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Acclimation capability inferred by metabolic performance in two sea cucumber species from different latitudes

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ABSTRACT

The notion that thermal specialists from tropical regions live closer to their temperature limits than temperate eurytherms, seems too generalized. Species specific differences in physiological and biochemical stress reactions are linked to key components of organism fitness, like metabolic capacity, which indicates that acclimation potential across latitudes might be highly diverse rather than simplistic. In this study the exposure of a tropical (*Holothuria scabra*) and a temperate (*Holothuria forskali*) sea cucumber species to identical cold- and warm-acclimation stress was compared using the key metabolic parameters, respiration rate, enzyme activity (ETS, LDH, IDH), and energy reserve fractions (lipid, carbohydrate and protein). Results show much broader respiratory adjustments, as response to temperature change, in *H. scabra* (2–30 $\mu gO_2*gww^{-1}*h^{-1}$) compared to *H. forskali* (1.5–6.6 $\mu gO_2*gww^{-1}*h^{-1}$). Moreover, the tropical species showed clearly pronounced up and down regulation of metabolic enzymes and shifts in energy reserves, due to thermal acclimation, while the same metabolic indicators remained consistent in the temperate species. In summary, these findings indicate enhanced metabolic plasticity in *H. scabra* at the cost of elevated energy expenditures, which seems to favor the tropical stenotherm in terms of thermal acclimation capacity. The comparison of such holistic metabolic analyses between conspecifics and congeners, may help to predict the heterogeneous effects of global temperature changes across latitudinal gradients.

1. Introduction

The emergence of thermal specialists and generalists implies a fundamental energetic trade-off between peak performance at optimal temperatures and thermal niche breadth (Verberk et al., 2015). Thermal specialists (stenotherms) can exhibit peak performance levels only within a very narrow thermal window, while thermal generalists (eurytherms) can maintain high performance levels across a wide thermal range (Schlichting and Pigliucci, 1998; Hochachka and Somero, 2002; Somero et al., 2008; Angilletta, 2009). Generally, tropical and polar species are considered stenothermic and temperate species eurythermic. This classification is based on the widely accepted 'Temperature Variability Hypothesis' that middle-latitude species exposed to high seasonal and diurnal temperature variations evolved a more marked thermal tolerance than species from low- and high-latitudes (Janzen, 1967; Addo-Bediako et al., 2000; Sunday et al., 2011). In the context of global climate change this theory predicts tropical specialists to experience the largest metabolic challenge due to rising temperatures, as they already live close to their upper temperature threshold level (Stillman, 2003; Tewksbury et al., 2008; Dillon et al., 2010; Huey et al., 2012). Consequently, tropical species may be especially vulnerable to oxygen uptake limitations at warmer temperature conditions (Somero, 2010; Rummer et al., 2014). Generalizations are, however, difficult as responses to thermal acclimation pressure, such as rapid changes in metabolic activity, can differ considerably among species.

The capability to optimize energy demand through respiratory adjustments has been associated with the adaptive response of thermal specialists to acclimatize to seasonal changes (Seebacher, 2015). Hence, the resilience of ectothermic animals, towards thermal challenge, can be linked to energy-physiological plasticity. Similar patterns were also found for the acclimatization potential of tropical stenothermic mangrove crabs (Fusi et al., 2014). These studies focused on species-specific metabolic plasticity and proposed that equatorial stenotherms can

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possess a higher acclimatization potential than eurytherms. Apparently, the adaptation of thermal generalists to variable environments reduces acclimatization capacity (Huey and Berrigan, 1996; Hoffmann, 1990; Van Buskirk and Steiner, 2009; Chown et al., 2010). The debate about 'winners and losers' in future global warming scenarios is ongoing, and contrasting metabolic plasticity patterns between tropical stenotherms and temperate eurytherms are central to determining acclimation capacities of species from different climatic zones.

Future global warming scenarios will intensify acclimatization pressure, especially for sessile and slow-moving benthic organisms like sea cucumbers, which do not exhibit fast distribution shifts. Sea cucumbers can significantly shape benthic communities through their high abundances (Crozier, 1918; Birkeland, 1988; Billett, 1991). They also provide important ecological functions, such as nutrient recycling, enhancing of sea water chemistry, supporting biodiversity as host of many symbiotic associations and transmission of primary food sources to higher trophic levels as prey (Purcell et al., 2016). The sea cucumbers *Holothuria scabra* and *Holothuria forskali* represent two widely distributed species inhabiting similar ecological niches in tropical and temperate regions, respectively.

