Journal of Experimental Marine Biology and Ecology 445 (2013) 116–119

Contents lists available at SciVerse ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Biotic interference in parasite transmission: Can the feeding of anemones counteract an increased risk of parasitism in amphipods at higher temperature?

A. Studer [a,*,1], L. Kremer [a,1], J. Nelles [a,1], R. Poulin [a,1], D.W. Thieltges [b]

[a] Department of Zoology, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand
[b] NIOZ Royal Netherlands Institute for Sea Research, P.O. Box 59, 1790 AB Den Burg, Texel, The Netherlands

ARTICLE INFO

Article history:
Received 13 July 2012
Received in revised form 24 January 2013
Accepted 18 April 2013
Available online xxxx

Keywords:
Cercariae
Endohelminths
Intensity-dependent mortality
Trematodes

ABSTRACT

The transmission of parasites is embedded in the complexity of natural systems and is influenced not only by prevailing abiotic conditions, but also by the composition of the ambient community. In particular, temperature affects the number of transmission stages (e.g. cercariae of trematodes) released into the environment as well as their survival and infectivity. Temperature, however, also influences the metabolic rate and feeding activity of non-host organisms. We tested the hypothesis that at higher temperatures, the predatory anemone *Anthopleura aureoradiata*, known to ingest cercariae, may interfere with the transmission of the intertidal trematode parasite *Maritrema novaezealandensis* hence reducing the number of cercarial transmission stages successfully infecting *Paracalliope novizealandiae* amphipod hosts. In a microcosm experiment with two temperatures (15 and 22 °C) and three densities of anemones (0, 5 and 10 per microcosm), however, only a significant effect of temperature was found, with more parasites infecting surviving amphipods at the higher temperature. The effect of anemone density was not significant, although there was a trend towards fewer parasites infecting amphipods when anemones were present at 22 °C. Our results show that the effect of non-host species on trematode transmission might not be strong enough to mitigate increased parasite transmission at higher temperatures. However, mortality of amphipods kept at 22 °C was highest with low and medium anemone density; possibly reflecting an increased transmission pressure and parasite-induced mortality, therefore masking the actual outcome of this experiment based on surviving amphipods. Despite this, all findings point towards a higher risk of parasite-induced mortality of small crustaceans with increasing temperature, which is of particular concern in the context of global warming.

1. Introduction

The transmission of free-living stages of parasites such as larval endohelminths takes place within the continuously changing complexity of the environment. During transmission, these larval parasite stages are directly affected by ambient biotic and abiotic conditions. For example, members of the community of organisms in which the transmission processes are embedded, can alter the transmission success of (or the disease risk from) a parasite through consumption of infective stages (Johnson and Thieltges, 2010; Johnson et al., 2010; Thieltges et al., 2008a). Simultaneously, prevailing abiotic conditions provide a framework in which biotic processes such as parasite transmission take place. These conditions not only directly affect a parasite, but also all the hosts and non-hosts in the community and therefore the interactions between them. In light of on-going and predicted climate changes, especially global warming, a better understanding of the complex interactions between abiotic and biotic factors and parasite transmission is of great importance.

Trematodes are the dominant parasite group in coastal ecosystems (Mouritsen and Poulin, 2002) and they are important and highly influential ecological components (e.g. Fredensborg et al., 2005; Kuris et al., 2008; Mouritsen and Poulin, 2005). Trematodes have complex life cycles involving several members of a community. The transmission from a first intermediate host to a second intermediate host occurs via free-living cercariae which are directly exposed to ambient conditions. In marine ecosystems, non-host predators and filter-feeders such as fish, crabs, anemones, barnacles and molluscs have been found to prey on these transmission stages (Kaplan et al., 2009; Mouritsen and Poulin, 2003; Prinz et al., 2009; Thieltges et al., 2008b, 2009). For instance, the anemone *Anthopleura aureoradiata* living on the shell of cockles (*Austrovenus stutchburyi*) has been shown to decrease parasite transmission to these cockles by preying on trematode cercariae which use cockles as intermediate hosts (Mouritsen and Poulin, 2003). The same anemone has also been shown to ingest cercariae of the trematode *Maritrema novaezealandensis* (the parasite species used in the present study) and thus reduce the transmission

---

* Corresponding author. Tel.: +64 3 479 7964; fax: +64 3 479 7584.
E-mail address: studeranja@gmail.com (A. Studer).
[1] Tel: +64 3 479 7964; fax: +64 3 479 7584.

