high nutrition will increase resilience in juvenile hydroids to seawater temperatures predicted for 2100 in the North Sea. This study illustrates that habitat energy availability is a major driver of species distribution ranges and should be considered in when predicting responses of marine invertebrates to future environmental stressors.

1. Introduction

Global climate change due to rising anthropogenic greenhouse gas emissions such as carbon dioxide (CO_2) is a major threat for marine ecosystems (IPCC, 2014). Since 1750, CO_2 concentrations increased by 40%, leading to atmospheric and ocean warming (0.11 °C per decade between 1970 and 2010; IPCC, 2014). By 2100, atmospheric CO_2 concentrations and ocean temperatures are predicted to rise higher than ever recorded. Globally, sea surface temperatures (SST) are predicted to increase by 1.5–2 °C (IPCC, 2014), and in the North Sea by 3 °C (Schrum et al., 2016) due to topographic conditions of shelf sea areas (e.g., shallow depths, high tidal ranges, large scale intertidal zones).

Further, future climate change scenarios predict a decrease in marine primary (> 6% since the early 1980) and secondary production in the northern Atlantic Ocean (Gregg et al., 2003), due to the influence of the North Atlantic Oscillation (Fromentin and Planque, 1996), as well as changes in ocean dynamics (e.g., wave action, storm events; Grabemann and Weisse, 2008; Woth et al., 2005). For example, the mean wave height of continuous waves in the North Sea is predicted to increase by up to +18% by 2100 (Grabemann and Weisse, 2008) due to projected wind changes. Prey-capture success of sessile filter feeders is suggested to be highly affected by wave action (Sebens, 2002). As a result, higher waves in addition to a reduced food availability might limit feeding time and prey-capture success in benthic suspension feeders in the future.

It is strongly debated whether the potential for acclimation or adaption in marine organisms will cope with the rate of ecosystem changes (reviewed in Hoffmann and Sgro, 2011), which might be highly species specific. Evidence accumulates that ocean warming can drive species turnover (Hillebrand et al., 2010) influencing species

* Corresponding author at: Carl von Ossietzky University Oldenburg, Carl von Ossietzky Str. 9-11, 26111 Oldenburg, Germany.
E-mail address: yvonne.eder@uni-oldenburg.de (Y. Eder).

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specific changes in phenology and trophic mismatches (Edwards and Richardson, 2004). This might lead to shifts in ecological communities and ecosystems in the future. Marine organisms living in intertidal habitats tend to have wider tolerances and higher potentials for phenotypic plasticity due to larger (daily and seasonal) fluctuations in temperature, salinity and oxygen availability compared to those living in the subtidal. However, due to recent changes in environmental conditions (e.g., rising summer SST, lower nutrition; Puce et al., 2009) in intertidal zones, several studies have shown a decrease in ecosystem biodiversity but an increase of sessile organisms, leading to higher competition (Hawkins et al., 2009).

Numerous impacts of rising SST on marine biota are reported in the literature ranging from restricted physiological performance to reduced reproduction, growth and even survival (e.g., Chomsky et al., 2004b; Hofmann and Todgham, 2010). Higher seawater temperatures can alter the kinetics of biochemical processes and disrupt enzyme functions and cofactor binding in poikilothermic organisms, leading to increasing metabolic fluxes, and higher standard metabolic rates and increasing cell damage (Hofmann and Todgham, 2010). This in turn can induce higher production rates of harmful reactive oxygen species (ROS). In aerobic animal cells, ROS are mainly formed in the mitochondrial electron transport chain during the course of oxidative phosphorylation (Harman, 1956, 1972). Oxidative stress occurs in case of rising ROS production rates and/or an imbalance in the pro-oxidant/antioxidant ratio, which turn favours pro-oxidants and results in oxidative damage accumulation (e.g., protein carbonyls, Halliwell and Gutteridge, 1999).

In marine invertebrates, phenotypic plasticity – in other words, phenotypic changes of morphological, physiological, life history or behavioural traits based on the experienced environmental conditions and at a given genotype (Garland and Kelly, 2006; Pigliucci, 2005; Scheiner, 1993; West-Eberhard, 2003) - is an important strategy to withstand rapid environmental changes. For example, high morphological plasticity is an effective mechanism in scleractinian corals to cope with the heterogenic nature of the reef environment or with external environmental forces (e.g., differences in light, sedimentation, gravity, water flow, competition, Flot et al., 2011; Shaish et al., 2007; Todd, 2008). Another well-known example for acclimation by a high level of phenotypic plasticity is shown in plants, which can achieve optimal reproduction and growth according to different environmental conditions through structural and physiological modifications (Todd, 2008; Weaver and Kramer, 1932).

A well-studied model organism with a high polymorphic potential (e.g., morphological plasticity; Blackstone, 1998; Frank et al., 2001) is the hydroid *Hydractinia echinata* (Fleming, 1828). This colony forming species mostly encrusts gastropod shells inhabited by the hermit crab *Pagurus bernhardus* (Linnaeus, 1758). They occur in the intertidal (< 1 m depth) as well as in the subtidal (> 50 m depth) of the North Atlantic Ocean, including the North Sea. The colonies consist of different types of polyps with distinct functions – gastrozooids (feeding), gonozooids (sexual reproduction), dactylozooids and tentaculozooids (interaction / protection / capture of prey) – which are connected through a gastrovascular system (Frank et al., 2001). Before the age of 3 months, the colony consists of gastrozooids exclusively, while the other polyp types can be found, when they become sexual mature (Frank et al., 2001). Due to the high polymorphic potential, the different polyp types possess limited availability to convert into each other (Müller, 1961) and the shape of the colonies is highly variable (runner like vs. sheet like, Blackstone, 1998), partly dependent on the external environmental conditions.

