The most primitive metazoan animals, the placozoans, show high sensitivity to increasing ocean temperatures and acidities.

Article:

Schleicherová, Dáša, Dulias, Katharina, Osigus, Hans-Jűrgen et al. (3 more authors) (2017) The most primitive metazoan animals, the placozoans, show high sensitivity to increasing ocean temperatures and acidities. Ecology and Evolution. pp. 895-904. ISSN 2045-7758

https://doi.org/10.1002/ece3.2678

Received: 15 March 2016 | Revised: 9 November 2016 | Accepted: 13 November 2016

DOI: 10.1002/ece3.2678

ORIGINAL RESEARCH

WILEY Ecology and Evolution Open Access

The most primitive metazoan animals, the placozoans, show high sensitivity to increasing ocean temperatures and acidities

Dáša Schleicherová* | **Katharina Dulias*** | **Hans-Jűrgen Osigus** | **Omid Paknia** | **Heike Hadrys** | **Bernd Schierwater**

ITZ, Ecology and Evolution, TiHo Hannover, Hannover, Germany

Correspondence
Dáša Schleicherová and Katharina Dulias, ITZ, Ecology and Evolution, TiHo Hannover, Hannover, Germany.
Emails: dasa.schleicherova@ecolevol.de, katharina.dulias@hud.ac.uk

Present address
Katharina Dulias, Department of Biological Sciences, School of Applied Sciences, University of Huddersfield, Huddersfield, UK

Funding information
Studienstiftung des deutschen Volkes; Leverhulme Trust; Deutsche Forschungsgemeinschaft, Germany, Grant/Award Number: DFG-Schi 277/29-1

Abstract

The increase in atmospheric carbon dioxide (CO_2) leads to rising temperatures and acidification in the oceans, which directly or indirectly affects all marine organisms, from bacteria to animals. We here ask whether the simplest—and possibly also the oldest—metazoan animals, the placozoans, are particularly sensitive to ocean warming and acidification. Placozoans are found in all warm and temperate oceans and are soft-bodied, microscopic invertebrates lacking any calcified structures, organs, or symmetry. We here show that placozoans respond highly sensitive to temperature and acidity stress. The data reveal differential responses in different placozoan lineages and encourage efforts to develop placozoans as a potential biomarker system.

KEYWORDS

biomarkers, evolutionary constraints, global warming, ocean acidification, placozoa

1 | INTRODUCTION

Global warming has been changing the phenology, abundance, and distribution of many taxa in marine and terrestrial ecosystems (e.g., Falkowski, 2012; Thackeray, Jones, & Maberly, 2008) and ultimately affects all living taxa on earth. The immediate outcomes of climate change include ocean acidification, ocean warming, sea level rise (and subsequent changes in ocean circulation), and decrease in salinity (Houghton et al., 2001). For the potentially particularly affected benthic marine invertebrates, very little data exist and more empirical data are urgently needed in order to better understand possible changes in marine benthic ecosystems (Chen, 2008; Törnroos et al., 2014).

Animal populations may respond to shifting conditions in different ways, for example, expanding their ecological niche and/or by moving to a new habitat (Hinder et al., 2014). How such demographic processes will develop in the future has become a crucial question in many areas of ecological research. Habitat suitability models, which aim to predict how species ranges might change, are a theoretical means to find answers (e.g., Paknia & Schierwater, 2015; Törnroos et al., 2014). On the other side, empirical measures may include the use of sensitive biomarkers in long-term monitoring studies and promise to be more sensitive and possibly also more reliable (cf. Feindt, Fincke, & Hadrys, 2014; Hadrys et al., 2005; Hardege et al., 2011; Schroth, Ender, & Schierwater, 2005).

1.1 | Effects of ocean warming

Increasing temperatures often disturb physiological processes by damaging proteins, membrane fluidity, or organ function (Hochachka & Somero, 2002). As many marine organisms live close to their thermal tolerance (Hughes et al., 2003; Somero, 2002), increase in temperature may have severe impact on their performance and survival. Many reef-building corals for example respond to warm episodes with widespread coral bleaching and show increased rates in mortality (Hughes et al., 2003; McWilliams et al., 2005). Often it is the planktonic larval or early benthic stages, which are especially

---

*Both authors have contributed equally and share first authorship.

sensitive (e.g., Foster, 1971; Pechenik, 1989). Rising water temperatures can also drive behavioral changes at the community level. To name just two out of many examples: The timing of spawning in the marine bivalve, *Macoma balthica*, is temperature dependent and so is the strength with which the sea star *Pisaster ochraceus* interacts with its principal prey (habitat forming mussels; Sanford, 1999). For the placozoans, which are found in most temperate and warm marine waters, nothing has been known yet about their sensitivity to temperature stress.

1.2 | Effects of ocean acidification

The rapidly increasing carbonic emissions into the atmosphere (e.g., Neftel et al., 1985) have led to a decrease in the seawater pH at a rate of 0.02 units per decade (IPCC, 2013). This acidification can cause serious problems to organismal functions with respect to metabolism, calcification, and others (Langenbuch & Pörtner, 2003; Munday, Crawley, & Nilsson, 2009; Munday, Dixson, et al., 2009; Nakamura et al., 2011; Pörtner, 2008; Pörtner & Peck, 2010; Uthicke, Soars, Foo, & Byrne, 2013; Uthicke, Pecorino, et al., 2013). As a long-term result, species communities may change, with some species simply disappearing (e.g., Goodwin et al., 2013; Pörtner, 2008) and others finding new niches (e.g., Foo et al., 2012; Parker et al., 2012; Sunday et al., 2011). No information is yet available for placozoans, which—in sharp contrast to the majority of other invertebrates—lack any kind of organs for homeostatic regulation.

Overall, the literature on documented effects of rising temperature and acidity on marine invertebrates is limited, but nonetheless covers a broad spectrum of levels of observation and sensitive taxa (Table 1). The shown summary table documents how fragmentary our current is. Evolutionary constraints are part of every organism, but the limitations for adaptation to environmental change are hard to foresee. Moreover, little is known about combined effects of ocean warming and acidification on the development of marine invertebrates. Combined effects of such stressors are not necessarily cumulative, because both additive and antagonistic (stress decreasing if combined) effects are known (Byrne & Przeslawski, 2013; Folt et al., 1999). Such effects have been studied in corals, mollusks, echinoderms, and crustaceans, across different ontogenetic stages. Additive negative effects on fertilization or growth rate, respectively, have for example been reported from the coral, *Acropora tenuis*, (Albright & Mason, 2013) and the oyster, *Crassostrea gigas* (Parker, Ross, & O'Connor, 2010). Antagonistic effects have been found for example in the sea urchins *Heliocidaris tuberculata* (Byrne et al., 2010) and *Sterechinus neumayeri* (Byrne et al., 2013; Ericson et al., 2011), where warming partially compensated for the negative effect of acidification on larval growth.

In this study, we investigate the effects of temperature and acidity stress on placozoan reproduction and report strong and differential effects for both factors on the population growth rate (PGR) in different lineages (species) of placozoans. The observed differential sensitivity of different placozoan species or lineages suggests that placozoans might be promising organisms for developing a new generation of biomonitoring systems.