0022-0981/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jembe.2013.04.014

success of this parasite from its first intermediate snail host to second intermediate crab hosts (Hopper et al., 2008).

Abiotic factors, in particular temperature, are well known to influence the survival and infectivity of trematode transmission stages (e.g. Pietrock and Marcogliese, 2003). Furthermore, the output of trematode cercariae from first intermediate mollusc hosts is also strongly influenced by temperature, with generally more cercariae emerging with increasing temperature (Poulin, 2006), at least up to an optimum temperature level (Studer et al., 2010; Thieltges and Rick, 2006). Poulin (2006) therefore suggested that trematodes may find favourable conditions for transmission more often in a warming world. However, in a warmer world, the feeding demands of predators and filter-feeders may also increase due to the temperature dependence of metabolic rates (e.g. Schmidt-Nielson, 1997; Stone and Johnston, 2000). Hence, an increase in transmission success could potentially be compensated by an increased consumption of infective stages under warmer conditions (Thieltges et al., 2008a).

The combined effect of high temperature and increased parasite transmission is known to have caused a massive parasite-induced die-off of amphipod hosts in an intertidal soft-sediment ecosystem with drastic ecosystem-wide consequences (Jensen and Mouritsen, 1992; Mouritsen et al., 1998). Given that the mortality of crustacean hosts, in particular *Paracalliope novizealandiae* amphipods parasitised by *M. novaezealandensis*, depends on infection intensity (Bates et al., 2010; Fredensborg et al., 2004), the transmission success of this parasite will also determine its impact on amphipod populations and possibly on the entire crustacean host community.

The objective of this study was to investigate the combined effect of temperature and the presence of anemones at different densities on the transmission of the trematode parasite *M. novaezealandensis* to the amphipod host *P. novizealandiae*. We determined whether the presence a known predator of these cercariae has the potential to protect amphipods from increasing parasite loads under increasing temperatures. If fewer cercariae successfully infect amphipods in treatments with anemones present – in particular at the higher temperature level – then this would indicate a buffering role for anemones, as increased anemone feeding activity may reduce the number of transmission stages remaining in the system.

2. Materials and methods

A microcosm experiment was conducted as a 2 × 3 design with two temperatures (15 and 22 °C) and three densities of *A. aureoradiata* anemones (0, 5 or 10 anemones per microcosm). Anemones were collected from Hooper's Inlet (Otago Peninsula, New Zealand; 45°52′S, 170°42′E) a week prior to the experiment in order to allow for adequate acclimatisation to laboratory conditions and in order to minimise their feeding. On local mudflats, these anemones are very patchily distributed, occurring in dense aggregations in some areas and being absent from others. Thus, our experimental densities reflected a range of natural densities observed in the field. Infected first intermediate snail hosts (*Zeacumantus subcarinatus*) were used from stock aquaria (snails collected from Lower Portobello Bay, Otago Harbour, New Zealand; 45°50′S, 170°40′E) in order to obtain the cercarial transmission stages (see below). Uninfected *P. novizealandiae* amphipods were also collected from Hooper's Inlet a few days prior to the experiment. In this locality, *Z. subcarinatus* snails are absent and amphipods have never been found infected by any trematode species (Bryan-Walker et al., 2007; Fredensborg et al., 2004; Studer et al., 2010). The temperature levels used in this experiment were chosen to mirror conditions where transmission is low (15 °C), and conditions where transmission should be optimal (Studer et al., 2010) and the feeding activity of anemones can be expected to be elevated due to increased metabolic demands at higher temperatures (22 °C) (see Chomsky et al., 2004).

Either 0, 5 or 10 anemones were placed into round plastic containers (300 ml total volume; 8.5 cm high; 7 cm diameter at the bottom; i.e. density of anemones per 38.5 cm^2), which were filled with 100 ml of seawater (five replicate containers per treatment). Containers were then incubated at either 15 or 22 °C, randomly assigned to one of two replicate water baths per temperature. Incubation was for 6 h according to the duration of a low tide period when transmission to amphipods is taking place in the field. Towards the end of this period, amphipods were added to the containers ($n = 10$ per container). Also, infected *Z. subcarinatus* snails ($n = 40$; five snails in eight replicate Petri dishes containing 7 ml of seawater) were incubated for 1 h at 25 °C under constant illumination, triggering the emergence of cercarial transmission stages. The seawater containing the free-swimming cercariae was combined in order to use a genetic mixture of parasites in the experiment. Approx. 400 cercariae (average age of about 1 h; max. life span < 24 h) from this cercarial mixture were then added to each container. Under constant illumination, containers with anemones, amphipods and cercariae were then incubated for 24 h at the respective temperature (i.e. 15 or 22 °C). Subsequently, amphipods were transferred into new plastic containers filled with 300 ml of aerated seawater, provided with a strip of sea lettuce (*Ulva* sp.) for food and shelter and stored at 15 °C for 2–3 days. Surviving amphipods were then sexed, measured (size classes: 2.5, 3.0, 3.5, 4.0 and 4.5 ± 0.25 and >4.75 mm) and dissected under a dissecting microscope to determine the number of parasites infecting each amphipod.