In a controlled aquarium experiment, we investigated both morphological and physiological plasticity of juvenile *H. echinata* colonies in response to different temperature and food regimes. The parental colonies of these juveniles originated either from the subtidal around Helgoland or the intertidal around Sylt in the German Bight. Juveniles from Helgoland and Sylt were exposed to ambient (18 °C) and high (21 °C) temperatures, the latter according to predicted summer SST by 2100 in the German Bight, cross-factored with two food regimes (high and low). In order to assess potentials for acclimation to future environmental conditions and to estimate energetic costs of phenotypic plasticity, we determined morphological (growth), biochemical (contents of proteins and protein carbonyls) and physiological (standard metabolic rates) traits in juvenile *H. echinata* after 50 days of experimental exposure. In this study, we investigated how nutrition can alter physiological performance to cope with thermal stress.

2. Material & methods

2.1. Collection of parental colonies

As *H. echinata* are distributed in different habitats in the North Sea (intertidal and subtidal), 8–10 wildtype colonies of the hydroid growing on gastropod shells were sampled in April 2016 at two different locations within the German Bight. Staff of the Alfred Wegener Institute collected animals i. at a depth of around 50 m (subtidal) at Helgoland (54°07′–54°15′ N; 07°48′–07°57′ E) using the research vessel Uthörn and ii. at a depth of 1–3.5 m (intertidal) at Sylt (55°02′ N; 08°28′ E) using the research vessel MYA II. Gastropod shells colonized by living *H. echinata* colonies from Helgoland and Sylt were shipped to the University of Oldenburg; hermit crabs (*Pagurus bernhardus*) inhabiting the shells had been removed. At Helgoland, the annual sea surface temperature (SST) ranges from −1 °C to 19 °C and the salinity ranges from 30.8–33.5 (Becker et al., 1997). Around Sylt, the mean annual SST is +1 °C higher compared to Helgoland and ranges from 1 °C to 20 °C (Musat et al., 2006), while salinity ranges from 25 to 33 (Hickel, 1980). In contrast to Helgoland with solid, rocky seabed, the seabed of Sylt is dominated by sand flats. The hydroids were transported to the University of Oldenburg, and cultured in artificial seawater (Aqua Medic, Germany) at 12 °C and a salinity of 34. The colonies were fed daily with two-day old living *Artemia salina* nauplii.

2.2. Reproduction & larval settlement

Following the *Helgoland manual of animal development* (Plickert, 2013), spawning in *H. echinata* colonies was induced by an artificial light source (12/12 h light/dark cycle, 10.8 ± 3.5 µmol photons $m^{-2} s^{-1}$, T5 Razor, Giesemann, Germany). Wildtype colonies started to release eggs and sperm daily approximately one h after the onset of light. Four h after spawning, fertilized eggs were collected and washed in a 70 µm pore filter (Cell Strainers, Falcon™, Thermo Fisher Scientific, USA) and transferred into 100 ml of autoclaved artificial seawater at 18 °C and a salinity of 34. After two days, fertilized eggs had transformed into planula larvae and were collected and rinsed in a 200 µm pore filter (Luer, Süd-Laborbedarf GmbH, Germany).

The transformation of the planula larvae was induced by incubating them for 3 h in 116 mM caesium chloride at 18 °C following the protocol of Seipp et al. (2007). Larvae were then rinsed and transferred into a glass bowl containing artificial seawater and black glass tiles (dimensions 10 mm × 10 mm × 2 mm, Mosaikstein GmbH, Germany) for settlement. Prior to the experiment, consecutive numbers were engraved at the bottom side of the glass tiles for identification (population and individual) of juvenile colonies during the experiment. Rates of survival of the juvenile colonies is low within the first two weeks (pers. comm. Daniel Tschink). Therefore, approximately 5 planula larvae were settled onto each plate to ensure the settlement of at least 2–3 larvae on each tile.

2.3. Experimental setup

Overall 300 glass tiles colonized by juvenile *H. echinata* were distributed randomly into 24 holding tanks (100 ml, 5–7 glass tiles per tank and source population, Fig. 1). To be able to balance for tank specific effects, six replicate tanks were exposed to one of the four