2 | MATERIALS AND METHODS

2.1 | Study organism

The phylum Placozoa holds a key position in the metazoan Tree of Life, close to the last common metazoan ancestor. Placozoans represent the simplest (not secondarily reduced) metazoan bauplan and have become an emerging model organism for understanding early metazoan evolution (Eitel et al., 2013; Schierwater, de Jong, & DeSalle, 2009; Schierwater, Eitel, et al., 2009; Schierwater et al., 2016; Signorovitch, Dellaporta, & Buss, 2006).

These tiny invertebrates are common in warm tropical and subtropical as well as in some temperate marine waters in different depths up to 20 m. Their preferred habitats are calm water areas with hard substrates like mangrove tree roots, rocks, corals, and other hard substrates in the eulittoral and littoral zone. Placozoans have occasionally also been found on sandy surfaces or in areas with high wave activity. Yet, the biodiversity and ecology of placozoans are poorly known (Eitel & Schierwater, 2010; Maruyama, 2004; Pearse & Voigt, 2007).

Recent genetic studies have revealed a high biodiversity and systematic complexity of the Placozoa. As no morphological differences are visible among placozoan haplotypes in light microscopy, the known haplotypes represent “cryptic” species (Eitel & Schierwater, 2010; Loenarz et al., 2011; Schierwater, 2005; Schierwater, de Jong, et al., 2009; Schierwater, Eitel, et al., 2009; Signorovitch et al., 2006). At present, the phylum Placozoa is the only monotypic phylum in the animal kingdom, with the only formally described species *Trichoplax adhaerens* (Schulze, 1883, 1891). Placozoans offer unique possibilities for experimental ecophysiological studies because of their small size, simple morphology, and fast vegetative reproduction (Eitel & Schierwater, 2010; Eitel et al., 2011, 2013; Schierwater, 2005). Vegetative reproduction through binary fission or budding is the usual mode of reproduction in the laboratory and in the field. In contrast, bisexual reproduction is rarely seen in the laboratory, but most likely present in all placozoans (Eitel et al., 2011; Signorovitch, Buss, & Dellaporta, 2007). The details of sexual reproduction and embryonic development in placozoans remain widely unknown, because all efforts to complete the sexual life cycle in the laboratory have been unsuccessful, because embryonic development has never gone beyond the 128 cell stage (Eitel et al., 2011). As the overall effects of physiological stress are best seen in the performance of vegetative reproduction by binary fission, we used overall PGR as the dependent and easily quantifiable variable for the subsequent experiments.

2.2 | Experimental setup for temperature experiments

All animal lineages used in the experiments have been cultured in our Institute of Animal Ecology and Cell Biology of the TiHo, Hannover (Germany), for several years:

1. H1—*Trichoplax adhaerens* (cosmopolitic), our so-called Grell lineage found by Karl Gottlieb Grell in an algal sample from the Red

TABLE 1 Summary of temperature and ocean acidification effects on marine biota in current literature

Major group	Studied organism	Effects of temperature	Effects of pH	Reference
Macroalgae	*Amphiroa fragillisima*		Decrease in calcification	Langdon et al. (2003)
	Chondria dasyphylla			
	Gelidiopsis intricate			
	Haptilon cubense			
	Sargassum muticum and *Cystoseira tamariscifolia*	Reduce in biomass of macroalgal assemblages	Reduce in biomass of macroalgal assemblages	Olabarria et al. (2013)
Cnidaria	*Acropora digitifera*		Reduced metabolic rates	Nakamura et al. (2011)
	Stylophora pistillata	Net photosynthesis affected	Cell-specific density affected	Reynaud et al. (2003)
	Aiptasia pulchella	Host cell adhesion dysfunction		Gates, Baghdasarian, and Muscatine (1992)
	Pocillopora damicornis			
	Diploria strigosa	Negative effect on larval development		Bassim, Sammarco, and Snell (2002)
Bryozoa	*Membranipora membranacea*	Capable of acclimating to elevated temperatures		Menon (1972)
	Electra pilosa			
	Conopeum reticulum			
	Myriapora truncata	Negative effect on calcification (combination of temperature rise and ocean acidification)	Negative effect on calcification (combination of temperature rise and ocean acidification)	Rodolfo-Metalpa et al. (2010)
			Corrosion of calcareous skeletons	Lombardi et al. (2011)
Mollusks	*Clio pyramidata*		Reduced calcification rates	Fabry et al. (2008)
	Crassostrea gigas		Calcification rates decrease	Gazeau et al. (2007)
	Haliotis laevigata		Affected specific growth rate	Harris et al. (1999)
	Haliotis rubra			
	Mercenaria mercenaria		Dissolution-induced mortality	Green et al. (2004)
	Mytilus edulis		Negative effects on growth	Berge et al. (2006)
			Calcification rates decrease	Gazeau et al. (2007)
	Saccostrea glomerata		Possibility to adapt	Parker et al. (2012)
		Decreased fertilization	Abnormal D-veligers	Parker, Ross, and O'Connor (2009)
	Strombus luhuanus		Affects growth	Shirayama and Thornton (2005)
Arthropods	*Acartia clausi*	Respiration and ammonia excretion		Gaudy, Cervetto, and Pagano (2000)
	Acartia erythraea		Reproduction rate and larval development	Kurihara, Shimode, and Shirayama (2004)
	Acartia steueri			
	Acartia tonsa	Respiration and ammonia excretion		Gaudy et al. (2000)
	Callinectes sapidus		Compensation of hypercapnia	Cameron and Iwama (1987)
Echinoderms	*Acanthaster planci*		Negative impacts on larval development	Uthicke, Soars, et al. (2013), Uthicke, Pecorino, et al. (2013)
	Centrostephanus rodgersii	Decrease in gastrulation	Decrease in cleavage stage embryos	Foo et al. (2012)
	Echinometra mathaei		Early development	Kurihara and Shirayama (2004)
			Affects growth	Shirayama and Thornton (2005)
			Male spawning ability	Uthicke et al. (2013)
	Hemicentrotus pulcherrimus		Early development	Kurihara and Shirayama (2004)
			Affects growth	Shirayama and Thornton (2005)
	Pisaster ochraceus	Affects keystone predation		Sanford (1999)
	Psammechinus miliaris		Hypercapnia and mortality	Miles et al. (2007)
	Strongylocentrotus franciscanus		Thermal stress	O'Donnell et al. (2009)

(Continues)

TABLE 1 (Continued)

Major group	Studied organism	Effects of temperature	Effects of pH	Reference
Chordata	*Amphiprion percula*		Impairs olfactory discrimination	Munday, Crawley, et al. (2009), Munday, Dixson, et al. (2009)
	Ictalurus punctatus		Compensation of hypercapnia	Cameron and Iwama (1987)
	Lepidonotothen kempi		Inhibition of protein biosynthesis	Langenbuch and Pörtner (2003)
	Ostorhinchus cyanosoma	Declines in aerobic scope	Declines in aerobic scope	Munday, Crawley, et al. (2009), Munday, Dixson, et al. (2009)
	Ostorhinchus doederleini			
	Pachycara brachycephalum		Inhibition of protein biosynthesis	Langenbuch and Pörtner (2003)
	Sillago japonica		Acute toxicity on juveniles	Kikkawa et al. (2006)

Sea in 1969, hereafter named “H1$_{gre}$.” For 30 years, this lineage had been cultured in Bochum (Wenderoth & Ruthmann laboratory), and in 1999, it was transferred to the Schierwater laboratory (Schierwater, 2005).

