

Large scale patterns of trematode parasitism in a bivalve host: no evidence for a latitudinal gradient in infection levels

A. Studer^{1,*}, M. Widmann¹, R. Poulin¹, M. Krkošek^{1,2}

¹Department of Zoology, University of Otago, PO Box 56, Dunedin 9054, New Zealand

²Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, Ontario M5S 3B2, Canada

ABSTRACT: Climate is a key driver of parasite transmission and disease dynamics. For trematode parasites, the high temperature sensitivity of transmission between first and second intermediate hosts may lead to higher infection rates with global warming, or spatially with warmer latitudes. However, spatial heterogeneities are common, and local factors are known to play crucial roles in determining infection levels. Using the latitudinal and sea temperature gradient along the New Zealand coastline, we assessed if this temperature sensitivity indeed translates into higher parasite abundance towards lower (i.e. warmer) latitudes in the cockle *Austrovenus stutchburyi* which serves as second intermediate host for several echinostome trematode species. Seventeen mudflats were sampled, and host densities and infection levels (i.e. metacercariae abundance) were measured for cockles, as well as for whelks and mudsnails (prevalence; first intermediate hosts). No evidence was found for a latitudinal pattern of metacercariae abundance in cockles. Instead, whelk prevalence per site and cockle foot size were found to be the main predictors. This highlights the importance of local factors—in particular, infection levels in first intermediate (i.e. source) hosts. These results indicate that, at least at large spatial scales, the temperature sensitivity of host-parasite systems may be offset by other ecological factors that confer resilience against on-going and predicted climate change.

KEY WORDS: Biogeography · Transmission · Host-parasite interaction · Climate change · Intertidal · Cockles

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Climate—in particular, temperature—is an important modulator of the transmission dynamics of parasites, and as a consequence, global climate change may have implications for the epidemiology of infectious diseases (Marcogliese 2001, Mouritsen & Poulin 2002a, Cattadori et al. 2005, Kutz et al. 2005). This is of relevance in all ecosystems, including marine ecosystems, where there have been concerns about an increase in infection levels and potential negative repercussions for affected populations and systems (Harvell et al. 2002, Ward & Lafferty 2004, Poulin &

Mouritsen 2006). These concerns stem from the risk of parasite-induced host mortality (e.g. Fredensborg et al. 2004), but also from the fact that parasites play broader ecological roles (Mouritsen & Poulin 2002b, Marcogliese 2004, Wood et al. 2007, Lafferty et al. 2008). Hence, changes to the distribution and abundance of parasites or their vital rates such as reproduction and survival may not only have important implications for affected hosts, but also cascading effects on ecosystem structure and function. Despite the importance of parasites in marine ecosystems and growing concerns about some increasing diseases, our understanding of marine host-parasite systems

*Email: studeranja@gmail.com

and dynamics remains limited. In particular, knowledge of baseline infection levels are often lacking, thus impeding any attempt to detect changes that may be, or may have been, occurring. Also, determinants of infection levels in natural host populations are often unknown, limiting our ability to identify areas at risk for strong host-parasite interactions such as mortality events.

Trematodes are an important parasite group in coastal ecosystems (Lauckner 1984, Mouritsen & Poulin 2002b). They usually have a complex life cycle involving several members of a community. Typically, their life cycle comprises a definitive host (usually a vertebrate), a first intermediate mollusc host and a second intermediate host (invertebrate or vertebrate). A range of environmental factors are known to influence the transmission of trematodes (Pietroock & Marcogliese 2003, Thieltges et al. 2008). However, in particular the transmission between the first and the second intermediate host is known to be very sensitive to temperature (e.g. Mouritsen & Jensen 1997, Fingerut et al. 2003). Not only is the production of transmission stages (i.e. cercariae) affected by temperature, but also their emergence (Mouritsen 2002), survival (Studer et al. 2010) and infectivity (Thieltges & Rick 2006). Under optimal conditions for transmission, cercariae leave a first intermediate host to infect a second intermediate host. In general, the number of cercariae emerging increases with increasing temperature up to a limit; at the same time, their survival decreases, although this decrease is offset by an increase in infectivity (but see also Morley 2011). These responses are not only important for a parasite's ability to complete its life cycle, but also in terms of the potential impact of a parasite on affected host populations. For parasites with temperature sensitive transmission processes, such as many trematodes, increasing temperature suggests that these parasites may have higher transmission rates in warmer areas or periods (Poulin 2006, Thieltges et al. 2009d), and therefore that infection levels in hosts (second intermediate hosts in the case of trematodes) may show a latitudinal pattern due to temperature gradients across large spatial scales (Thieltges et al. 2009d).