In a previous study, Kühnhold et al. established the combined assessment of key energetic enzymes activity (iso-citrate dehydrogenase (IDH) and lactate dehydrogenase (LDH)), respiration through direct (whole-organism oxygen consumption rate (OCR)) and indirect (activity of the electron transport system (ETS)) measurements, and changes in individual energy depots (carbohydrate, protein and lipid), as a comprehensive tool to assess energy-metabolic changes related to thermal acclimation, in the tropical sea cucumber *H. scabra* (Kühnhold et al., 2017). The same biomarker approach was now applied on the temperate sea cucumber *H. forskali*, following identical experimental design. The aim of this study is to compare the new data from *H. forskali* (eurytherm) with published data from *H. scabra* (stenotherm) to investigate whether thermal acclimation capacity, solely based on metabolic adjustments, differs consistently in two congeners from different latitudes. The assessment of thermal acclimation capacities in these two ecologically and commercially relevant sea cucumbers is pivotal to predict the effects of ocean warming, due to global climate change, and to minimize thermal stress in aquaculture scenarios.

2. Materials and methods

2.1. Model species and sampling area

Holothuria scabra distribution is tropical to subtropical (Hamel et al., 2001) within a preferred temperature window ranging from 25 to 29 °C (OBIS, 2017). *Holothuria forskali* occurs in temperate regions, along the entire Atlantic Coast of Europe and the United Kingdom, and in parts of the Mediterranean Sea (Mercier and Hamel, 2013). Due to factors, such as seasonality, *H. forskali* is exposed to a broader thermal variability, hence, its preferred temperature window ranges from 11 to 19 °C (OBIS, 2017). In this study, optimal temperature conditions were considered as centre of each species-specific thermal window. Therefore, control temperatures of 27 °C and 15 °C where chosen for *H. scabra* and *H. forskali*, respectively. For both species, juvenile animals of similar size (10–30 g) were collected by SCUBA divers near shore in water depth between 3 and 10 m. *H. forskali* were collected in February 2015 at Carreiro de Joannes (39°21′14.3″N, 9°23′43.6″W) off the coast of Peniche, Portugal. In the previous study by Kühnhold et al. (2017), *H. scabra* were collected in August 2014 at Pantai Sira di Pagi Hari (8°22′5.42″S, 116°6′58.37″E) off the coast of Lombok, Indonesia.