Due to mortality of amphipods after experimental exposures, we tested for the effect of temperature and anemone density on the number of amphipods per replicate container surviving until dissections, using a Generalised Linear Model fitted with a Poisson error structure. Amphipods from the different replicates that survived until dissection had to be pooled for the subsequent analysis of infection levels. A General Linear Model (Gaussian error structure) was used to test for the effects of temperature, anemone density, sex and size class of amphipods on the number of parasites infecting the surviving amphipods (square root transformed). In both analyses, effects were considered significant at $p < 0.05$.

3. Results

The number of surviving amphipods was significantly affected by temperature, but not by anemone density, with a mean number (± standard error) of 7.27 (± 0.42) and 4.40 (± 0.55) amphipods surviving at 15 and 22 °C, respectively (Generalised Linear Model, Poisson; temperature: $\chi^2 = 11.57$, df = 1, p = 0.001; anemone density: $\chi^2 = 2.16$, df = 2, p = 0.339). The number of surviving amphipods was lowest in the low and medium anemone density treatments at 22 °C (Fig. 1). In total, 175 amphipods (74 females, 99 males, 2 unknown) were recovered; at 15 °C: $n = 31$ (0 anemone/container), $n = 43$ (5 anemones/container), $n = 35$ (10 anemones/container); at 22 °C: $n = 22$ (0 anemone/container), $n = 14$ (5 anemones/container) and $n = 30$ (10 anemones/container), of which 96.6% were infected.

Temperature, but not anemone density (or their interaction), had a significant effect on the number of parasites infecting surviving amphipods (Table 1). At 22 °C, amphipods harboured on average 9.5 (± 0.7) parasites, whereas those at 15 °C had 6.3 (± 0.4) (Fig. 2). At 15 °C, the presence of anemones had no influence on the transmission, whereas a trend, albeit not significant, was apparent at 22 °C with slightly reduced numbers of parasites in treatments where anemones were present (Fig. 2). Sex and size of amphipods did not significantly influence the number of parasites infecting the amphipods (Table 1).

4. Discussion

We hypothesised that in treatments with *A. aureoradiata* anemones present, their potentially higher feeding activity at 22 °C compared to 15 °C might compensate or reduce the increased transmission pressure of *M. novaezealandensis* cercariae to amphipods, hence resulting in

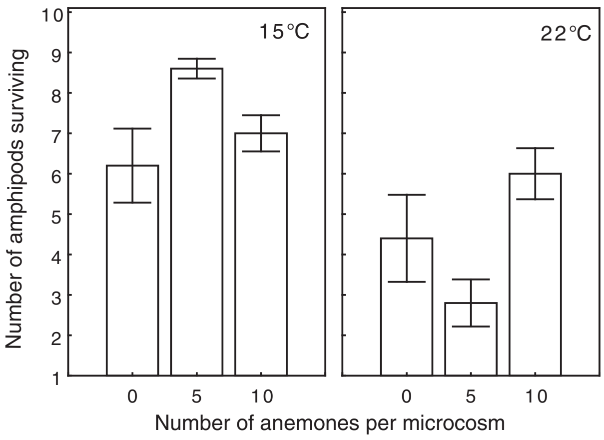

Fig. 1. Mean number (± standard error) of amphipods surviving until dissection at two temperatures (15 and 22 °C) and three densities of *Anthopleura aureoradiata* anemones (0, 5 or 10 anemones per microcosm). There were five replicate containers in each treatment to which 10 amphipods were originally added.