Indeed, there is an increasing number of studies investigating latitudinal patterns of parasitism, and in particular trematodes, in marine and other systems (Rohde 1992, Rohde & Heap 1998, Rohde 2002, Poulin & Mouritsen 2003, Poulin & Morand 2004, Thieltges et al. 2009b, Thieltges et al. 2009d). While Poulin & Mouritsen (2003) found no evidence for a latitudinal gradient in trematode infection levels in

first intermediate snail hosts, Thieltges et al. (2009d) found latitude to be an important predictor of infection levels and trematode richness in second intermediate amphipod hosts. Their meta-analysis indicated that levels of infection (intensity and abundance) decreased with increasing latitude, consistent with the pattern expected based on the temperature sensitivity of the transmission from first to second intermediate hosts. However, as transmission dynamics are influenced by a complex interplay between biotic as well as abiotic environmental parameters (Thieltges et al. 2008), the importance of local, small scale factors causing heterogeneity and influencing infection levels has also been strongly emphasised (Poulin & Mouritsen 2003, Thieltges & Reise 2007, Thieltges et al. 2009c).

Here, we investigated latitudinal trends of trematode infections in the New Zealand cockle *Austrovenus stutchburyi*. This suspension-feeding bivalve is endemic to New Zealand, occurs in soft sediment ecosystems where it can reach densities of several hundred individuals per square meter, and has substantial value for commercial and recreational fisheries. The cockle serves as a second intermediate host for a number of parasites (Poulin et al. 2000), with echinostomes, namely *Curtuteria* spp. (Allison 1979, Leung et al. 2009a) and *Acanthoparyphium* spp. (Martorelli et al. 2006, Leung et al. 2009a) infecting the feet of cockles. These echinostomes are characterised by a spiny collar surrounding their oral sucker, with different numbers of spines in different species. The echinostomes targeted in this study use birds as definitive hosts (oystercatchers, possibly other species), whelks (e.g. *Cominella glandiformis* for *Curtuteria* spp.) and mudsnails (e.g. *Zeacumantus subcarinatus* for *Acanthoparyphium* spp.) as first intermediate hosts. In terms of their transmission mode and their impact on cockles, the echinostome species are roughly equivalent (Babirat et al. 2004). Cercariae emerge from infected snail hosts (temperature dependent; Allison 1979, Koprivnikar & Poulin 2009) and infect a cockle, encysting as metacercariae in the cockle's foot. Metacercariae accumulating in a cockle's foot eventually impair the burrowing ability of the cockle, leaving heavily infected individuals exposed on the sediment surface (Thomas et al. 1998, Mouritsen & Poulin 2005). While this increases the likelihood of avian predation and hence transmission to definitive hosts, the manipulation of the cockle's burrowing ability also affects the structure and function of the entire benthic community and ecosystem; these parasites are thus considered ecosystem engineers