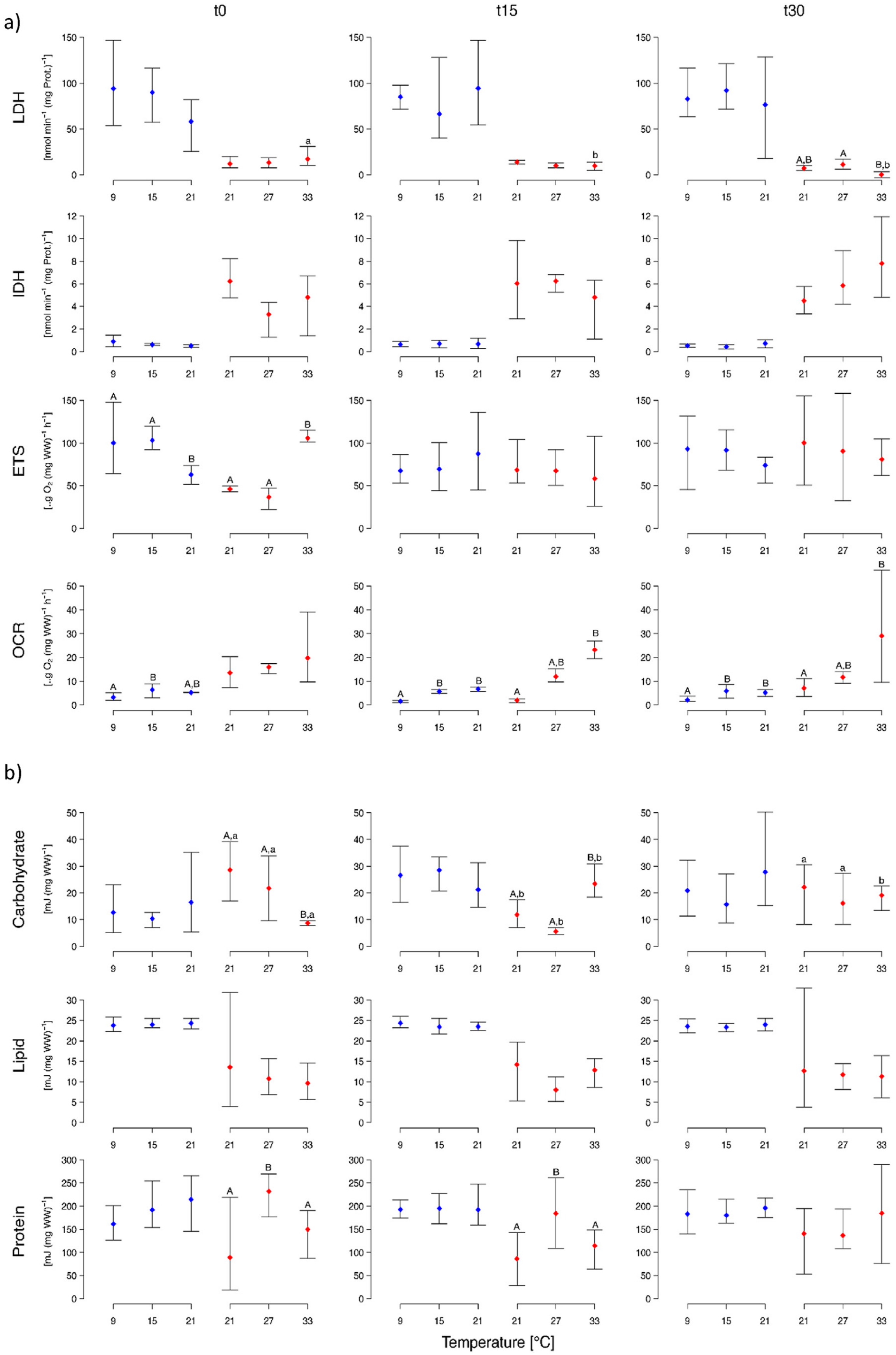
Holothuria scabra (Jaeger, 1833) were exported from hatchery facilities at the Indonesian Research Centre for Oceanography (LIPI) on Lombok, Indonesia. These were transported by air-freight in sealed seawater containers to the Alfred Wegener Institute, Helmholtz-Centre for Polar and Marine Research (AWI) in Bremerhaven, Germany-Import procedures (invertebrate import from registered hatchery) were completed by the AWI import office to EU standards. Wild-caught *Holothuria forskali* were exported from the aquaculture research facilities of MARE – Marine and Environmental Sciences Centre of the Polytechnic Institute of Leiria in Peniche, Portugal, and transported by air-freight in sealed seawater containers to the Alfred Wegener Institute, Helmholtz-Centre for Polar and Marine Research (AWI) in Bremerhaven, Germany. Import procedures were completed at airport point of entry verbally as per transports within the European Customs Union – upon arrival both species were maintained in closed recirculation systems for 14 days for acclimation and to ensure quarantine standard were maintained.

2.2. Experimental design

Thermal acclimation experiments were conducted sequentially for each species. Culture tanks and experimental design conditions were identical for both species. A total of 72 animals were randomly assigned to three separate high density polyethylene (HDPE) tanks (24 animals per tank), filled with 100 L aerated seawater (15 °C (*H. forskali*) and 27 °C (*H. scabra*), 33 ppt) and a layer (approx. 7–10 cm) of autoclaved sea sand. The photoperiod was 12 h:12 h (light:dark) and animals were fed *ad libitum*, with the commercial feed 'Algamac' (39% protein, 20.4% lipid and 20.6% carbohydrate) (www.aquafauna.com). The water of each tank circulated through individual filter units, containing a biofilter and a protein skimmer. Sea cucumber feces and excess feed were syphoned every day from the sediment to prevent sediment fouling and build-up of decomposing matter. After an acclimation period of 28 days, the animals in the treatment tanks were exposed to incrementally increasing (+1 °C/day) and decreasing temperatures (-1 °C/day), until the designated treatment temperatures (*H. forskali*: cold – 9 °C, control – 15 °C, warm – 21 °C; *H. scabra*: cold – 21°; control – 27 °C; warm – 33 °C) were reached. Seven animals ($n = 7$) were sampled after 6 days, when the desired treatment temperatures were reached (t0), and subsequently after an acclimation time of 15 (t15) and 30 days (t30). At each sample time, longitudinal muscle (for LDH, IDH and energy depots) and respiratory tree (for ETS) tissues were removed, immediately shock frozen and stored at −80 °C. In addition, at each sample interval, marked animals ($n = 3$; photo identified) were placed in gas-tight acrylic chambers, placed in the culture tanks. In this way, the real-time oxygen consumption of the living animals could be measured while being exposed to the experimental temperatures (see Kühnhold et al., 2017 for more details). The ETS measurements for cellular oxygen consumption as well as the enzyme assays (LDH, IDH, ETS) and analyses of energy depots (protein, carbohydrate and lipids), were conducted as per Kühnhold et al. (2017) at 25 °C, in triplicates and using a Synergy H1 Hybrid Multi-Mode Microplate Reader (BioTek Instruments, Vermont, USA).