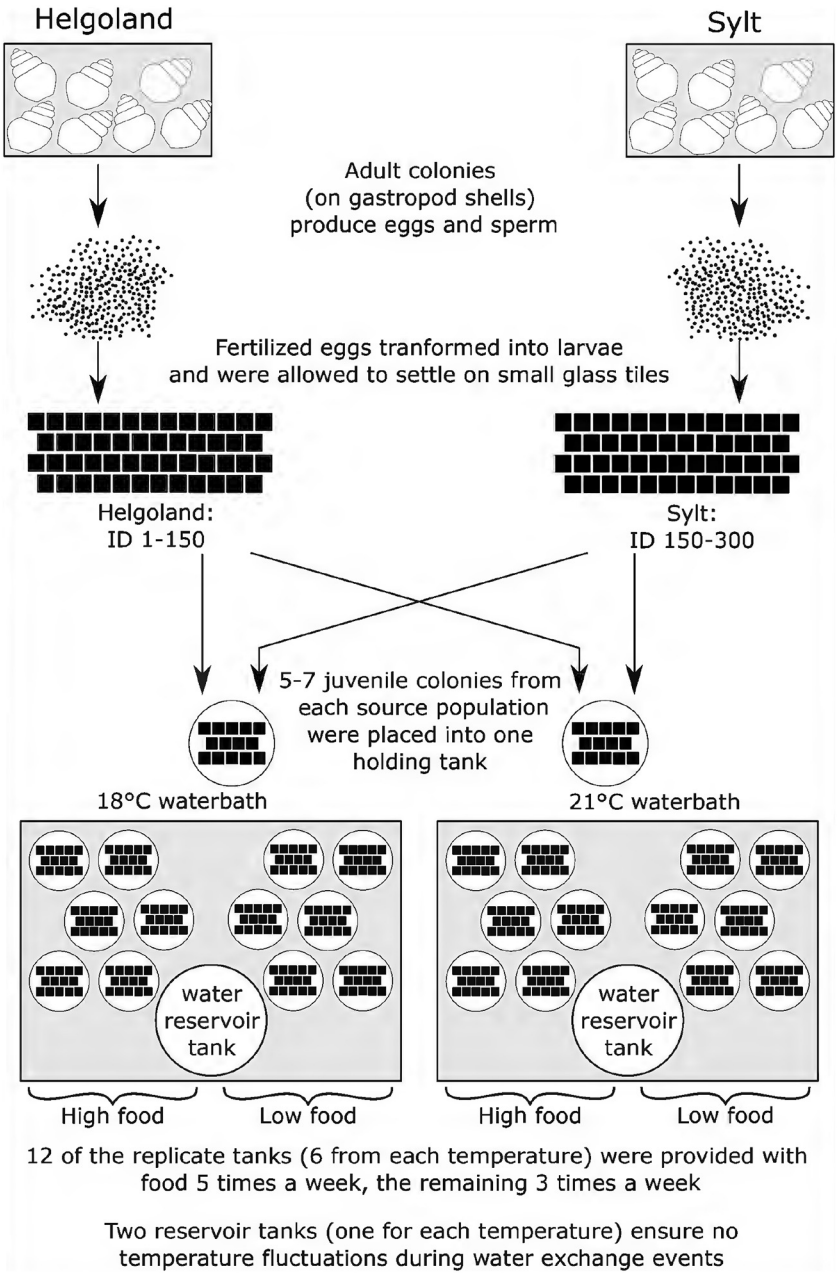

Fig. 1. Schematic diagram of the experimental design. Black glass tiles, with juvenile colonies were placed into 24 tanks (six tanks per treatment; five to six glass tiles of every source population in each tank (12 glass tiles per tank in total)). 24 replicate tanks containing the juvenile hydroids were placed in two water baths with either 18 °C or 21 °C. Half of the juveniles in each temperature treatment were fed three times a week (low food), and the other half five times a week (high food) with two-day old living *A. salina* nauplii. For 50 days juvenile *H. echinata* colonies were exposed to two different temperatures cross-factored with two different food conditions: *I* = 18 °C/high food; II = 18 °C/low food; III = 21 °C/high food; IV = 21 °C/low food.

experimental treatments (see below treatments I-IV, Fig. 1) for eight weeks. At day five post settlement, both Helgoland and Sylt juveniles were fed for the first time with two-day old living *Artemia salina* nauplii. After three weeks, the juvenile colonies growing closer to the edges were removed, to obtain one colony per tile.

Juvenile *H. echinata* colonies were exposed to two different temperatures cross-factored with two different food conditions: I. control temperature - high food (18 °C/high food), II. control temperature - low food (18 °C/low food), III. high temperature - high food (21 °C/high food), IV. high temperature - low food (21 °C/low food) (Fig. 1).

The control temperature of 18 °C simulated the actual summer sea surface temperature (SST) in in the German Bight (data from Helgoland Roads, 2010–2014, http://www.st.nmfs.noaa.gov), while the high temperature of 21 °C corresponded to the predicted increasing SST by the end of the century in the North Sea (IPCC, 2014). The tiles were placed in the holding tanks using tweezers without touching the colonies. The 24 replicate tanks containing the juvenile hydroids were placed in two water baths with water volumes of 40 l covered with a glass plate in a frame of polystyrene to reduce evaporation and cooling. One thermostatic heater (Thermo control 300, Eheim, Germany) and two circulation pumps (Voyager NANO, Sicce, Italy) in each water baths kept temperatures constant at 18 °C or 21 °C (12 tanks and a reservoir tank containing 2 l of seawater in each water bath / temperature regime). Each replicate tank was provided with air through an air stone connected to a pump (HP-40, Hiblow, Japan). To evaluate the impact of food availability on the acclimatization potential in, half of the juveniles in each temperature treatment were fed three times a week (treatments II & IV), and the other half five times a week (treatments I & III) with two-day old living *A. salina* nauplii (> 1000 nauplii/ml per tank per feeding event). Since adult colonies were able to maintain their own metabolic needs and additionally were able to spawn daily at control temperatures when being fed 5 times a week (pers. comm. Daniel Tschink), we defined this regime as high food condition. The juvenile colonies were exposed to a 14 h-light and 10 h-dark cycle according to in situ summer (July/August) conditions in the German Bight. The conditions in treatment I-IV were monitored throughout the

experiment. The temperature was constantly measured with a HOBO Tidbit v2 Temp Logger (Onset®, USA) and the salinity was controlled prior to every water exchange (3 h after every feeding event) with a handheld refractometer (ARCARDA GmbH, Germany). Twice a week, the pH was measured with a pH controller (Aqua Medic, Germany), and concentrations of ammonium, nitrite and nitrate were monitored with test kits (JBL, Germany). Glass tiles were cleaned once a week without touching the colonies to minimize the influence of biofilm formation. This experimental design allowed us to assess the role of food availability on physiological performance of *H. echinata* under ambient and future seawater temperatures. An experimental duration of 50 days was chosen, because at this age, the juvenile colonies start to develop a layer containing chitin (pers. comm. Daniel Tschink). These morphological shifts might affect the physiological processes investigated in the present study, therefore the experiment was limited to 50 days.