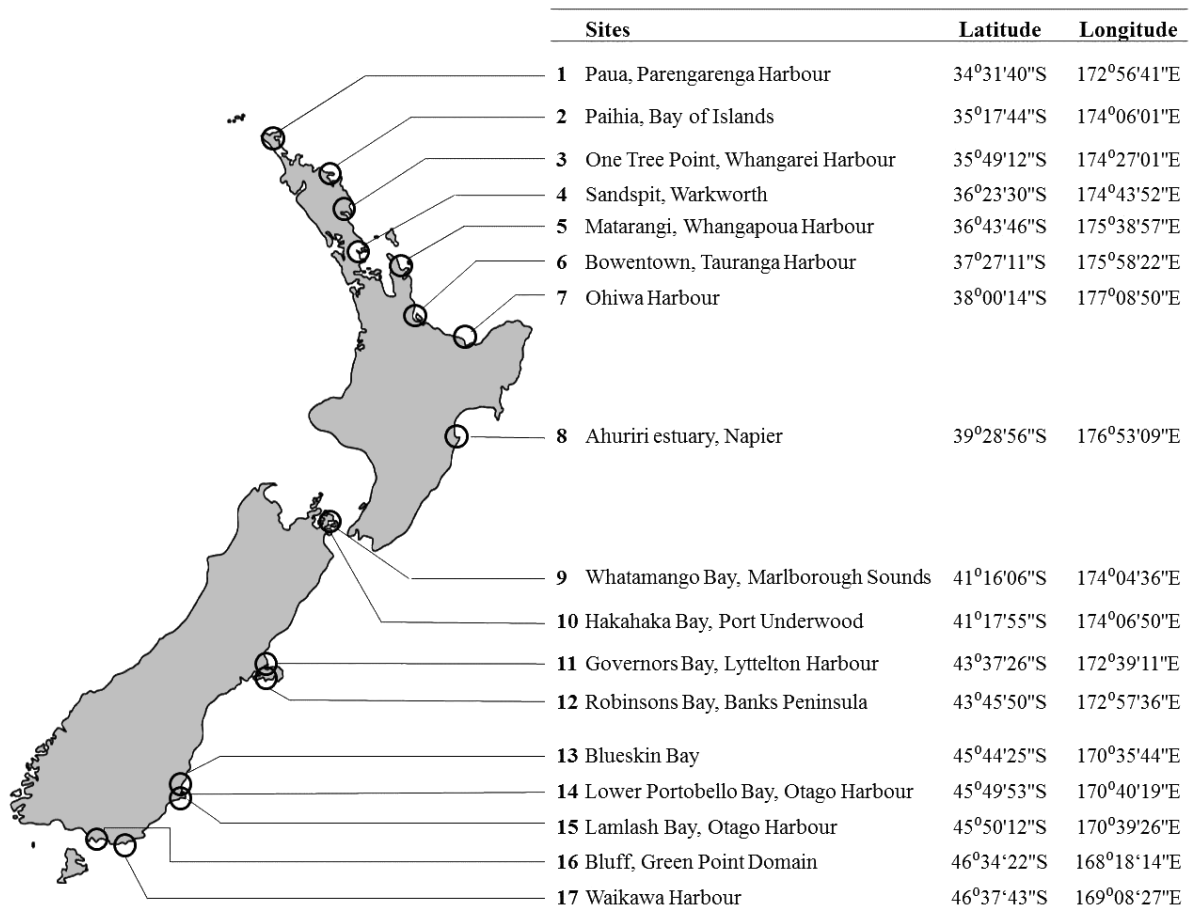


Fig. 1. Location of the 17 sampling sites and their grid references (latitude, longitude) in New Zealand

(Mouritsen & Poulin 2005, Mouritsen & Poulin 2006, Mouritsen & Poulin 2010).

This trematode host-parasite system may be a good candidate for evaluating whether the high temperature-sensitivity of transmission that has been measured in laboratory experiments translates into spatial patterns of infections associated with temperature. Spanning about 12° latitude, mean annual sea surface temperatures along the North and South Islands of New Zealand range from about 11°C in the South to 18°C in the North (Figs.1 & 2) (Chiswell 1994, Uddstrom & Oien 1999). However, temperatures on mudflats tend to be more extreme than temperatures in nearby main water bodies (Studer & Poulin 2012). Therefore, the temperature ranges covered in laboratory experiments — on which our understanding of the temperature effects is mainly based — should be expressed along this latitudinal and hence climatic gradient. The aim of the present study was therefore to investigate levels of cockle parasitism along the latitudinal range of New Zealand, while simultane-