2.3. Statistical analysis

Subsequent to testing the data for normality and homogeneity (Kolmogorov–Smirnov; Levene tests), statistical differences between temperature treatments (cold, control and warm) and measurement times (t0, t15 and t30) were evaluated by two-way ANOVA (Microsoft Excel 2011 with "StatPlus" for Mac). Significant multiple comparisons were determined using the Fisher-LSD post-hoc test followed by a Bonferroni p-value correction to account for random effects due to multiple comparisons against temperature. For all statistical tests, the significance level was set at the Bonferroni adjusted p-value (p_{adj}) $p_{adj} < 0.048$. Data are expressed as mean ± minimum and maximum values. For an overall comparison, a 'Principal Component Analysis' (PCA) was also conducted using the statistical program R-Studio (package: "vegan"), where missing replicates (if any) were replaced by group means.



(caption on next page)

Fig. 1. Data for *Holothuria scabra*[25] temperature treatments of 21 °C, 27 °C, and 33 °C (red diamonds). *H. forskali* temperature treatments of 9 °C, 15 °C, and 21 °C (blue diamonds). Measured parameters, activity of lactate dehydrogenase (LDH), iso-citrate dehydrogenase (IDH), electron transport system (ETS), oxygen consumption rate (OCR) (a); total amounts of carbohydrate, lipid, and protein (b). Measurement times, day 0 (t0), day 15 (t15), and day 30 (t30) are shown from left to right. Data points represent mean values with error bars of minimum and maximum values. $^{\mathrm{A,B}}$ indicate significant differences between temperature treatments within one measurement time and $^{\mathrm{a,b}}$ indicate significant differences between measurement times at one temperature (two-way ANOVA, Fisher LSD, $p = 0.048$).

3. Results

The results of this study revealed no clear thermal acclimation response, in form of marked changes in oxygen consumption, metabolic enzyme activity and energy reserve fractions, in the temperate species *H. forskali*. This pattern is in stark contrast with the previous findings for the topical species *H. scabra*, which showed a clear trend of thermal acclimation, foremost through adjustments of metabolic enzyme activity and increased respiration, especially at warmer temperatures. Throughout the experiment, none of the temperature treatments led to animal mortalities for neither of the two species.

3.1. Enzymes related to energy metabolism

Specific LDH activity in *H. forskali* did not differ significantly between temperature treatments and time points. In *H. scabra*, LDH activity reduced over time with significant differences between t0 and t15 ($p = 0.0111$) and t0 and t30 ($p = 0.0001$) in warm acclimated animals (Fig. 1 a). The lowest LDH activities in *H. scabra* were measured under warm conditions after 30 days, with significant differences between warm (33 °C) and control (29 °C) treatment ($p = 0.025$). Specific IDH activities in *H. forskali* exhibited no significant changes over time or between temperature treatments. In *H. scabra*, after 30 days (t30) of acclimation to 33 °C, IDH activities exceeded LDH activities in absolute numbers (IDH = 7.8 ± 2.7 nmol*min^{-1}*mg Prot.$^{-1}$; LDH = 4 ± 0.64 nmol*min^{-1}*mg Prot.$^{-1}$) (Fig. 1 a). This switch in dominance of energy related key enzymes, was only observed under warm conditions in *H. scabra.*