2. H2—“Roscoff” (cold-water population): This haplotype derived from a single animal collected from the coast of Roscoff (France) in 2009 and is hereafter named “H2$_{ros}$” (von der Chevallerie, Eitel, & Schierwater, 2010).
3. H2—“Panama” (warm-water population): This haplotype culture derived from a single animal collected in 2002 in Bocas del Toro (Panama), hereafter named “H2$_{pan}$” (Eitel et al., 2013).

H1 and H2 represent different species (Schierwater, Osigus, Kamm K, Eitel M, & DeSalle, in preparation), while the two H2 lineages are different populations of the same species.

All experiments were carried out in glass Petri dishes (Ø: 14 cm) placed at three different temperatures (low = 21°C, medium = 25°C, and high = 29°C). About 21°C (room temperature) was maintained in the laboratory by means of an air-conditioning system (DC Inverter, Fujitsu). Experimental groups tested at 25 and 29°C were placed in separate aquaria (in the same room), filled with ASW (artificial seawater), and heated to the desired temperature by two heaters (ProTemp S200, accuracy: ±0.5°C). To keep the water temperature evenly distributed within aquaria, a water pump was installed to circulate the water (Figure 1).

At the start of the experiment, 360 individuals per lineage were randomly assigned to nine experimental groups (Table S1). Testing three lineages of placozoans, each for three different temperatures, we performed eight replicates with each five specimens as a starting point. After an acclimation period of 2 days (the chosen placozoan species adapt very quickly to new culture conditions), and in order to measure the PGR over the 3 weeks experimental period, the total number of individuals per plate was counted every 3 days (nine censuses).

2.3 | Experimental setup for pH experiments

We used the same lineages as described above. The aquarium was setup with a CO$_2$ reactor (JBL ProFlora), a pH meter, and an aeration system for the seawater carbon dioxide (CO$_2$) and the manipulation of the pH (for further details, see also Riebesell et al., 2000 and Figure 2).

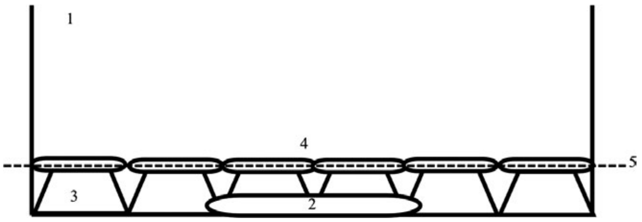

FIGURE 1 The experimental setup for the temperature experiment. 1—Aquarium filled with artificial seawater, 2—heater, 3—glass bowls turned over, 4—covered Petri dishes with the experimental animals placed on the glass bowls, 5—surface line of artificial seawater

At the start of the experiment, 80 specimens per lineage were randomly assigned to six experimental groups (Table S2). Food was provided ad libitum by placing one slide covered with algae inside the Petri dish. After an acclimation period of 2 days, the placozoans were left in one of two 160-L aquaria, one with a constant pH of 7.6, and the other with a pH of 8.0 (control; normal pH conditions in the laboratory cultures). In order to measure the PGR during the experimental period (12 days), the total number of individuals per plate was counted every 2 days (five censuses).

2.4 | Statistical analysis

The Kolmogorov–Smirnov one-sample test was used to test for normality distribution. As none of the data sets showed normal distribution (Kolmogorov–Smirnov test; $p < .05$), the data were normalized by log-transformation for the temperature experiment. Differences in PGR between the three different temperature settings were tested for by one-way ANOVA with the total number of individuals as a dependent variable and treatment as a fixed factor. Differences in PGR between the two different pH settings were tested for by means of the Mann–Whitney *U*-test. Thus, PGRs were compared between treatments (three different temperatures—experiment 1; two different pH—experiment 2) in the three clonal lineages (H1$_{gre}$, H2$_{ros}$, and H2$_{pan}$). Statistical analyses of both experiments were performed using the statistical software Minitab 16 and PAST (Hammer, Harper, & Ryan, 2001). Descriptive statistics are reported as means ± *SE*

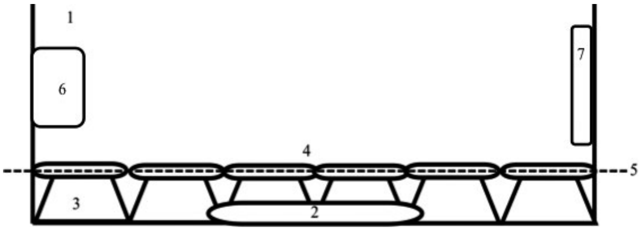

FIGURE 2 The experimental setup for the pH experiment. 1—Aquarium filled with artificial seawater, 2—heater, 3—glass bowls turned over, 4—covered Petri dishes with the experimental animals placed on the glass bowls, 5—surface line of artificial seawater, 6—CO_2-reactor, and 7—pH meter

3 | RESULTS

Both factors, temperature and pH, affected the PGR of different placozoan lineages significantly.

3.1 | The effect of temperature

The three lineages $H1_{gre}$, $H2_{ros}$, and $H2_{pan}$ responded in sharply different ways to changes in water temperature:

1. The cosmopolitic $H1_{gre}$:

   One-way ANOVA revealed highly significant differences in the PGR for the three different temperatures ($F_{2,27} = 14.89$, $df = 2$, $p < .001$). Post hoc tests revealed highly significant differences in the PGR between 25 and 29°C ($p < .001$) and also between 21 and 25°C ($p = .013$). Between 21 and 29°C, no significant difference was observed ($p > .05$); at both temperatures, the PGR was low compared to the "optimal" temperature of 25°C (Figure 3a).

2. The cold-water $H2_{ros}$:

   Also here, the effect of temperature on the PGR was significant ($F_{2,27} = 8.04$, $df = 2$, $p = .002$; one-way ANOVA). Post hoc tests revealed significant differences in the PGR between 21 and 29°C ($p = .002$) and also between 21 and 25°C ($p = .033$), while between 25 and 29°C, no significant difference was observed ($p > .05$). At both higher temperatures, the PGR of the cold $H2_{ros}$ was low suggesting the lower temperature of 21°C to be preferred (Figure 3b).

3. The warm-water $H2_{pan}$:

   The $H2_{pan}$ clone behaved similar to the $H1_{gre}$ clone, showing significant changes in PGR when moving away from the "optimal" temperature of 25°C ($F_{2,27} = 6.08$, $df = 2$, $p = .007$; one-way ANOVA). The harmful effect of higher temperature even on the warm-water population seems particularly notable (Figure 3c).

Profound effects of slight changes in pH value were found for the lineages $H1_{gre}$ and $H2_{ros}$. After about 5 days into the experiment, the PGR in the acidified water slowed down significantly compared to the control (pH 8.0) cultures, with the effect becoming more and more substantial over time (Figure 4a–c and Table 2). The Panama lineage showed an unusual slow PGR under the given conditions (room temperature—21°C) already at "normal" pH conditions. As we do not know the reasons for the unusual slow reproductive activity, we excluded these data from further analyses. The observation that under more acid conditions, the PGR was higher than under pH 8.0 conditions maybe an artifact or may indeed be a lineage-specific adaptive response, but at this point, any further conclusions would be premature.