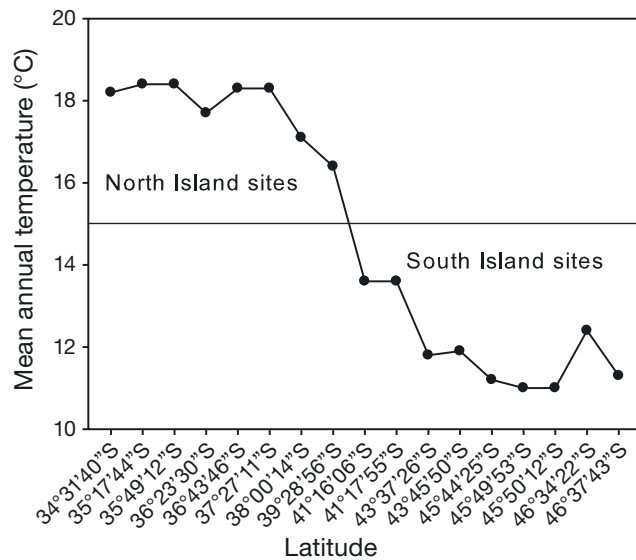


Fig. 2. Mean annual temperatures (°C) at each sampling location's latitudinal position. Temperature data was extracted from the World Ocean Atlas selecting the grid references closest to the sampling locations

ously controlling for the effects of relevant local factors. If temperature is an important environmental factor determining levels of parasitism in these second intermediate hosts, then we would expect higher infection levels at lower latitudes, i.e. northern and warmer, sites. If no such spatial pattern is detected, then other local ecological factors may be the key drivers of trematode parasitism in *Austrovenus stutchburyi*. Establishing such baseline data across large spatial scales, identifying determinants of infection levels and hence identifying hotspots of host-parasite interactions are all crucial, especially in the context of global climate change.

MATERIALS AND METHODS

Field sampling

The sampling was conducted on tidal mudflats along the east coast of both North and South islands of New Zealand from late February to early April 2012 (Fig. 1; total of 17 sites). Mudflats were, as much as logistically feasible, not sampled in a latitudinal order, and differences in times between sampling are considered negligible. Sampling locations were chosen to span the maximum latitudinal gradient logistically feasible (approx. 12°C latitude; 46° 37' S to 34° 31' S). On each mudflat, three 50 m transects were laid out in the mid-upper intertidal area at least 30 m apart and 30 m away from the upper shore (except in Hakahaka Bay: 30 m transects spanning the entire mudflat; Bluff: 50 m transects spanning the entire mudflat). The mid-upper area was targeted, as infection levels in cockles tend to be higher at high shore compared to low shore sites (Poulin et al. 2000, Mouritsen et al. 2003). Transects were laid out perpendicular to the low tide water line (except in Ahuriri estuary: sampling area enclosed by channels on either side). Along each transect, 3 randomly placed quadrats (0.45 × 0.45 m) were sampled (i.e. 9 replicate quadrats per location).

The upper 10 cm of the sediment within a quadrat were sieved (5 mm mesh size) and all cockles *Austrovenus stutchburyi*, whelks *Cominella* spp. and mudsnails *Zeacumantus subcarinatus* or *Z. lutulentus* were collected and stored in separate 2 l plastic containers. All individuals were counted for density estimates. From each quadrat, 10 cockles (min. size 15 mm), 10 whelks (min. size 15 mm) and 10 mudsnails (min. size 10 mm) were haphazardly selected for dissections. In situations when there were insufficient individuals available from the initial sampling,