3.2. Direct and indirect oxygen consumption

At the first measurement time (t0) *H. forskali* exhibited significantly lower ETS activities at warmer (21 °C) temperatures, compared to the activities measured at control (15 °C) and cold (9 °C) conditions ($p = 0.0155$; $p = 0.0226$). Thereafter *H. forskali* maintained stable ETS activities at all temperatures and throughout the acclimation time. *Holothuria scabra* initially (t0) showed a significant peak in ETS activity at warm conditions compared to the control ($p = 0.001$) and cold ($p = 0.005$) treatment. Over the remaining experimental time mean ETS activities of *H. scabra* did not vary significantly between treatment conditions (Fig. 1 a). Throughout the experiment, *H. forskali* maintained its OCR within a mean range of 1.5–6.6 μgO$_2$*gww^{-1}*h^{-1}, and showed an overall trend of similar oxygen consumption rates for control (15 °C) and warm (21 °C) exposed animals, while cold (9 °C) exposed animals showed significantly lower OCR levels compared to controls (t0: $p = 0.0376$; t15: $p = 0.0078$; t30: $p = 0.0142$). The experiment with *H. scabra* revealed consistently higher mean OCR levels in warm (33 °C) exposed specimens, and lower consumption rates under cold (21 °C) conditions. This trend was noticeable throughout the entire experiment, although significant differences were only measured at t15 (33 °C vs. 21 °C: $p = 0.019$) and t30 (33 °C vs. 21 °C: $p = 0.047$). For *H. scabra* the overall range of mean OCR levels was between 2 and 30 μgO$_2$*gww^{-1}*h^{-1} (Fig. 1 a).

3.3. Energy reserve fractions

Mean carbohydrate levels in the muscle tissue of *H. forskali* did not differ significantly between the three treatment temperatures or between measurement times. Significant variations in carbohydrate levels were observed in *H. scabra* over time and between treatment temperatures. At the first sampling (t0), warm (33 °C) treated *H. scabra* exhibited significantly lower carbohydrate levels compared to the specimens cultured under cold (21 °C; $p = 0.00001$) and control (27 °C; $p = 0.0023$) conditions (Fig. 1 b). Over time, *H. scabra* that were acclimated under warm conditions showed a significant increase in carbohydrate level ($p = 0.0004$) between the first (t0) and the second measurement time (t15). Whereas, control and cold animals showed a significant drop in carbohydrate levels between the first two measurement times ($p = 0.0002$; $p = 0.0001$), which led to significantly lower levels relative to the warm conditions ($p = 0.00003$; $p = 0.0043$). After 30 days of acclimation (t30), no significant differences in carbohydrate level in *H. scabra* were observed (Fig. 1 b). Neither *H. forskali* nor *H. scabra* showed significant differences or trends between treatment temperatures and measurement times in the lipid fraction (Fig. 1 b). No significant differences or trends were visible in the protein levels measured in *H. forskali*. Muscle protein levels in *H. scabra* were significantly different at the first two measurement times (t0 and t15), as control animals had higher protein levels compared to the levels measured at cold (t0: $p = 0.0004$; t15: $p = 0.0092$) and warm (t0: $p = 0.0027$; t15: $p = 0.0486$) treatments. At t30, however, the significant differences between the three treatments were no longer observed. In *H. forskali* total mean protein levels ranged from 160 to 220 mJ*mg^{-1}, while in *H. scabra* total mean protein varied markedly and ranged from 80 to 240 mJ*mg^{-1} (Fig. 1 b).

3.4. Principal component analysis (PCA)

All parameters measured for both species, at each measurement time and temperature treatment, are summarized with a principal component analysis (PCA) (Fig. 2). This data view revealed that PCA 1 (x-axis) is driven primarily by differences between species and explains 47.7% of all differences between the data. The associated vectors along the x-axis, which tend in opposite directions, indicate that the differences between the species is mainly explained by the parameters IDH, LDH, OCR, and lipids. While IDH and OCR are consistently higher in *H. scabra*, and LDH and the lipid fraction were higher in *H. forskali.* PCA 2 (y-axis) explains merely 17% of the differences between all measured parameters. In this dimension the vectors of ETS and carbohydrate are responsible for most of the variation.

4. Discussion

This study investigated the potential connectivity between adaptation to regional thermal variability and metabolic plasticity in two congenic sea cucumbers, representing a tropical stenotherm (*H. scabra*), and a temperate eurytherm (*H. forskali*). The results revealed species specific adjustments of the metabolic system, as response to identical thermal acclimation. *Holothuria forskali* exhibited a relatively consistent metabolic performance over a broad temperature range (12 °C), indicative for a thermal generalist. In contrast to that, *Holothuria scabra* showed a pronounced metabolic response over a temperature change of the same magnitude, especially during warm-acclimation, which is characteristic for a thermal specialist. However, next to these expected thermal acclimation trends, the lower thermal responsiveness exhibited by *H. forskali*, relative to *H. scabra*, may suggest a lower capability towards temperature change in the temperate species, rather than enhanced thermal resilience. This comparative analysis may provide important insights into the heterogeneous effects of future ocean