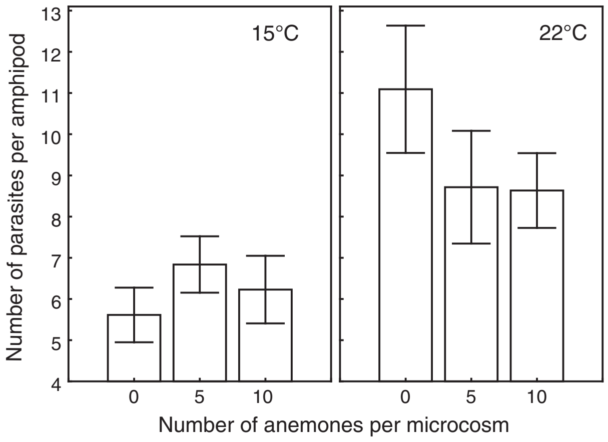

Fig. 2. Mean number (± standard error) of *Maritrema novaezealandensis* parasites infecting amphipod hosts (*Paracalliope novizealandiae*) at two temperatures (15 and 22 °C) and three densities of *Anthopleura aureoradiata* anemones (0, 5, or 10 anemones per microcosm).

no differences in the numbers of parasites infecting amphipod hosts. In contrast to our expectation, the results from our study did not completely support this: the effect of anemones was not strong enough to interfere with the greater transmission success of the parasite to surviving amphipods at higher temperatures. Amphipods at 22 °C compared to 15 °C were more infected and the presence of anemones did not significantly reduce the number of parasites successfully infecting surviving amphipods.

Given that *A. aureoradiata* anemones were shown to cause a more than four-fold reduction in the number of *M. novaezealandensis* acquired by crab hosts and that they were by far the most effective interfering community member investigated in a previous study (Hopper et al., 2008), it is surprising that we could not conclusively confirm this role for the transmission to amphipod hosts. A direct assessment of the feeding activity of anemones at the experimental temperatures was, however, not conducted as part of our study and thus it remains unclear to what extent temperature indeed influenced the feeding of anemones on cercariae. The lack of this data considerably hinders our ability to draw conclusions about actual feeding rates under experimental conditions and hence still requires further assessment. However, increasing feeding rates with increasing temperatures up to an optimum level have been conclusively shown for many marine invertebrates (e.g. Newell, 1979; Sanford, 1999, 2002; Yukihira et al., 2000 and references therein). Anemones likely follow a similar pattern, but surprisingly little is known about their feeding ecology at different temperatures (Chomsky et al., 2004 and references therein). The number of parasites infecting amphipods in our experiment was highest at 22 °C and when no anemones were present (Fig. 2), suggesting that the presence of anemones may have had at least some influence on the transmission, which was clearly not the case at 15 °C. Irrespectively, the result suggests that anemones might not be capable of substantially interfering with the transmission of *M. novaezealandensis* by reducing the number of cercariae present in a system at higher temperatures.

Moreover, it is likely that amphipods that died after the experimental exposure and could not be included in the dissections due to fast decomposition, may have been the ones that were most infected. The highest number of amphipods died at 22 °C when zero or five anemones were present per container (see Results, Fig. 1). These should be the treatments with the highest expected infection levels, and mortality of amphipods may therefore directly reflect the high transmission pressure under these conditions, i.e. intensity-dependent mortality coupled with an exposure to 22 °C for 24 h. This may provide some indirect evidence for a buffering role of anemones if they are present in high densities. Parasite-induced amphipod mortality may thus be responsible for the non-significant outcome of the statistical analysis which only included infection levels of surviving amphipods; actual infection levels of all amphipods right after the experimental exposure may thus be considerably underestimated. While the differential survival of amphipods is a result in itself, the missing data regarding infection levels in amphipods that died prior to dissection further limit our ability to conclusively pinpoint the role of anemones in interfering with the transmission process of *M. novaezealandensis*.

Additionally, other mechanisms may also be responsible for the observed lack of an effect of anemone density. It is possible that the feeding activity or the densities of the anemones were not high enough to interfere with the number of cercariae added to the microcosms. However, densities adequately reflected the natural range and anemones have been shown to feed on *M. novaezealandensis* cercariae in similar experiments conducted in our lab (Hopper et al., 2008). Alternatively, the number of cercariae added to the experimental units may have been too high for the anemones to clearly reduce, therefore masking any effect the anemones feeding activity may have. However, infected *Z. subcarinatus* snails are known to release much higher numbers of cercariae (Studer et al., 2010) and the number of cercariae added in this experiment is probably rather conservative for natural systems. During the main transmission window of *M. novaezealandensis*, i.e. on warm sunny days when the water in tide pools warms up, several snails can simultaneously release readily developed cercarial transmission stages within a relatively small area, leading to a “burst” of cercariae emerging (Fredensborg et al., 2004; Keeney et al., 2007). Under such conditions, amphipods and any other crustaceans may be exposed to hundreds if not thousands of parasites at a time. In our experiment, the same number of cercariae was added to each microcosm regardless of treatment. In reality,

Table 1
Results from a General Linear Model assessing the effects of temperature and density of anemones (including their interaction) as well as amphipod sex and size class, on the number of *Maritrema novaezealandensis* parasites infecting amphipods (square root transformed).