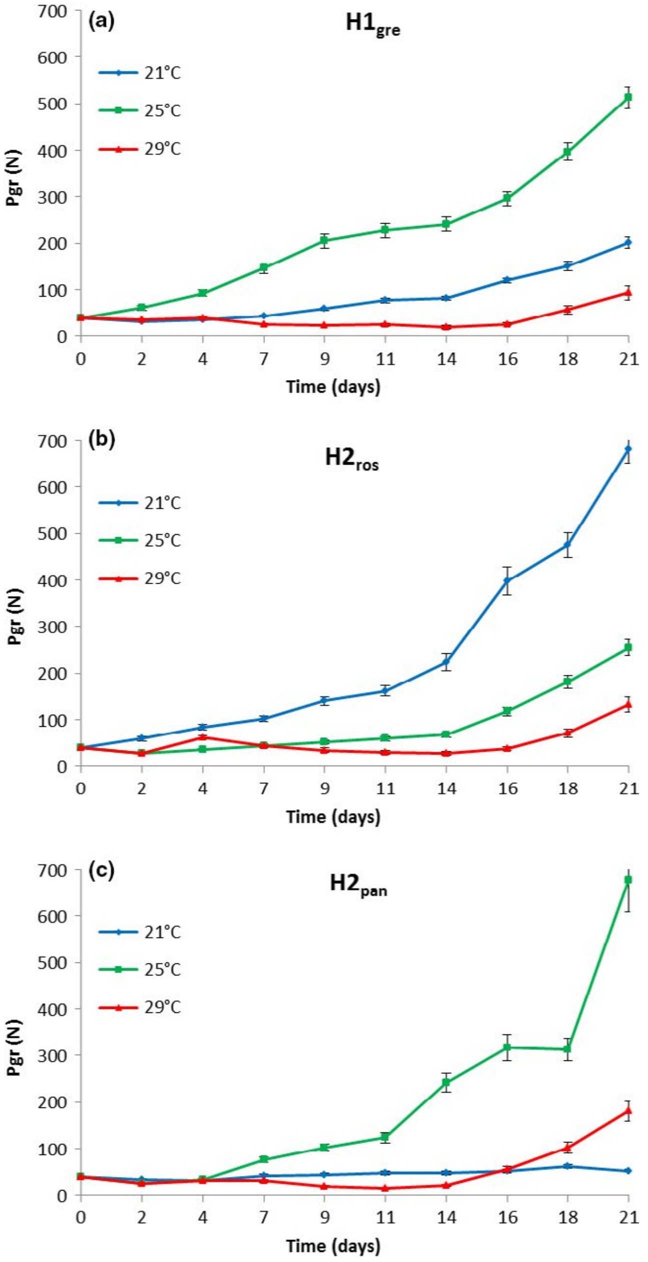

FIGURE 3 Population growth rate (PGR) at different temperatures for the three placozoan lineages (a) $H1_{gre}$, (b) $H2_{ros}$, and (c) $H2_{pan}$

4 | DISCUSSION

Climate change is directly or indirectly affecting the distribution, abundance, breeding, and migration of marine plants and animals (e.g., Doney et al., 2009; Hoegh-Guldberg & Bruno, 2010; Ji et al., 2007; Jiao et al.,

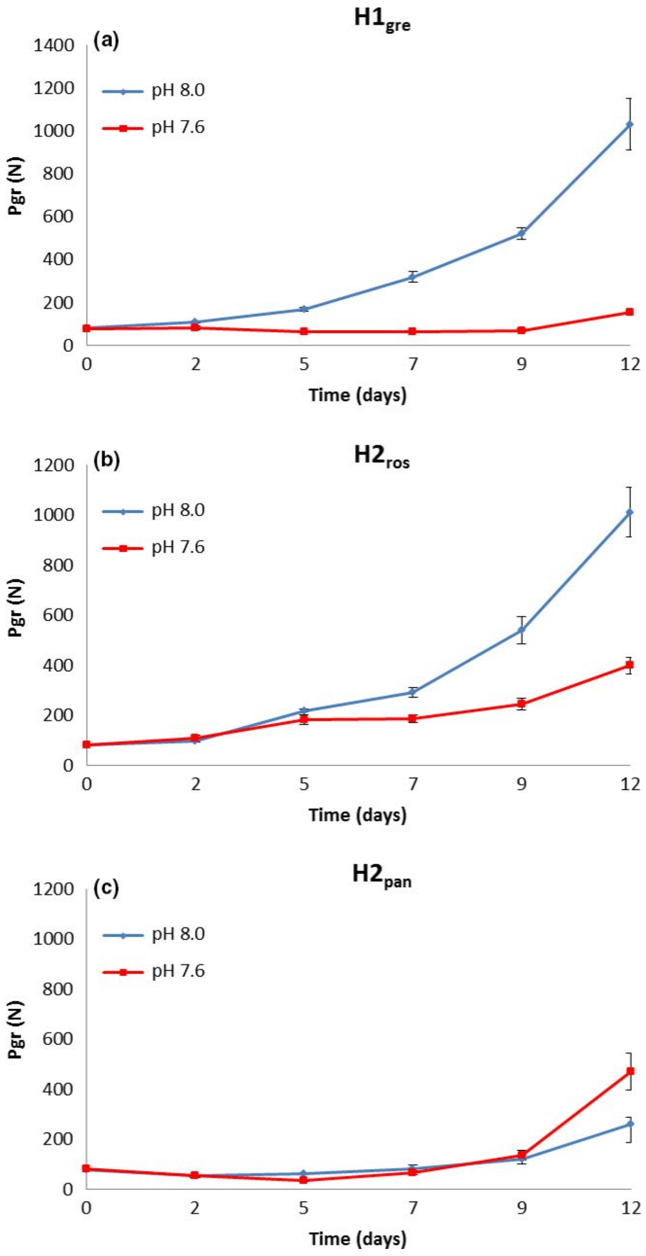

FIGURE 4 Population growth rate (PGR) at the two different pH levels for the lineages (a) $H1_{gre}$, (b) $H2_{ros}$, and (c) $H2_{pan}$

TABLE 2 Influence of increased water acidity on the PGR in the placozoan lineages $H1_{gre}$, $H2_{ros}$, and $H2_{pan}$ (Mann–Whitney *U*-test at the different observation points; bold = significant values)

Lineage	Time (days)	*p* Value (>.050)	Monte Carlo *p*	Exact *p*
$H1_{gre}$	2	.3123	.3461	.3429
$H1_{gre}$	5	**.0294**	**.0289**	**.02857**
$H1_{gre}$	7	**.0294**	**.0288**	**.02857**
$H1_{gre}$	9	**.03038**	**.0323**	**.02857**
$H1_{gre}$	12	**.03038**	**.0282**	**.02857**
$H2_{pan}$	2	.8852	1	1
$H2_{pan}$	5	**.0294**	**.0296**	**.02857**
$H2_{pan}$	7	.8852	1	1
$H2_{pan}$	9	.8852	.8877	.8857
$H2_{pan}$	12	.3123	.3441	.3429
$H2_{ros}$	2	.5614	.5405	.5429
$H2_{ros}$	5	.3123	.3496	.3429
$H2_{ros}$	7	.1124	.1153	.1143
$H2_{ros}$	9		.0588	.05714
$H2_{ros}$	12	.0606	.0546	.05714

PGR, population growth rate.