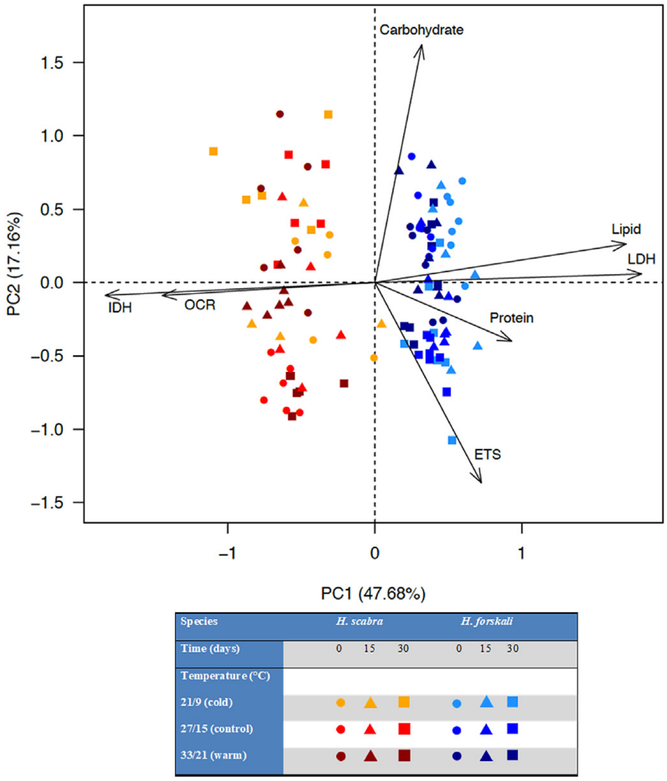


Fig. 2. Principal Component Analysis (PCA). *Holothuria scabra* and *Holothuria forskali,* and the different temperature treatments are indicated by different colors (see legend). Time points are presented in different shapes: Circle (t0), triangle (t15), and square (t30). Principal component 1 (PC 1) is given in the dimension of the x-axis and explains 47.68% of all differences between the parameters. Principal component 2 (PC2) is represented as y-axis dimension and explains 17.16% of all data variation. The visualization in a simplified two-dimensional model shows that data disparities are caused to 64.84% by the three conditions 1) species, 2) temperature and 3) time.

warming, driven by species-specific metabolic susceptibility patterns.

The metabolic enzyme lactate dehydrogenase (LDH) is crucial in anaerobic energy pathways (Hochachka and Somero, 2002). LDH activity levels have been identified as an adaptive trait to distinct thermal variability and geographical latitudes (Fields and Somero, 1998). In sea cucumbers, increased LDH activity can indicate stress response to hypoxia (Guo et al., 2014) and transportation stress (Tonn et al., 2016). The activity of iso-citrate dehydrogenase (IDH) represents the energetic antagonist of LDH, reflecting aerobic energy turnover. While *H. forskali* showed no significant trends in metabolic key enzymes activities of LDH and IDH across temperatures or time, a noticeable but not significant elevated IDH level was seen in cold treated (9 °C) *H. forskali* at t0 and at warm conditions (21 °C) after 30 days of acclimation (t30). The same pattern was observed in *H. scabra* and may indicate increased aerobic performance after the initial temperature drop of 6 °C (15 °C down to 9 °C (*H. forskali*) and 27 °C down to 21 °C (*H. scabra*)), due to acutely elevated energy requirements. The enhanced IDH activity under warmer conditions at the final measurement time (t30) appears to indicate a growing relevance of aerobic energy turnover over time, due to warm-acclimation in both species. Conversely LDH levels in warm treated *H. scabra* exhibited significantly lower LDH expression levels at t15 and t30 compared to t0.

Activity changes in LDH represent switches in energy turnover due to challenging conditions (Dahlhoff, 2004). At t30 the LDH activity level of warm acclimated *H. scabra* dropped below total values of IDH, indicating augmented aerobic metabolism induced by warm-acclimation. Determining the capability to sustain an appropriate aerobic metabolism under acute thermal variability is a cornerstone principle to understand thermal tolerance in different species (Pörtner and Knust, 2007; Pörtner and Farrell, 2008; Pörtner and Giomi, 2013). In this sense the ability of *H. scabra* to switch from an anaerobic (LDH dominated) to an aerobic (IDH dominated) driven metabolism indicates a pronounced acclimation capacity in the form of high metabolic plasticity to respond to anomalous temperature events. Supporting this hypothesis is the difference in total IDH and LDH activity within each species. *Holothuria forskali* exhibited consistently higher mean LDH activity levels relative to the activity levels of mean IDH, which is in line with the data obtained by Tonn et al. (2016), for *H. forskali*. In comparison *Holothuria scabra* showed only slightly lower ranges of mean IDH- compared to mean LDH- activities, which enabled the tropical species even to switch towards an IDH dominated energy turnover during warm acclimation. In general, this indicates that sea cucumbers are facultative anaerobes, in line with their generally slow-moving lifestyle as bottom dwellers.