2.4. Morphological, biochemical and physiological analysis

To be able to determine morphological parameters, oxygen consumption rate and total protein content for the same colonies, animals were used in a certain order. First, living animals were placed in the respiration chambers to measure individual oxygen consumption rates. Thereafter, the same animals were photographed individually for the analysis of polyp number and colony area (for details see method description and results in the supplement). Finally, the animals were scraped off the tiles (which kills the animals), weighed and snap frozen in liquid nitrogen to analyse biomass and total protein content. The remaining animals contained in the experimental tanks were snap frozen in liquid nitrogen to determine protein carbonyl content. Due to fewer fertilized eggs/larvae, more variable mortality rates and therefore lower number of colonies from Helgoland, the protein carbonyl content could only be determined in juveniles from Sylt.

2.4.1. Respiration

The oxygen (O_2) consumption rate as a proxy for standard metabolic rates was measured at the respective treatment temperature (18 °C and 21 °C) following the protocol of Strahl et al. (2015). The colonies were placed individually in incubation chambers containing 2.9 ml of O_2 saturated artificial seawater. Magnetic glass stirrer in the bottom of each incubation chamber ensured continuous mixing (500 rpm) of the seawater to avoid a build-up of an O_2 concentration gradient. Colonies were incubated for approximately one hour in incubation chambers (including 15–20 min of acclimation time, which was excluded from the calculation of standard metabolic rates). They were kept in a water bath on top of a magnetic stir plate (Multi-point Magnetic Stirrer MS-MP8, Witeg, Germany). During incubation, O_2 saturation was recorded every 15 s by a Multi-channel fibre optic oxygen transmitter system (Oxy-4-Mini and associated software OXY-4 mini version 2.30FB, PreSens, Germany) connecting to oxygen sensor spots (PreSens, Germany) inside the chambers. After measurement, colonies were scraped off as described above and standard metabolic rates of colonies were calculated in mg O_2 h^{-1} mg^{-1} biomass.

2.4.2. Fresh weight

The fresh weight was determined as a proxy for biomass. The colonies on the glass tiles were patted gently with a precision wipe (Kimtech science, Kimberly-Clark Professional, UK) to remove excess seawater. The glass tiles were weighed (weight A) with a precision balance (QUINTIX124-1S, Satorius, Germany), and individual colonies were scraped off the tiles with a surgical blade, transferred into Eppendorf tubes, weighed (weight B) and directly frozen at −80 °C for protein content determination. The tiles were cleaned and dried carefully, weighed again (weight C); the net weight of the colony was calculated by subtraction of the weights A and C. The weight B was used for calculating of the total protein content, whereas the difference between weights A and C gave the exact weight of the colony, which was used for determining the oxygen consumption rate.

2.4.3. Total protein content

The total protein content as an indicator for energy storage capacity, was determined using the DC™ Protein Assay (Bio-Rad Laboratories, USA) based on the Lowry assay (Lowry et al., 1951). Both, a serial of diluted protein standards (Bovine Serum Albumin, Bio-Rad Laboratories) and hydroid samples were prepared referring to Leuzinger et al. (2003). The frozen colony tissues were diluted in 1 M NaOH 1:80 (w:v). Glass beads (diameter 0.1 mm) were added and samples were vortexed (Vortex-Genie 2, Scientific Industries, Inc., USA) for 20–30 s and further homogenized on a rotating heating block (Thermomixer comfort, Eppendorf, Germany) for 20 min at 50 °C and 200 × *g*. These two steps were repeated three times, respectively. The sample homogenates were then centrifuged for 10 min at RT and 1500 × *g* and the protein content of the supernatant was determined using the DC™ Protein Assay (Bio-Rad Laboratories; Hercules, California, USA). Absorbance was measured at 750 nm with a microplate reader (Infinite 200® Pro, Tecan, Switzerland). Total protein content was calculated in mg, referring to the standard curve.

2.4.4. Protein carbonyls

The protein carbonyl content, which is an indicator for production rates of harmful reactive oxygen species (ROS), was assessed in hydroids from Sylt by using an OxiSelect protein carbonyl enzyme-linked immunosorbent assay (ELISA) kit (Cell Biolabs, USA) according to the manufacturer's protocol. Briefly, samples (each tissue from one individual colony) were mixed with chilled 1 × phosphate buffered saline (PBS, pH 7.4) at 1:50 (w/v) and homogenized in a MagNA Lyser Beadbeater (Roche Diagnostics, Germany) for 40 s at 5000 rpm. The homogenate was centrifuged for 3 min at 16100 × *g* and 4 °C, and the protein content of the supernatant was determined. The supernatant was diluted to 10 mg protein ml^{-1} in 1 × PBS, and protein carbonyl-bovine serum albumin standards were prepared. 100 ml of sample or standard was transferred into wells of a 96-well protein binding plate and incubated overnight at 4 °C. Subsequently, the samples were incubated for 45 min in 2,4-dinitrophenylhydrazine working solution and for 1.5 h in blocking solution. Immunodetection was performed using 2,4-dinitrophenol and horseradish peroxidase-conjugated antibodies provided by the manufacturer. Samples were incubated for 10 min in substrate solution before measuring the absorbance at 450 nm with a microplate reader (Infinite 200® Pro, Tecan, Switzerland). The protein carbonyl content of the samples was standardized to the protein content of the sample.

2.4.5. Data analysis

Since the treatments had cross-factored conditions, the effect of temperature, food availability and location was evaluated with a general linearized model (GLM). The distribution pattern of each dataset differed, so the GLM was fitted to each dataset and the respective distribution was tested with a Kolmogorov-Smirnov test (weight = Poisson distribution; respiration rate = binomial distribution; total protein content = binomial distribution; protein carbonyls = Gaussian distribution). The most parsimonious model was accomplished by backward elimination with a Chi-square test. Post-hoc pairwise Wilcoxon Rank Sum Test with a Bonferroni correction was performed to identify differences between the single treatments. Statistical analyses were conducted with R Studio (R Version 3.2.2., R Core Team (2016)). Since no tank effects were recorded in the replicate tanks, all colonies for each treatment and source population were pooled. The fresh weight, respiration rate and the total protein content was investigated in the same animals (Helgoland treatment I. $n = 19$, II. $n = 27$, III. $n = 11$, IV. $n = 14$; Sylt treatment I. $n = 20$, II. $n = 22$, III. $n = 23$, IV. $n = 16$). Due to high assay costs, animals were randomly selected out of each treatment from one source population to investigate the protein carbonyl content, (Sylt treatment I. n = 16, II. $n = 13$, III. $n = 12$, IV. n = 13).