2015). Mean global temperatures will continue to rise even if greenhouse gas emissions are stabilized at present levels (IPCC, 2001, 2013). Some of the most affected ecosystems are the oceans, which show rising temperature and acidity. Sensitive organisms, which respond to such changes early and are restrained from quick adaptations by evolutionary constraints, might be useful biomarkers for biomonitoring studies (e.g., Dallas & Jha, 2015; Moschino, Del Negro, & De Vittor, 2016; Natalotto et al., 2015) .

Our experiments revealed strong and differential effects of both, temperature and pH, on the PGR of placozoans, with temperature showing the strongest effects. Interestingly, but not surprisingly, the lineage which had been found in relatively cold Atlantic waters ($H2_{ros}$) showed a thermal preference for the low temperature setting, whereas higher temperatures significantly reduced the PGR. The other two lineages performed best at 25°C, which has been regarded as the "normal" temperature for placozoans (Schierwater, 2005). Both, *T. adhaerens* (species $H1_{gre}$, which has been collected from the Red Sea) and $H2_{pan}$ (collected from Panama), only performed well at 25°C. Interestingly, for clones adapted to tropical waters, both species almost cease propagation at the high temperature of 29°C. As all clones sharply reduce propagation rates at the highest temperature, we assume harmful effects of such high temperatures for placozoans in general.

Placozoans behave like most marine species, which show thermal preferences for a well-defined temperature range (IPCC, 2007; Nakano, 2014). In many locations, ocean temperatures have either increased (Bethoux, Gentili, & Tailliez, 1998; Freeland, 1990; IPCC, 1996, 2001, Ji et al., 2007; Scranton et al., 1987) or decreased in short time (IPCC, 1996, 2001, Ji et al., 2007; Read & Gould, 1992), and demographic effects on many marine species, including placozoans, must have occurred recently. According to Hiscock et al. (2004), the ocean temperature will continue to show significant short-term variations, with maximum ocean-surface temperatures close to 28°C (with a trend toward even higher temperatures). As the natural habitat of placozoans is mainly surface waters, we must predict ongoing demographic changes and differential effects on placozoan communities. Such differential effects mark placozoans as potential biomarkers for monitoring studies on the effects of ocean warming.

The sharp decline in propagation rate observed in *T. adhaerens* ($H1_{gre}$) and $H2_{ros}$ mirrors a quite sensitive response to increasing water acidity. This sensitivity is also highlighted by quite extreme changes in morphology toward the end of the experiments (Figure 5). These dramatic and harmful effects forced us to end the experiments after 12 days. Although the experiments on the $H2_{pan}$ clone were not

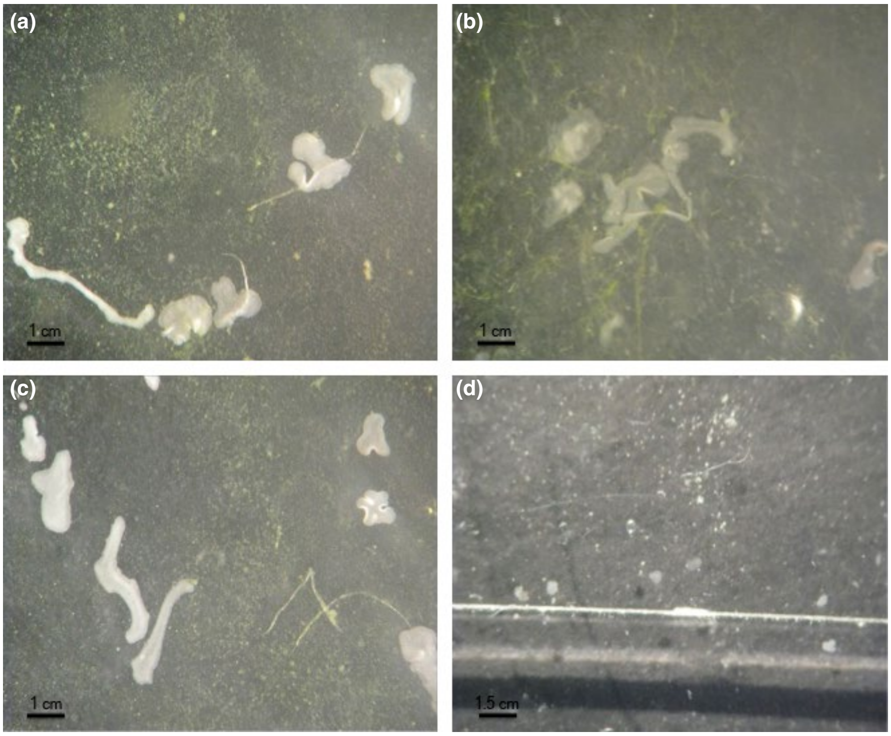

FIGURE 5 Changes in morphology of *Trichoplax adhaerens* under acidity stress. (a) Unusually enlarged specimens, (b) extremely long specimen, (c) normal to very long shaped specimens, (d) very tiny, round-shaped specimens

conclusive, the relative increase in PGR toward the end of the experiment as well as the differences between the other two clones suggests that different placozoan lineages differ in their sensitivity and response to change in water acidity. These observations not only highlight the sensitivity of placozoans to water acidity but also point to the potential of combining different sympatric placozoan species into a multiple-species biomarker system. Several other examples of sympatric species complexes might be available also from different other invertebrate taxa (e.g., Azevedo et al., 2015; Hoegh-Guldberg & Bruno, 2010; Kroeker et al., 2013; Nakamura et al., 2011; Navarro et al., 2013; O'Donnell, Hammond, & Hofmann, 2009; Schmidt, Power, & Quinn, 2013).

As Malakoff (2012) points out, understanding the ecological and evolutionary implications of acidifying oceans requires extended experiments and long-term monitoring studies. Kelly and Hofmann (2012) review empirical studies on adaptability and acclimatization of marine organisms to elevated $p\mathrm{CO}_2$ values (e.g., in algae, positive trends for photosynthesis were found), including adaptation reports from some cnidarians, which increase their biomass with increasing $p\mathrm{CO}_2$. What may look like a "favorable" response and quick adaptation here certainly does not apply to placozoans. Here, each factor by itself, temperature and acidity, can bring growth rate to die down and a combination of both factors must be even more detrimental. On the other hand, this sensitivity can open new avenues for using placozoans as sensitive biosensors in long-term biomonitoring studies.

4.1 | Final conclusions

Placozoans, the most simple organized and possibly also the oldest metazoan animals (cf. Schierwater, de Jong, et al., 2009; Schierwater, Eitel, et al., 2009), are highly sensitive to temperature and acidity stress and thus might be explored as potential biosensors. They offer the unique advantage of showing differential response patterns in different but sympatrically occurring placozoan species. The potential of a multiple "cryptic" species monitoring system has not been explored yet, but in practice should be based upon high-throughput genetic assays of community diversity and stress gene expression. Furthermore, the quantified differences in niche parameters must also be relevant for species descriptions following the taxonomic circle approach in a large group of cryptic placozoan species.