Factors	df	MS	F	p
Temperature	1	13.96	16.23	<0.001
Anemone density	2	0.62	0.72	0.490
Temperature × density	2	1.26	1.47	0.234
Amphipod sex	1	0.05	0.06	0.815
Amphipod size	4	0.48	0.56	0.690
Error	162	0.86		

the number of cercariae released into the environment by first intermediate snail hosts would be substantially higher at 22 than at 15 °C (Fredensborg et al., 2005; Studer et al., 2010) and therefore also the density of cercariae to which crustaceans are exposed to. Hence, even if the anemones were capable of interfering with the transmission through their feeding activity, the high numbers of cercariae released at higher temperatures may easily overcome any losses to predation by anemones and thus increase the risk of parasite-induced amphipod mortality under optimal conditions for transmission.

We conclude that despite the fact that community members have the potential to strongly interfere with and regulate the transmission of trematodes and consequently influence infection levels in host organisms, this effect might not be strong enough to counteract increased transmission of trematode cercariae at higher temperatures. This supports that host species such as amphipods are at an increased risk of intensity-dependent, parasite-induced mortality under increased temperatures, and that this may be particularly the case in an ecosystem with low species diversity and/or densities. The role of co-inhabiting organisms with on-going and predicted global changes clearly needs to be further investigated. The combined study of abiotic and biotic factors in disease ecology is relatively novel despite the fact that climate change, the unprecedented loss of biodiversity, species invasions and increasing diseases and parasitism have all been recognised as major challenges of global concern (e.g. Sala et al., 2000). The acknowledgement that host–parasite interactions are integrated within complex and dynamic ecological communities exposed to a network of fluctuating abiotic conditions must now be followed by targeted research to provide a more realistic understanding of how these components are functionally linked.

Acknowledgements

We are grateful for the constructive comments made by the reviewers on an earlier version of this manuscript. We also wish to thank staff at the Department of Zoology, University of Otago, for their support in running the experiment. This research was funded by the University of Otago including a postgraduate scholarship to A.S. **[ST]**

References

Bates, A.E., Poulin, R., Lamare, M.D., 2010. Spatial variation in parasite-induced mortality in an amphipod: shore height versus exposure history. Oecologia 163, 651–659.

Bryan-Walker, K., Leung, T.L.F., Poulin, R., 2007. Local adaptation of immunity against a trematode parasite in marine amphipod populations. Mar. Biol. 152, 687–695.

Chomsky, O., Kamenir, Y., Hyams, M., Dubinsky, Z., Chadwick-Furman, N.E., 2004. Effects of temperature on growth rate and body size in the Mediterranean Sea anemone *Actinia equina*. J. Exp. Mar. Biol. Ecol. 313, 63–73.

Fredensborg, B.L., Mouritsen, K.N., Poulin, R., 2004. Intensity-dependent mortality of *Paracalliope novizealandiae* (Amphipoda: Crustacea) infected by a trematode: experimental infections and field observations. J. Exp. Mar. Biol. Ecol. 311, 253–265.

Fredensborg, B.L., Mouritsen, K.N., Poulin, R., 2005. Impact of trematodes on host survival and population density in the intertidal gastropod *Zeacumantus subcarinatus*. Mar. Ecol. Prog. Ser. 290, 109–117.

Hopper, J.V., Poulin, R., Thieltges, D.W., 2008. Buffering role of the intertidal anemone *Anthopleura aureoradiata* in cercarial transmission from snails to crabs. J. Exp. Mar. Biol. Ecol. 367, 153–156.

Jensen, K.T., Mouritsen, K.N., 1992. Mass mortality in two common soft-bottom invertebrates, *Hydrobia ulvae* and *Corophium volutator* — the possible role of trematodes. Helgol. Meeresunters. 46, 329–339.

Johnson, P.T.J., Thieltges, D.W., 2010. Diversity, decoys and the dilution effect: how ecological communities affect disease risk. J. Exp. Biol. 213, 961–970.

Johnson, P.T.J., Dobson, A., Lafferty, K.D., Marcogliese, D.J., Memmott, J., Orlofske, S.A., Poulin, R., Thieltges, D.W., 2010. When parasites become prey: ecological and epidemiological significance of eating parasites. Trends Ecol. Evol. 25, 362–371.