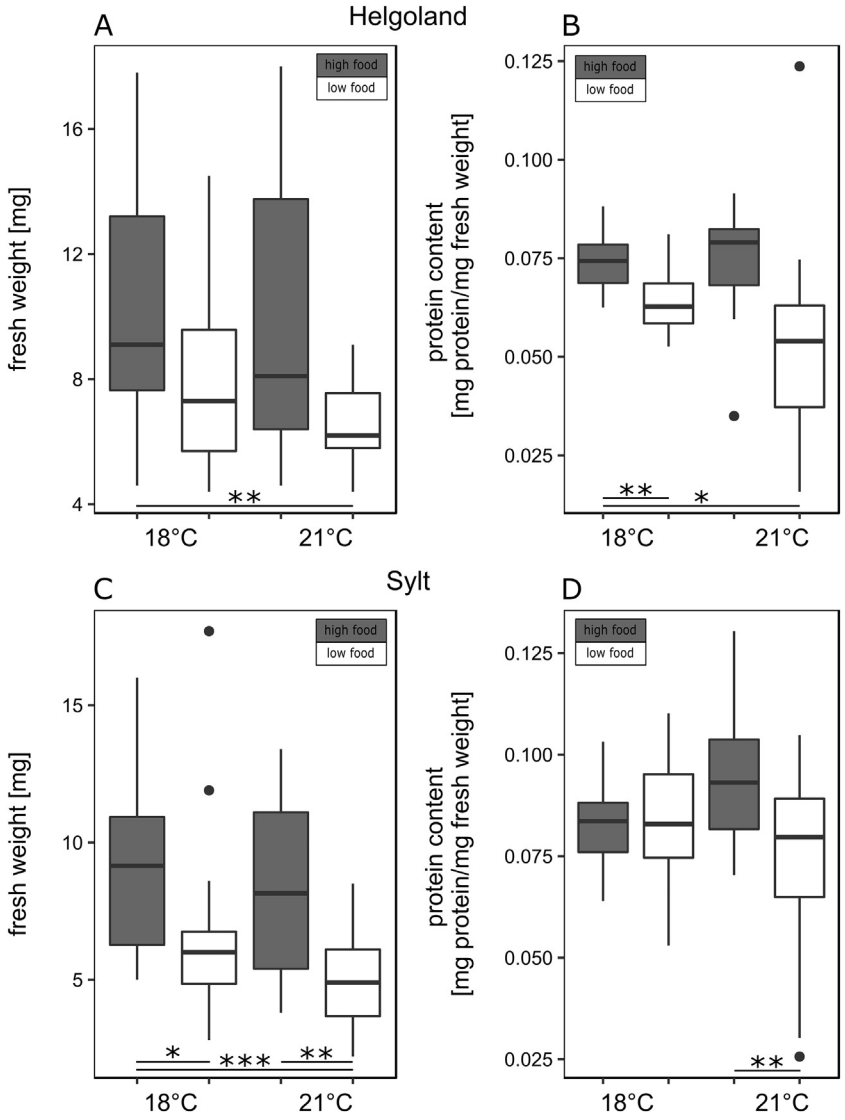

Fig. 2. Growth performance of *Hydractinia echinata* from Helgoland (A, B) and Sylt (C, D) exposed to two different temperatures (18 °C (left), 21 °C (right)) cross-factored with two different food conditions (high (grey), low (white)). The parameters fresh weight (A, C) and protein content (B, D) were measured after 50 days of exposure to experimental conditions. Helgoland n = 11–27 (per treatment, median ± SD) and Sylt n = 16–23 (per treatment, median ± SD). Significant differences between single treatments (Mann-Whitney-U, * p-value < .05, ** p-value < .01, *** p-value < .001).

3. Results

3.1. Fresh weight

The fresh weight of *H. echinata* colonies was significantly affected by food, temperature and location (Fig. 2, Table 1). The colonies exposed to 21 °C/low food were smaller compared to colonies from all other treatments. The mean fresh weight of colonies from Helgoland was 38% (Mann-Whitney-U, $p < .01$) and from Sylt 48% (Mann-Whitney-U, $p < .001$) lighter in the 21 °C/low food than in the 18°/high food treatment (Fig. 2). Further, the colonies from Sylt in the 21 °C/high food treatment were 41% heavier compared to those exposed to the 21 °C/low food (Mann-Whitney-U, $p < .005$).

3.2. Total protein content

The total protein content of *H. echinata* colonies was significantly affected by location and the interaction between food and temperature (Fig. 2, Table 1). The colonies from Helgoland, had a 25% lower total protein content at 21 °C/low food and 14% lower total protein content at 18 °C/low food compared to colonies exposed to 18 °C/high food conditions (Mann-Whitney-U, $p < .05$). For the colonies from Sylt, the mean total protein content was 21% higher in the colonies exposed to 21 °C/high food compared to those exposed to the 21 °C/low food treatment (Mann-Whitney-U, p < .05).