ACKNOWLEDGMENTS

We are particularly grateful to Fabio Polesel and Fabian Műller for their help with the experiments and to Jutta Bunnenberg and Nicole Bartkowiak for their most valuable technical support in the laboratory. Hans-Jürgen Osigus acknowledges a doctoral fellowship of the Studienstiftung des deutschen Volkes. Katharina Dulias acknowledges a doctoral scholarship of the Leverhulme Trust. We are grateful to Stefano for his valuable comments on the manuscript. This project was funded by the Deutsche Forschungsgemeinschaft, Germany (DFG-Schi 277/29-1).

CONFLICT OF INTEREST

None declared.

REFERENCES

Albright, R. A., & Mason, B. (2013). Projected near-future levels of temperature and pCO2 reduce coral fertilization success. *PLoS One*, *8*, e56468.

Azevedo, L. B., Schryver, A. M., Hendriks, A. J., Huijbregts, M. A. (2015). Calcifying species sensitivity distributions for ocean acidification. *Environmental Science & Technology*, *49*, 1495–1500.

Bassim, K., Sammarco, P., & Snell, T. (2002). Effects of temperature on success of (self and non-self) fertilization and embryogenesis in *Diploria strigosa* (Cnidaria, Scleractinia). *Marine Biology*, *140*(3), 479–488.

Berge, J. A., Bjerkeng, B., Pettersen, O., et al. (2006). Effects of increased sea water concentrations of CO_2 on growth of the bivalve *Mytilus edulis* L. *Chemosphere*, *62*(4), 681–687.

Bethoux, J. P., Gentili, B., & Tailliez, D. (1998). Warming and fresh-water budget change in the Mediterranean since the 1940s, their possible relation to the greenhouse effect. *Geophysical Research Letters*, *25*, 1023–1026.

Byrne, M., Ho, M. A., Koleits, L., et al. (2013). Vulnerability of the calcifying larval stage of the Antarctic sea urchin *Sterechinus neumayeri* to near-future ocean acidification and warming. *Global Change Biology*, *19*(7), 2264–2275.

Byrne, M., & Przeslawski, R. (2013). Multistressor impacts of warming and acidification of the ocean on marine invertebrates' Life histories. *Integrative and Comparative Biology*, *53*(4), 582–596.

Byrne, M., Soars, N. A., Ho, M. A., et al. (2010). Fertilization in a suite of coastal marine invertebrates from SE Australia is robust to near-future ocean warming and acidification. *Marine Biology*, *157*, 2061–2069.

Cameron, J. N., & Iwama, G. K. (1987). Compensation of progressive hypercapnia in channel catfish and blue crabs. *Journal of Experimental Biology*, *133*(1), 183–197.

Chen, T. C. A. (2008). Effects of climate change on marine ecosystems. In K. Tsukamoto, T. Kawamura, T. Takeuchi, T. D. Beard, & M. J. Kaiser (Eds.), *Fisheries for Global Welfare and Environment, 5th World Fisheries Congress 2008* (pp. 307–316). Yokohama, Japan: Terrapub.

von der Chevallerie, K., Eitel, M., & Schierwater, B. (2010). Focus on an unexpected discovery in Roscoff—A warm water species of the phylum Placozoa. *Cahiers de Biologie Marine*, *51*, 212–213.

Dallas, L. J., & Jha, A. N. (2015). Applications of biological tools or biomarkers in aquatic biota: A case study of the Tamar estuary, South West England. *Marine Pollution Bulletin*, *95*, 618–633.

Doney, S. C., Fabry, V. J., Feely, R. A., et al. (2009). Ocean acidification: The other CO_2 problem. *Annual Review of Marine Science*, *1*, 169–192.

Eitel, M., Guidi, L., Hadrys, H., et al. (2011). New insights into placozoan sexual reproduction and development. *PLoS One*, *6*, e19639.

Eitel, M., Osigus, H. J., DeSalle, R., et al. (2013). Global diversity of the Placozoa. *PLoS One*, *8*, e57131.

Eitel, M., & Schierwater, B. (2010). The phylogeography of the Placozoa suggests a taxon rich phylum in tropical and subtropical waters. *Molecular Ecology*, *19*, 2315–2327.

Ericson, J. A., Ho, M. A., Miskelly, A., et al. (2011). Combined effects of two ocean change stressors, warming and acidification on fertilization and early development of the Antarctic echinoid *Sterechinus neumayeri*. *Polar Biology*, *35*, 1027–1034.

Fabry, V. J., Seibel, B. A., Feely, R. A., et al. (2008). Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES Journal of Marine Science: Journal du Conseil*, *65*(3), 414–432.

Falkowski, P. (2012). Ocean science: The power of plankton. *Nature*, *483*, S17–S20.

Feindt, W., Fincke, O., & Hadrys, H. (2014). Still a one species genus? Strong genetic diversification in the world's largest living odonate, the Neotropical damselfly *Megaloprepus caerulatus*. *Conservation Genetics*, *15*, 469–481.

Folt, C. L., Chen, C. Y., Moore, M. V., et al. (1999). Synergism and antagonism among multiple stressors. *Limnology and Oceanography*, *44*, 864–877.

Foo, S. A., Dworjanyn, S. A., Poore, A. G., et al. (2012). Adaptive capacity of the habitat modifying sea urchin *Centrostephanus rodgersii* to ocean warming and ocean acidification: Performance of early embryos. *PLoS One*, *7*, e42497.

Foster, B. A. (1971). On the determinants of the upper limit of intertidal distribution of barnacles (*Crustacea: Cirripedia*). *Journal of Animal Ecology*, *40*, 33–48.

Freeland, H. J. (1990). Sea surface temperatures along the coast of British Columbia: Regional evidence for a warming trend. *Canadian Journal of Fisheries and Aquatic Sciences*, *47*, 346–350.

Gates, R. D., Baghdasarian, G., & Muscatine, L. (1992). Temperature stress causes host cell detachment in symbiotic cnidarians: Implications for coral bleaching. *The Biological Bulletin*, *182*(3), 324–332.

Gaudy, R., Cervetto, G., & Pagano, M. (2000). Comparison of the metabolism of *Acartia clausi* and *A. tonsa*: Influence of temperature and salinity. *Journal of Experimental Marine Biology and Ecology*, *247*(1), 51–65.

Gazeau, F., Quiblier, C., Jansen, J. M., et al. (2007). Impact of elevated CO_2 on shellfish calcification. *Geophysical Research Letters*, *34*(7), L07603.

Goodwin, C., Rodolfo-Metalpa, R., Picton, B., et al. (2013). Effects of ocean acidification on sponge communities. *Marine Ecology*, *35*, 41–49.

Green, M. A., Jones, M. E., Boudreau, C. L., et al. (2004). Dissolution mortality of juvenile bivalves in coastal marine deposits. *Limnology and Oceanography*, *49*(3), 727–734.

Hadrys, H., Schroth, W., Streit, B., et al. (2005). Tree hole odonates as environmental monitors: Non-invasive isolation of polymorphic microsatellites from the neotropical damselfly. *Conservation Genetics*, *6*, 481–483.

Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, *4*, 1–9.