Kaplan, A.T., Rebhal, S., Lafferty, K.D., Kuris, A.M., 2009. Small estuarine fishes feed on large trematode cercariae: lab and field investigations. J. Parasitol. 95, 477–480.

Keeney, D.B., Waters, J.M., Poulin, R., 2007. Diversity of trematode genetic clones within amphipods and the timing of same-clone infections. Int. J. Parasitol. 37, 351–357.

Kuris, A.M., Hechinger, R.F., Shaw, J.C., Whitney, K.L., Aguirre-Macedo, L., Boch, C.A., Dobson, A.P., Dunham, E.J., Fredensborg, B.L., Huspeni, T.C., Lorda, J., Mababa, L., Mancini, F.T., Mora, A.B., Pickering, M., Talhouk, N.L., Torchin, M.E., Lafferty, K.D., 2008. Ecosystem energetic implications of parasite and free-living biomass in three estuaries. Nature 454, 515–518.

Mouritsen, K.N., Poulin, R., 2002. Parasitism, community structure and biodiversity in intertidal ecosystems. Parasitology 124, S101–S117.

Mouritsen, K.N., Poulin, R., 2003. The mud flat anemone–cockle association: mutualism in the intertidal zone? Oecologia 135, 131–137.

Mouritsen, K.N., Poulin, R., 2005. Parasites boost biodiversity and changes animal community structure by trait-mediated indirect effects. Oikos 108, 344–350.

Mouritsen, K.N., Mouritsen, L.T., Jensen, K.T., 1998. Change of topography and sediment characteristics on an intertidal mud-flat following mass-mortality of the amphipod *Corophium volutator*. J. Mar. Biol. Assoc. U. K. 78, 1167–1180.

Newell, R.C., 1979. The Biology of Intertidal Animals, 3rd ed. Marine Ecological Surveys Ltd, Kent, UK.

Pietrock, M., Marcogliese, D.J., 2003. Free-living endohelminth stages: at the mercy of environmental conditions. Trends Parasitol. 19, 293–299.

Poulin, R., 2006. Global warming and temperature-mediated increases in cercarial emergence in trematode parasites. Parasitology 132, 143–151.

Prinz, K., Kelly, T.C., O'Riordan, R.M., Culloty, S.C., 2009. Non-host organisms affect transmission processes in two common trematode parasites of rocky shores. Mar. Biol. 156, 2303–2311.

Sala, O.E., Stuart Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M., Wall, D.H., 2000. Global biodiversity scenarios for the year 2100. Science 287, 1770–1774.

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

Sanford, E., 2002. The feeding, growth, and energetics of two rocky intertidal predators (*Pisaster ochraceus* and *Nucella canaliculata*) under water temperatures simulating episodic upwelling. J. Exp. Mar. Biol. Ecol. 273, 199–218.

Schmidt-Nielson, K., 1997. Animal Physiology: Adaptation and Environment, 5th ed. Cambridge University Press Cambridge.

Stone, P.W.G., Johnston, I., 2000. Environmental Physiology of Animals. Blackwell Science, Oxford.

Studer, A., Thieltges, D.W., Poulin, R., 2010. Parasites and global warming: net effects of temperature on an intertidal host–parasite system. Mar. Ecol. Prog. Ser. 415, 11–22.

Thieltges, D.W., Rick, J., 2006. Effect of temperature on emergence, survival and infectivity of cercariae of the marine trematode *Renicola roscovita* (Digenea: Renicolidae). Dis. Aquat. Organ. 73, 63–68.

Thieltges, D.W., Jensen, K.T., Poulin, R., 2008a. The role of biotic factors in the transmission of free-living endohelminth stages. Parasitology 135, 407–426.

Thieltges, D.W., Bordalo, M.D., Hernandez, A.C., Prinz, K., Jensen, K.T., 2008b. Ambient fauna impairs parasite transmission in a marine parasite–host system. Parasitology 135, 1111–1116.

Thieltges, D.W., Reise, K., Prinz, K., Jensen, K.T., 2009. Invaders interfere with native parasite–host interactions. Biol. Invasions 11, 1421–1429.

Yukihira, H., Lucas, J.S., Klumpp, D.W., 2000. Comparative effects of temperature on suspension feeding and energy budgets of the pearl oysters *Pinctada margaritifera* and *P. maxima*. Mar. Ecol. Prog. Ser. 195, 179–188.