additional specimens of above minimum size were collected haphazardly from the immediate vicinity of a quadrat or from the wider area of the mudflat to ensure, where possible, the collection of a total of 90 cockles, whelks and mudsnails per location. Cockles were measured (shell length and foot length in mm) using callipers, dissected and the foot of each cockle was cut off and put into a 1.5 ml tube filled with 99% ethanol for sample preservation and later assessment of parasite (i.e. metacercariae) abundance in the laboratory (see next subsection). The focus on the cockles' feet was based on available knowledge on the echinostome species targeted—especially the temperature sensitivity of cercarial emergence from snail first intermediate hosts as well as the important ecological role that these parasites play by affecting the cockles' burrowing behaviour (see 'Introduction'). Whelks and mudsnails were measured (shell length in mm) under a dissecting microscope and dissected alive to assess their infection status and to identify, where possible, the trematode species or family. Identification was based on morphological differences between sporocysts/rediae (larval forms infecting a snail, which asexually produces cercariae) as well as morphological and behavioural differences between cercariae (e.g. swimming behaviour) (Schell 1970, Allison 1979, Martorelli et al. 2006). Trematodes were categorised as either 'echinostomes' or 'other' trematode species (including microphallids, philophthalmids, opisthorchids, and unknown species).

Laboratory analysis

To determine the number of metacercariae in each cockle foot, the foot was rinsed in distilled water and then transferred into tubes filled with 1.5 ml of pepsin solution (6 g pepsin powder and 7 ml conc. HCl in 1000 ml Milli-Q water). Tubes containing individual cockle feet were incubated at 40°C for 2 to 3 h to allow digestion of the cockle tissue. After incubation, metacercariae were rinsed by removing the pepsin solution and adding distilled water 3 times, and then transferred back into 99% ethanol for sample preservation. Subsequently, metacercariae were counted under a dissecting microscope. Species differentiation for metacercariae based on morphology (i.e. not considering cryptic species) would require the careful examination of individual cysts under high magnification for a count of the collar spines of each larval worm; this was not done as part of the present study.

Statistical analyses

Generalised linear mixed effect models (GLMM) were used to account for the nested structure of the sampling design. As a random factor, we included quadrat nested within transect, nested within site. All continuous variables were centred. Error structures were chosen to best account for the type of data analysed (see below). Models were fit to the data using maximum likelihood methods, and models were compared using Akaike’s Information Criterion (AIC; Burnham & Anderson 2002). All analyses were done using R 2.15.1 (R Development Core Team; www.R-project.org).

Metacercariae abundance in cockles, i.e. number of parasites per cockle foot including uninfected cockles, was analysed using the glmmADMB package (Skaug et al. 2012) and a negative binomial error structure. This analysis included only data on cockles collected from within quadrats (i.e. no cockles collected additionally were included). Two sets of models were assessed. The first set included density of infected whelks and mudsnails per quadrat as predictor variables. Model 1 included latitude as a predictor; Model 2, sea surface temperatures; Model 3, neither latitude nor temperature. The second set included the total density of whelks and mudsnails (i.e. infected and uninfected snails combined) per quadrat and their respective prevalence per site as predictor variables. Model 4 included latitude as a predictor; Model 5, sea surface temperatures; Model 6, neither latitude nor temperature. Temperature data used in the models were retrieved as mean annual temperatures over 1/4 degree-fields from the World Ocean Atlas (www.nodc.noaa.gov/OC5/WOA09/pr_woa09.html); values from the grid references closest to the sampling locations were extracted. All models also included cockle density and length of each cockle foot as predictor variables. Models were compared using AIC values. A GLMM (Gaussian error structure) was used to assess the relationship between latitude and cockle shell length (log transformed). Separate GLMMs (fitted with a Poisson error structure) were run to assess the effect of latitude on the density of cockles, whelks, echinostome-infected whelks, mudsnails, echinostome-infected mudsnails, and metacercariae per quadrat. Metacercariae per quadrat was calculated based on the mean parasite abundance per cockle in a quadrat times the density of cockles in that quadrat. Separate GLMMs with a binomial error structure were used to assess the effect of latitude and shell length on the echinostome infection status of whelks or mudsnails. These analyses were done using the package lme4 (Bates & Maechler 2009).