Hardege, J. D., Rotchell, J. M., Terschak, J., et al. (2011). Analytical challenges and the development of biomarkers to measure and to monitor the effects of ocean acidification. *Trends in Analytical Chemistry*, *30*, 1320–1326.

Harris, J. O., Maguire, G. B., Edwards, S. J., et al. (1999). Effect of pH on growth rate, oxygen consumption rate, and histopathology of gill and kidney tissue for juvenile greenlip abalone, *Haliotis laevigata* Donovan and blacklip abalone, *Haliotis rubra* Leach. *Journal of Shellfish Research*, *18*(2), 611–619.

Hinder, S. L., Gravenor, M. B., Edwards, M., et al. (2014). Multi-decadal range changes vs. thermal adaptation for north east Atlantic oceanic copepods in the face of climate change. *Global Change Biology*, *20*, 140–146.

Hiscock, K., Southward, A., Tittley, I., et al. (2004). Effects of changing temperature on benthic marine life in Britain and Ireland. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *14*, 333–362.

Hochachka, P. W., & Somero, G. N. (2002). *Biochemical adaptation: Mechanism and process in physiological evolution*. New York, NY: Oxford University Press.

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The impact of climate change on the world's marine ecosystems. *Science*, *328*, 1523–1528.

Houghton, J. T., Ding, Y., & Griggs, D. J. et al. (2001). The scientific basis. In J. T. Houghton, Y. Ding, D. J. Griggs, M. Noguer, van der Linden P. J., X. Dai, K. Maskell & C. A. Johnson (Eds.), *Climate Change 2001 Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change* (881 pp). Cambridge, UK and New York, NY, USA: Cambridge University Press.

Hughes, T. P., Baird, A. H., Bellwood, D. R., et al. (2003). Climate change, human impacts, and the resilience of coral reefs. *Science*, *301*, 929–933.

IPCC (1996). *Intergovernmental Panel on Climate Change 1996*. Cambridge, UK: Cambridge University Press.

IPCC (2001) *Intergovernmental Panel on Climate Change 2001. Contribution of Working Groups I, II, and III to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK: Cambridge University Press.

IPCC (2007) The physical science basis. In S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor & H. L. Miller (Eds), *Intergovernmental Panel on Climate Change 2007. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK: Cambridge University Press.

IPCC (2013) *Intergovernmental Panel on Climate Change 2013*. Cambridge, UK: Cambridge University Press.

Ji, R., Davis, C. S., Chen, C. S., et al. (2007). Influence of ocean freshening on shelf phytoplankton dynamics. *Geophysical Research Letters*, *34*, L24607.

Jiao, N. Z., Chen, D. K., Luo, Y. M., et al. (2015). Climate change and anthropogenic impacts on marine ecosystems and countermeasures in China. *Advances in Climate Change Research*, *6*, 116–125.

Kelly, M. W., & Hofmann, G. E. (2012). Adaptation and the physiology of ocean acidification. *Functional Ecology*, *27*, 980–990.

Kikkawa, T., Sato, T., Kita, J., et al. (2006). Acute toxicity of temporally varying seawater CO 2 conditions on juveniles of Japanese sillago (*Sillago japonica*). *Marine pollution bulletin*, *52*(6), 621–625.

Kroeker, K. J., Kordas, R. L., Crim, R., et al. (2013). Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming. *Global Change Biology*, *19*, 1884–1896.

Kurihara, H., Shimode, S., & Shirayama, Y. (2004). Effects of raised CO_2 concentration on the egg production rate and early development of two marine copepods (*Acartia steueri* and *Acartia erythraea*). *Marine Pollution Bulletin*, *49*(9), 721–727.

Kurihara, H., & Shirayama, Y. (2004). Effects of increased atmospheric CO_2 on sea urchin early development. *Marina Ecology Progress Series*, *274*, 161–169.

Langdon, C., Broecker, W. S., Hammond, D. E., et al. (2003). Effect of elevated CO_2 on the community metabolism of an experimental coral reef. *Global Biogeochemical Cycles*, *17*(1), 1011.

Langenbuch, M., & Pörtner, H. O. (2003). Energy budget of hepatocytes from Antarctic fish (*Pachycara brachycephalum* and *Lepidonotothen kempi*) as a function of ambient CO_2: pH-dependent limitations of cellular protein biosynthesis? *Journal of Experimental Biology*, *206*, 3895–3903.

Loenarz, C., Coleman, M. L., Boleininger, A., et al. (2011). The hypoxia-inducible transcription factor pathway regulates oxygen sensing in the simplest animal, *Trichoplax adhaerens*. *EMBO Reports*, *12*, 63–70.

Lombardi, C., Rodolfo-Metalpa, R., Cocito, S., et al. (2011). Structural and geochemical alterations in the Mg calcite bryozoan *Myriapora truncata* under elevated seawater pCO2 simulating ocean acidification. *Marine Ecology*, *32*(2), 211–221.

Malakoff, D. (2012). Researchers struggle to assess responses to ocean acidification. *Science*, *338*, 27–28.

Maruyama, Y. K. (2004). Occurrence in the field of a long-term, year-round, stable population of placozoans. *Biological Bulletin*, *206*, 55–60.

McWilliams, J. P., Coté, I. M., Gill, J. A., et al. (2005). Accelerating impacts of temperature induced coral bleaching in the Caribbean. *Ecology*, *86*, 2055–2060.

Menon, N. R. (1972). Heat tolerance, growth and regeneration in three North Sea bryozoans exposed to different constant temperatures. *Marine Biology*, *15*(1), 1–11.

Miles, H., Widdicombe, S., Spicer, J. I., et al. (2007). Effects of anthropogenic seawater acidification on acid–base balance in the sea urchin *Psammechinus miliaris*. *Marine Pollution Bulletin*, *54*(1), 89–96.

Moschino, V., Del Negro, P., & De Vittor, C. (2016). Biomonitoring of a polluted coastal area (Bay of Muggia, Northern Adriatic Sea): A five-year study using transplanted mussels. *Ecotoxicology and Environmental Safety*, *128*, 1–10.

Munday, P. L., Crawley, N. E., & Nilsson, G. E. (2009). Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral reef fishes. *Marine Ecology Progress Series*, *388*, 235–242.

Munday, P. L., Dixson, D. L., Donelson, J. M., et al. (2009). Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proceedings of the National Academy of Sciences*, *106*, 1848–1852.

Nakamura, M., Ohki, S., Suzuki, A., et al. (2011). Coral larvae under ocean acidification: Survival, metabolism, and metamorphosis. *PLoS One*, *6*, e14521.

Nakano, H. (2014). Survey of the Japanese coast reveals abundant placozoan populations in the Northern Pacific Ocean. *Scientific Reports*, *4*, 5356.

Natalotto, A., Sureda, A., Maisano, M., et al. (2015). Biomarkers of environmental stress in gills of *Pinna nobilis* (Linnaeus 1758) from Balearic Island. *Ecotoxicology and Environmental Safety*, *122*, 9–16.

Navarro, J. M., Torres, R., Acuña, K., et al. (2013). Impact of medium-term exposure to elevated pCO_2 levels on the physiological energetics of the mussel *Mytilus chilensis*. *Chemosphere*, *90*, 1242–1248.

Neftel, A., Moor, E., Oeschger, H., et al. (1985). Evidence from polar ice cores for the increase in atmospheric CO_2 in the past two centuries. *Nature*, *315*, 45–47.