3.3. Respiration

The respiration rate of *H. echinata* colonies was significantly affected by food, temperature and location (Fig. 3, Table 1). In colonies from both locations, the respiration rate of the colonies in the 18 °C/high food treatment was 63% and 53% (Helgoland and Sylt, Mann-Whitney-U, $p < .002$) lower than in those exposed to 21 °C/low food. Furthermore, the respiration rate of the colonies from Sylt was 40% lower in the 21 °C/high food compared to the 21 °C/low food treatment (Mann-Whitney-U, $p < .01$). In addition, the respiration rate of the colonies from Sylt was 35% lower in the 18 °C/high food compared to the 18 °C/low food treatment (Mann-Whitney-U, $p < .05$). This was supported by an additional data set relating the respiration rate to total protein content [mg O_2/mg Protein/h] as an indicator for biomass (see

Table 1
Statistical results. Effect of temperature and food on fresh weight, respiration and total protein content of *H. echinata* from Helgoland and Sylt and protein carbonyls of *H. echinata* from Sylt exposed to two different temperatures (18 °C, 21 °C) cross-factored with two food regimes (high, low). General linearized model; significant differences ($p < .05$) are highlighted in bold; d.f. degrees of freedom.

	d.f.	*F*	p-value
Fresh weight			
Food	156	27.4877	< **.0001**
Temperature	157	5.4081	< **.05**
Location	155	10.3206	< **.01**
Respiration			
Food	158	20.4860	< **.0001**
Temperature	159	5.8710	< **.05**
Location	157	8.7384	< **.01**
Total protein content			
Food	150	0.8644	.35
Temperature	151	0.1501	.69
Location	149	4.9276	< **.05**
Food:Temperature	148	4.1667	< **.05**
Protein carbonyl content			
Food	50	7.5805	< **.01**
Temperature	51	7.9136	< **.01**
Food:Temperature	49	0.1791	.67

supplement Fig. I, Table I), which showed similar trends.

3.4. Protein carbonyls

The protein carbonyl content in *H. echinata* from Sylt was significantly affected by food and temperature (Fig. 3, Table 1). Highest protein carbonyl contents were detected in colonies at 21 °C/low food, while values were significantly lower (−26%) at 18 °C/high food (Mann-Whitney-U, $p < .001$). Protein carbonyl contents were similar at 18 °C/low food and 21 °C/high food.

4. Discussion

We investigated the effect of increased seawater temperature at different nutrition levels in juvenile *H. echinata* colonies, originated from Helgoland (subtidal) and Sylt (intertidal). To our knowledge, this is one of the first controlled aquarium studies adding defined food ratios to the experimental tanks when studying physiological responses in marine invertebrates to temperature stress. Our results suggest that food availability will significantly influence the resilience of hydroids to future environmental conditions, such as increasing seawater temperatures.

High nutrition seems to mitigate the effects of elevated temperature in *H. echinata*. When well fed, energy budgets of *H. echinata* were sufficient to maintain growth rates and protein contents at both temperatures, despite increasing metabolic and cell maintenance costs (e.g., up-regulation of cell protective capacities or protein turnover to 'recycle' protein carbonyls) at 21 °C. Our results are supported by earlier studies that suggested that high energy availability will increase the resilience of marine invertebrates to future climate change conditions (e.g., Han and Uye, 2010; Thomsen et al., 2013). Han and Uye (2010) showed that somatic growth increased with higher food availability under temperature stress in the scyphozoan *Aurelia aurita*. And Sokolova et al. (2012) stated that energy availability is directly linked to fitness-related functions such as development and growth in aquatic invertebrates and to their potential for acclimation to a broad range of environmental stressors. For example, high food availability mitigated acidification stress in marine bivalves. Thomsen et al. (2013) found seven times higher growth rates in the blue mussel *Mytilus edulis* at high acidic compared to low acidic conditions (pCO_2 1000 µatm vs. 600 µatm) in the Kiel fjord due to high nutrition. And in an experimental approach, growth in the blue mussel significantly decreased at low food concentrations and CO_2 stress (Melzner et al., 2011). In two other studies anemones were observed to be more resilient to artificially induced hypoxic conditions when being able to feed on other, dying invertebrates (Stachowitsch et al., 2007; Riedel et al., 2014)., Our results and previous studies illustrate that food supply and habitat energy availability have to be taken into consideration when predicting responses of marine invertebrates to future environmental stressors.

In the present study, juvenile *H. echinata* were significantly affected by temperature stress at low food conditions. The reduced weight and the lower total protein content in colonies from Helgoland and Sylt exposed to 21 °C/low food compared to those at the control temperature (18 °C) and high food regime point towards an energy deficiency in the former animals. Restricted growth rates under future environmental conditions (e.g., increasing SST and decreasing primary and secondary production in the North Atlantic, Gregg et al., 2003; IPCC, 2014) might reduce fitness in marine hydroids and benefit other competing ectosymbionts with a wider temperature tolerance window. In the present study, biomass rather than colony structure seem to be affected by high temperature and low food conditions, as experimental treatments had similar effects on area and polyp number of juvenile hydroids (see supplement Fig. II). In *H. echinata,* stressful temperature conditions seem to induce an energy allocation to more vital processes such as cell/tissue maintenance, while less energy is available to support growth and to maintain protein stores. For example, the total protein content in the Helgoland colonies decreased by 25% in the 21 °C/low food compared to the 18 °C/high food treatment. Accordingly, some field and laboratory studies showed that growth rates and total protein content in marine invertebrates decreased in response to high temperature and low food stress. Borell et al. (2008) and Bayne and Thompson (1970) reported this response in corals (*Stylophora pistillata* and *Galaxea fascicularis*) and in blue mussels and assumed that the breakdown of protein stores balanced increasing energetic demands at high seawater temperatures. Similarly, total protein contents in the soft coral *Heteroxenia fuscescens* declined at increasing seawater temperatures in the Red Sea between 1994 and 1998 (Ben-David-Zaslow and Benayahu, 1999). Two laboratory experiments with sea anemones *Actinia equina* showed that individuals exposed to either high temperatures (25–30 °C) or low feeding regime lost up to 50% of their body mass, in contrast to those exposed to ambient seawater temperatures (15–20 °C) or high nutrition (Chomsky et al., 2004a, 2004b). Also different field studies showed the coherence of food availability and the severity of stress responses in marine invertebrates, particularly to increasing seawater temperature. For example, a diverse epiphytic hydroid community showed a reduction in biomass during summer (Cunha and Jacobucci, 2010). Chomsky et al. (2004b) observed that the body size of adult *Actinia equina* decreased (0.39% day^{-1}) with increasing seawater temperature of +3 °C in summer. In contrast, *A. equina* built-up energy reserves in spring and fall, probably due to the combined effects of lower environmental temperatures and higher food availability (e.g., due to plankton blooms, Chomsky et al., 2004a). This confirms our findings, where growth rates and protein contents were highest in *H. echinata* at 18 °C/high food.