RESULTS

Infected cockles were found throughout the entire range covered by the study (Fig. 3A). Prevalence (the percentage of infected individuals) ranged from 29.2% (Waikawa Harbour) to 100% (several locations), and metacercariae abundance from a minimum range of 0 to 2 (Waikawa Harbour) to a maximum range of 1 to 2260 (Lower Portobello Bay) metacercar-

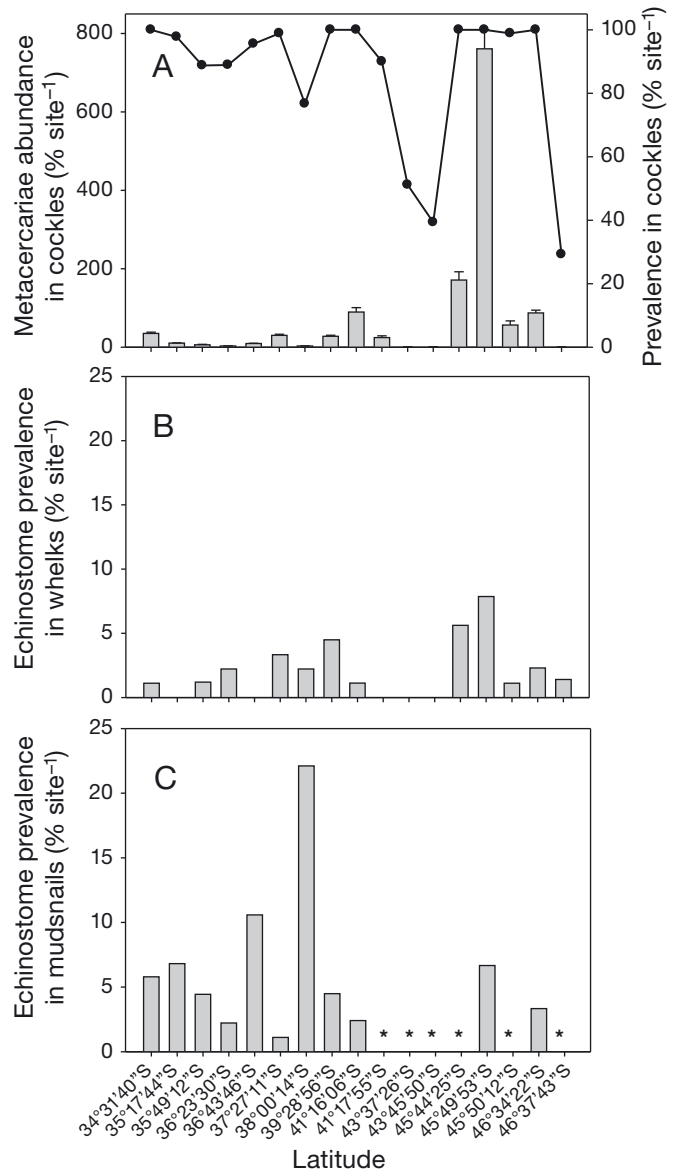


Fig. 3. Parasitism of cockles, whelks and mudsnails in relation to latitude. (A) Abundance of metacercariae (mean number ± SE of metacercariae per cockle foot including uninfected cockles; bar graph). Prevalence in cockles (% of infected individuals) per site (black circles). (B) Prevalence of echinostome infections in whelks. (C) Prevalence of echinostome infections in mudsnails. * denotes locations where no mudsnails were found

iae per cockle foot (Fig. 3A). Whelks were collected in all sampling locations, with overall trematode prevalence ranging from 0 (Robinsons Bay) to 30.3% (Ahuriri estuary) and echinostome prevalence from 0 (several locations) to 7.9% (Lower Portobello Bay) (Fig. 3B). Mudsnaileds were absent from 5 sampling locations (Fig. 3C). Overall trematode prevalence in mudsnails ranged from 1.1 (Bowentown) to 65.6% (Lower Portobello Bay) and from 1.1 (Bowentown) to 22.1% (Ohiwa Harbour) for echinostome infections (Fig. 3C). For more details see Table S1 in the Supplement at www.int-res.com/articles/suppl/m491p125_supp.pdf.