O'Donnell, M. J., Hammond, L. M., & Hofmann, G. E. (2009). Predicted impact of ocean acidification on a marine invertebrate: Elevated CO_2 alters response to thermal stress in sea urchin larvae. *Marine Biology*, *156*, 439–446.

Olabarria, C., Arenas, F., Viejo, R. M., et al. (2013). Response of macroalgal assemblages from rockpools to climate change: Effects of persistent increase in temperature and CO2. *Oikos*, *122*(7), 1065–1079.

Paknia, O., & Schierwater, B. (2015). Global habitat suitability and ecological niche separation in the phylum Placozoa. *PLoS One*, *10*, e0140162.

Parker, L. M., Ross, P. M., & O'Connor, W. A. (2009). The effect of ocean acidification and temperature on the fertilization and embryonic development of the Sydney rock oyster *Saccostrea glomerata* (Gould 1850). *Global Change Biology*, *15*(9), 2123–2136.

Parker, L. M., Ross, P. M., & O'Connor, W. A. (2010). Comparing the effect of elevated pCO2 and temperature on the fertilization and early development of two species of oysters. *Marine Biology*, *157*, 2435–2452.

Parker, L. M., Ross, P. M., O'Connor, W. A., et al. (2012). Adult exposure influences offspring response to ocean acidification in oysters. *Global Change Biology*, *18*, 82–92.

Pearse, V. B., & Voigt, O. (2007). Field biology of placozoans (*Trichoplax*): Distribution, diversity, biotic interactions. *Integrative and Comparative Biology*, *47*, 677–692.

Pechenik, J. A. (1989). Environmental influences on larval survival and development. In A. C. Giese, J. S. Pearse, & V. B. Pearse (Eds.), *Reproduction of marine invertebrates* (pp. 551–608). Palo Alto, CA: Blackwell Scientific Publications.

Pörtner, H. O. (2008). Ecosystem effects of ocean acidification in times of ocean warming: A physiologist's view. *Marine Ecology Progress Series*, *373*, 203–217.

Pörtner, H. O., & Peck, M. A. (2010). Climate change effects on fishes and fisheries: Towards a cause-and-effect understanding. *Journal of Fish Biology*, *77*, 1745–1779.

Read, J. F., & Gould, W. J. (1992). Cooling and freshening of the subpolar North Atlantic Ocean since the 1960s. *Nature*, *360*, 55–57.

Reynaud, S., Leclercq, N., Romaine-Lioud, S., et al. (2003). Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. *Global Change Biology*, *9*(11), 1660–1668.

Riebesell, U., Zondervan, I., Rost, B., et al. (2000). Reduced calcification of marine plankton in response to increased atmospheric CO_2. *Nature*, *407*, 364–367.

Rodolfo-Metalpa, R., Lombardi, C., Cocito, S., et al. (2010). Effects of ocean acidification and high temperatures on the bryozoan *Myriapora truncata* at natural CO2 vents. *Marine Ecology*, *31*(3), 447–456.

Sanford, E. (1999). Regulation of keystone predation by small changes in ocean temperature. *Science*, *283*, 2095–2097.

Schierwater, B. (2005). My favorite animal, *Trichoplax adhaerens*. *BioEssays*, *27*, 1294–1302.

Schierwater, B., de Jong, D., & DeSalle, R. (2009). Placozoa, and the evolution of Metazoa and intrasomatic cell differentiation. *The International Journal of Biochemistry & Cell Biology*, *41*, 370–379.

Schierwater, B., Eitel, M., Jakob, W., et al. (2009). Concatenated analysis sheds light on early metazoan evolution and fuels a modern "Urmetazoon" hypothesis. *PLoS Biology*, *7*, 36–44.

Schierwater, B., Miller, D., Holland, P. W., et al. (2016). Next generation analysis of a century old evolutionary debate: How to avoid "unringing" the urmetazoon bell. *Frontiers in Ecology and Evolution*, *4*, 5. doi:10.3389/fevo.2016.00005

Schmidt, W., Power, E., & Quinn, B. (2013). Seasonal variations of biomarker responses in the marine blue mussel (*Mytilus* spp.). *Marine Pollution Bulletin*, *74*, 50–55.

Schroth, W., Ender, A., & Schierwater, B. (2005). Molecular biomarkers and adaptation to environmental stress in moon jelly (*Aurelia* spp.). *Marine Biotechnology*, *7*, 449–461.

Schulze, F. E. (1883). *Trichoplax adhaerens*, nov. gen., nov. spec. *Zoologischer Anzeiger*, *6*, 92–97.

Schulze, F. E. (1891). Über *Trichoplax adhaerens*. In G. Reimer (Ed.), *Abhandlungen der Königlichen Preuss Akademie der Wissenschaften zu Berlin* (pp. 1–23). Berlin, Germany: Verlag der königlichen Akademie der Wissenschaften.

Scranton, M. I., Sayles, F. L., Bacon, M. P., et al. (1987). Temporal changes in the hydrography and chemistry of the Cariaco Trench. *Deep Sea Research Part A. Oceanographic Research Papers*, *34*, 945–963.

Shirayama, Y., & Thornton, H. (2005). Effect of increased atmospheric CO_2 on shallow water marine benthos. *Journal of Geophysical Research: Oceans*, *110*, C09S08.

Signorovitch, A. Y., Buss, L. W., & Dellaporta, S. L. (2007). Comparative genomics of large mitochondria in placozoans. *PLoS Genetics*, *3*, e13.

Signorovitch, A. Y., Dellaporta, S. L., & Buss, L. W. (2006). Caribbean placozoan phylogeography. *Biological Bulletin*, *211*, 149–156.

Somero, G. N. (2002). Thermal physiology and vertical zonation of intertidal animals: Optima, limits, and costs of living. *Integrative and Comparative Biology*, *42*, 780–789.

Sunday, J. M., Crim, R. N., Harley, C. D. G., et al. (2011). Quantifying rates of evolutionary adaptation in response to ocean acidification. *PLoS One*, *6*, e22881.

Thackeray, S. J., Jones, I. D., & Maberly, S. C. (2008). Long-term change in the phenology of spring phytoplankton: Species-specific responses to nutrient enrichment and climatic change. *Journal of Ecology*, *96*, 523–535.

Törnroos, A., Bonsdorff, E., Bremner, J., et al. (2014). Marine benthic ecological functioning over decreasing taxonomic richness. *Journal of Sea Research*, *98*, 49–56.

Uthicke, S., Pecorino, D., Albright, R., et al. (2013). Impacts of ocean acidification on early life-history stages and settlement of the coral-eating sea star *Acanthaster planci*. *PLoS One*, *8*, e82938.

Uthicke, S., Soars, N., Foo, S., & Byrne, M. (2013). Effects of elevated pCO_2 and the effect of parent acclimation on development in the tropical Pacific sea urchin *Echinometra mathaei*. *Marine Biology*, *160*(8), 1913–1926.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Schleicherová D, Dulias K, Osigus H-J, Paknia O, Hadrys H, and Schierwater B. The most primitive metazoan animals, the placozoans, show high sensitivity to increasing ocean temperatures and acidities. *Ecol Evol*. 2017;7:895–904. doi: 10.1002/ece3.2678