The metabolic rate was inversely correlated to growth in hydroids from Helgoland and Sylt. This supports the hypothesis of an energy deficiency and energy allocation towards cell maintenance and repair in *H. echinata* exposed to temperature stress and low nutrition. Highest oxygen consumption rates were found in juvenile hydroids with lowest growth rates at 21 °C/low food, while lower metabolic rates were found at 18 °C. This is in agreement with the universal temperature dependence of metabolic rates in marine invertebrates (Newell, 1973). Nevertheless, food outweighed the temperature effect in the present study. At both 18 °C and 21 °C, oxygen consumption rates were 22–55% higher in colonies at low compared to high nutrition, probably due to the utilization of all food components including lipids and the burning

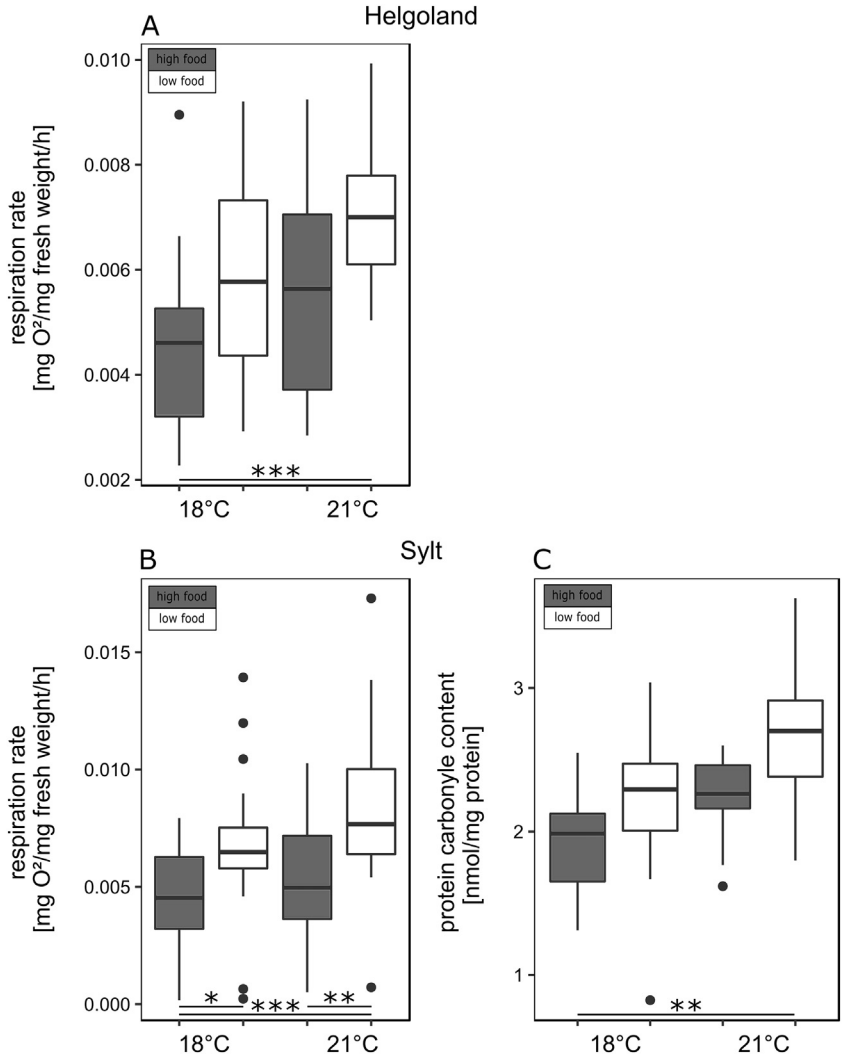

Fig. 3. Physiological responses of *Hydractinia echinata* from Helgoland (A) and Sylt (B, C) exposed to two different temperatures (18 °C (left), 21 °C (right)) cross-factored with two different food conditions (high (grey), low (white)). The parameters respiration rate (A, B) and protein carbonyl content (C) were measured after 50 days of exposure to experimental conditions. Helgoland n = 11–27 (per treatment, median ± SD) and Sylt *n* = 12–23 (per treatment, median ± SD). Significant differences between single treatments (Mann-Whitney-U, * *p*-value < .05, ** p-value < .01, *** p-value < .001).

of energy storages (e.g., lipids and proteins). The breakdown of lipids resulted in higher energy yields than that of proteins and carbohydrates, but it consumes more than twice the amount of oxygen needed for the oxidation of carbohydrate or protein (Schmidt-Nielsen, 1990). Our results suggest rising energetic requirements in *H. echinata* at low nutrition and elevated environmental temperatures, probably due to higher investment into cell maintenance and repairing task. Meanwhile, under high food conditions the primary use of proteins and carbohydrates (which consumes comparatively lower amounts of oxygen), might be sufficient to balance energy requirements in juvenile hydroids for somatic cell maintenance (e.g., cell protective capacities, protein turn over), as well as for tissue growth and maintaining protein stores. Correlations between the amount of oxygen consumption and the breakdown of major energy reserves (proteins, carbohydrates or lipids) have been previously observed in invertebrates (Selvan et al., 1993).