A coherent metabolic strategy across a broad temperature range is typical for a thermal generalist[16]. The more prominent difference between LDH- and IDH activity in *H. forskali* indicates exclusive LDH dominated energy turnover and can be interpreted as an energetic attribute of the temperate eurytherm. In contrast, *H. scabra* seems to be able to switch between anaerobic and aerobic dominated energy turnover, driven by changes in LDH and IDH activity. This enables *H. scabra* to increase its metabolic efficiency under challenging conditions such as critical temperatures, and more importantly, may reveal pronounced warm-acclimation in the warm tropical stenotherm.

Rate of oxygen consumption and adjustments in respiratory physiology are crucial to buffering environmental stress (Verberk et al., 2015; Verberk and Bilton, 2013). It is predicted that tropical stenotherms face higher energy expenditures than eurytherms especially when approaching their upper temperature limit. Increased respiration and electron transport system (ETS) activity, along with depleted carbohydrate and protein reserves at t0 warm treated *H. scabra* show the initial increase in temperature clearly promoted oxygen demand, which led to higher energy turnover and depletion of energy reserves. The consistently elevated respiration rate of *H. scabra* acclimated to warm conditions, accompanied by ETS levels and energy reserves that stabilized at levels equal to control and cold conditions shows successful warm-acclimation of the thermal specialist through elevated respiration in combination with adjustments in metabolic enzyme activity. Enhanced foraging activity was observed in the warm exposed *H. scabra*, implying increased food intake as another important warm-acclimation factor, not quantified in this study. The eurytherm species *H. forskali* did not show an increased oxygen demand due to increased temperature. At t0 *H. forskali* exhibited the lowest ETS activity under warm conditions. Throughout the experiment *H. forskali* acclimated to control and warm conditions showed very similar respiration and ETS activities. Clear signs of respiratory depression due to cold-acclimation were visible in *H. forskali*. According to Bao et al. (2010), cold induced metabolic loss (hibernation) was much more pronounced in the temperate sea cucumber *Apostichopus japonicus* than reduced energy demand driven by warm temperature (estivation). Hibernation and estivation are well described for sea cucumbers inhabiting the same latitude as *H. forskali*, such as *Holothuria tubulosa* (Coulon and Jangoux, 1993). Hence, our findings of enhanced down-regulation of respiration in cold-acclimated *H. forskali* could be associated to a hibernation behavior, which has not been studied yet in this species. In *H. scabra*, both up- and down –regulation of 6 °C caused clear deflections in respiration, while in *H. forskali* only the 6 °C temperature drop led to a clear adjustment of the respiratory system. Summarized, this points towards a broader aerobic scope in *H. scabra* than in *H. forskali*, which might be indicative for more advanced metabolic plasticity and, thus, higher acclimation capacity of the thermal specialist. To confirm this hypothesis, further studies on the thermal tolerance levels such as aerobic scope and associated species-specific critical temperature levels need to be implemented.

The nomination of polar- and tropical-stenotherms as undisputed losers in terms of ocean warming, due to adaptation to more stable thermal environments appears over-simplified[1,37]. As described by Williams (2008), a multilevel integrated approach is essential when assessing species-specific susceptibility and plasticity towards climate change at different latitudes. This also requires the detection of thermal acclimation stress at different organization levels (Kamyab et al., 2017). In the current study, different levels of the metabolic system were compared between two sea cucumbers from contrasting thermal environments, to determine distinct patterns in thermal-acclimation capacity. The results of this study, which reveal a prominent metabolic response due to warm acclimation in the tropical species, confirm the prediction that metabolic adjustments come with the burden of increased energetic costs, especially in tropical species (Stillman, 2003; Tewksbury et al., 2008; Dillon et al., 2010; Huey et al., 2012). However, costly adjustments of the aerobic system have also been associated with enhanced acclimation capacity to cope with extreme temperature events (Seebacher, 2015; Fusi et al., 2014). Seen from this perspective, the enhanced metabolic plasticity in *H. scabra* may point towards higher thermal tolerance and to greater resilience to future warming scenarios in this tropical stenotherm.