The protein carbonyl content in *H. echinata* mirrored the pattern of the respiration rate suggesting higher production rates of harmful reactive oxygen species (ROS) and the onset of oxidative stress at higher experimental temperature and high cellular oxygen turnover rates (e.g., highest rates of oxidative damage accumulation were found in juvenile hydroids exposed to the 21 °C/low food). The correlation between high oxygen turnover and ROS formation was first proposed by Fleming et al. (1981) in the 'Free-Radical-Rate-of-living' theory and recently supported by several studies (e.g., Abele et al., 2009; Basova et al., 2012; Philipp et al., 2005; Richier et al., 2005). Furthermore, modifications of environmental conditions such as temperature and Ultra Violet Radiation are known to induce the reduction of oxygen into ROS, leading to protein oxidation, lipid peroxidation and DNA degradation (Halliwell and Gutteridge, 1999). For example, protein carbonyl contents and lipid peroxidation (= Malondialdehyde content) in sea anemones *Anemonia viridis* and *Actinia schmidti* increased three to tenfold under experimental exposure to hyperoxia (100% O_2) or thermal stress (elevated temperature + 7 °C above normal, Richier et al., 2005). Here, elevated prooxidant conditions induced a depletion of antioxidant defences (e.g., superoxide dismutase activity) and cellular oxidative stress (Richier et al., 2005). Similarly, higher oxidative damage accumulation in juvenile *H. echinata* at low compared to high food conditions at the respective temperature can result either from a higher level of oxidative stress, and/or from a failure to repair or replace damaged biomolecules (Halliwell, 2006) due to a lack of energy. Thus, the combined effect of stressful temperature and nutrition induced highest protein carbonyl contents in hydroids observed at 21 °C/low food. Meanwhile, oxidative damage accumulation was lower in hydroids in the high food treatments at both temperatures, probably due to higher energy availability

and to the induction of cell protective capacities. It has been shown that cells are able to adapt to enhanced production rates of ROS by an up-regulation of energy-consuming defence and/or repair systems (e.g., antioxidants or chaperons, Downs et al., 2002; Halliwell, 2006). For example, increasing seawater temperatures and UV radiation induced an up-regulation of homeostatic processes such as production of heat-shock proteins and antioxidant enzymes in corals (Downs et al., 2013; Lesser, 1996; Suggett et al., 2008). Irreparable cellular damage by ROS can induce apoptotic or necrotic processes (Halliwell and Gutteridge, 1999), and chronic or repeated exposure to oxidative stress may decrease the fitness and genomic integrity of the individual and its offspring (Beckman and Ames 1998). On the other hand, researchers have suggested that some cnidarian species are 'immortal' due to high tissue renewal capacities and a lack of deteriorative processes associated with aging (reviewed in Martinez, 1998). Therefore, future studies should investigate whether additional adaptations in *H. echinata* to thermal stress such as an up-regulation of chaperons (e.g., heat shock proteins) and enzymatic activity.

The majority of morphological and physiological parameters determined in the present study showed similar treatment effects in juveniles originating from parental colonies from Helgoland (=subtidal) or Sylt (=intertidal). Nevertheless, slight differences between the two populations might be based on epigenetic acclimatization to different local environmental conditions. For example, the total protein content was higher in all treatments in offspring of the parental colonies from Sylt. This might be due to environmentally induced differences in percentage of the main body components (protein, lipids and carbohydrates) or in the soft-body-to-chitin-skeleton-ratio between the colonies. In the intertidal at Sylt, hydroids are exposed to higher fluctuations in temperature and salinity compared to the subtidal due to strong tides and the influence of the Elbe river (Hickel, 1980). While subtidal habitats at Helgoland are dominated by rocky substrate, mudflats and high contents of suspended sediment can be found in the intertidal at Sylt. It is already known, that environmental factors such as temperature contribute to DNA methylation variations (Pu and Zhan, 2017) and alter gene expression, which could explain the observed location specific (Helgoland vs. Sylt) differences in biochemical parameters in *H. echinata*.

5. Conclusion

It is strongly debated whether the potential for acclimation in marine organisms will keep pace with the rate of ecosystem changes predicted for the future (reviewed in Hoffmann and Sgro, 2011). The results of the present study suggest, that food supply and habitat energy availability have to be taken into consideration when predicting responses of marine invertebrates to future environmental stressors. In agreement, recent studies about spatial species shifts identified habitat energy availability (e.g., phytoplankton abundance; Singer et al., 2016) as one of the major drivers of species distribution (e.g., Puce et al., 2009). Our results point towards an energy deficiency in *H. echinata* exposed to high temperature and low nutrition indicated by restricted growth, high rates of oxygen consumption and oxidative damage accumulation. On the other hand, high nutrition seems to mitigate the negative effects of stressful temperature conditions in juvenile hydroids. Energy budgets of *H. echinata* at 21 °C/high food conditions were sufficient to maintain growth rates and protein store as well as cell protective and repair capacities. Thus, high nutrition will most likely improve potentials for physiological resilience in juvenile *H. echinata* to increasing seawater temperatures predicted for 2100 in the North Sea, whereas hydroids will suffer at low food availability. This suggests, that high energy availability will increase the resilience of marine invertebrates to future environmental conditions. The present study shows that physiological studies based on multi-factorial laboratory experiments will improve our understanding of species specific distribution limits and ecosystem shifts in the face of climate change.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Animal rights

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2018.07.009.

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