Whole organism respiration and key metabolic enzymes activities of IDH and LDH were identified as main drivers of thermal acclimation and detected clear higher warm-responsiveness in *H. scabra*. The total values of oxygen consumption (OCR) and metabolic enzyme activities (LDH, IDH) were also the most prominent factors that determined differences between the two species. This pattern was summarized descriptively using a PCA plot (Fig. 2), which shows clear dominance of aerobic performance parameters (OCR and IDH) in the tropical species while indicators for an anaerobic metabolism (LDH) were prevalent in the temperate species. Multivariate testing using redundancy analysis (RDA) revealed that species differences dominate significantly relative to the effects of temperature and time (Fig A1-A3 and Table A1 in the supplementary information). It is important to reiterate, however, that the focus of this study is the differences in individual, species-specific changes in performance parameters tested through the univariate analyses, rather than comparison of absolute values. The PCA plot is solely used as a descriptive tool as potential deviations in the level of technical, chemical and human factors limit the comparison and, most specifically, hypothesis testing of absolute values between these two experiments.

While the present findings show differences in energetic strategies between the two sea cucumber species, these may not represent thermal adaptation alone, but also other adjustment- or behavioral-patterns matching local environmental requirements. Both species are slow-moving bottom dwellers, well adapted to be enclosed by sediment and rocky crevices, which involves continuous adjustments of the predominant pathway for oxygen uptake: 1) ventilation through the posterior opening and 2) cutaneous respiration (Newell and Courtney, 1965). In contrast to *H. forskali*, *H. scabra* exhibits a diurnal burying cycle that requires a clear switch of the respiratory system to adjust to the contrasting conditions above and below the sediment. In this study, however, potential differences in terms of the relative contribution of the two oxygen-uptake pathways can be neglected as the oxygen consumption rate measurements accounted for both, posterior and cutaneous respiration. Similarly, direct effects of the burrowing behavior on the biochemical analyses can be excluded as tests were only conducted on unburied animals. The potential connection between a diurnal burrowing cycle and enhanced metabolic flexibility, which may lead to increased acclimation capacity, is of interest for future studies. The current findings are only representative for juvenile sea cucumbers. The metabolic response towards thermal acclimation may deviate significantly in adult species as energy allocation will be seasonally affected by gonad development. Both sea cucumber species are considered relevant aquaculture candidates. While the grow-out of hatchery sourced *H. scabra* in shallow intertidal zones is well established in many tropical regions (Hamel et al., 2001; Purcell et al., 2014), the species *H. forskali* has been recently identified as promising newcomer for the aquaculture sector in temperate regions (Santos et al., 2015). In general, sea cucumber production at shallow culture sites provide obvious advantages, in terms of management practices (e.g. animal accessibility for monitoring and harvesting), compared to deeper culture sites. However, the downside of shallow water farming is the increased thermal acclimation challenge, due to more rapid diurnal and seasonal temperature fluctuations. This kind of cultivation might expose the animals to significantly higher temperature variations than across their usual range. Moreover, water temperature changes are expected to be even more difficult to predict under the influence of climate change, which may follow as yet unascertained geographical variations (Shears and Bowen, 2017). A detailed understanding of thermal acclimation capacities is, therefore, of prime importance for the management of sea cucumber farms, including potential changes in the production cycle (i.e. earlier or later seeding of farm sites) and/or repositioning of culture sites. *H. scabra* metabolic response towards acute thermal maxima (Kühnhold et al., 2019) and longer-term thermal acclimation (Kühnhold et al., 2017; Kamyab et al., 2017) has been extensively studied. The current study provides first insights into the metabolic efficiency of *H. forskali* in response to thermal acclimation, and brings in the dimension of latitudinal differences through the comparative analyses with *H. scabra*. In conclusion, this detailed analysis of different metabolic system levels in two sea cucumber species enhances our knowledge of thermally driven metabolic changes. Assumptions about latitudinal effects, however, need to be validated in further studies. Population level studies with these two sea cucumber congeners, as suggested by Fusi et al. (2014) are recommended. Both species have a wide distribution breadth across different latitudes. Therefore, comparing metabolic plasticity within one species, between distribution limits, will help to assess thermal tolerance levels along latitudinal gradients and to predict the heterogeneous effects of future ocean warming scenarios.

Contributions

H.K. and A.K. conceived the study. H.K., E.K and M.S. conducted the experiments. S.C.N and M.F.L.L. supervised and assisted the laboratory assays and data analyses. H.K., L.M.F.A. and E.K. conducted the laboratory work. H.K. and S.C.N. drafted the manuscript, which was then completed with contributions from all authors.

Conflicts of interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2019.07.019.

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