Metacercariae abundance in cockles

Of the models compared, the model with density of snail hosts and their prevalence per site (as opposed to the density of infected snail hosts) and excluding temperature and latitude as a predictor emerged as the best model (i.e. the model with the lowest AIC; Table 1). For the abundance of metacercariae per cockle foot, whelk prevalence per site and cockle foot length emerged as the only important predictors (Table 2) (the same results apply to analyses with infection status of cockles as response variable, i.e. prevalence; results not shown). Latitude was also not a strong predictor for the shell length of dissected cockles, and the model was not significantly different from the respective null model ($\lambda^2 = 0.554$, $p = 0.457$). Cockle shell and foot size correlated linearly ($y = -0.904 + 0.459x$; $r^2 = 0.780$) (see Fig. S1 in the Supplement).

Densities

Average density of cockles in our sampling locations ranged from 64.2 (Waikawa Harbour) to 1001.4 m^{-2} (Ahuriri estuary) (Fig. 4A). Cockle density showed an increase towards lower latitudes, i.e. northern sites (Table 2). The average density of whelks ranged from 1.6 (Robinsons Bay) to 39.5 m^{-2} (Matarangi) (Fig. 4B). Whelk density increased towards lower latitudes (Table 2). However, no trend was found for the density of echinostome infected whelks, and the model including latitude was not significantly different from the null model ($\lambda^2 = 0.004$, $p = 0.953$). Mudsnail density in locations where mud-

Table 1. Candidate models on metacercariae abundance. Log likelihoods (Log Lik), Akaike's information criterion (AIC) and Δ AIC values of the models investigated with metacercariae abundance as the response variable (generalized linear mixed effect model with negative binomial error structures). Models 1, 2 and 3 included density of infected snail hosts as predictors. Models 4, 5 and 6 included densities of snails and prevalences per site as predictors. All models also included cockle foot size and cockle density as predictors. Bracketed information indicates whether the model included latitude, temperature or neither. The model with the lowest AIC is shown in **bold**

| Candidate models | Log Lik | AIC | Δ AIC |
|---------------------------------|----------------|-----------------|--------------|
| Model 1 (with latitude) | -5612.5 | 11 244.9 | 6.1 |
| Model 2 (with temperature) | -5612.5 | 11 245.0 | 6.2 |
| Model 3 (without either) | -5612.6 | 11 243.2 | 4.4 |
| Model 4 (with latitude) | -5608.3 | 11 240.6 | 1.8 |
| Model 5 (with temperature) | -5608.4 | 11 240.7 | 1.9 |
| Model 6 (without either) | -5608.4 | 11 238.8 | 0.0 |

snails were found ranged from 0.5 (Lamlash Bay) to 186.6 m^{-2} (Matarangi) (Fig. 4C). Mudsnail density also increased towards lower latitudes (Table 2), but the density of infected mudsnails did not, with the model including latitude not being significantly different from the null model ($\lambda^2 = 2.536$, $p = 0.111$). The density of metacercariae per quadrat was also not affected by latitude, with the model including latitude not being significantly different from the null model ($\lambda^2 = 0.187$, $p = 0.666$).

Table 2. Analyses of metacercariae abundance (Model 6), cockle density, whelk density, mudsnail density and echinostome prevalence in whelks (all generalized linear mixed effect models, GLMM). Table showing parameter estimates and standard errors. Bracketed information indicating error structure of the model. Significant predictors are in **bold**

| Analysis | Predictor variables | Estimate | SE |
|--|----------------------------------|---------------|---------------|
| Metacercariae abundance GLMM (negative binomial) | (Intercept) | 2.579 | 0.367 |
| | Foot length | 0.256 | 0.04 |
| | Cockle density | 0.053 | 0.06 |
| | Whelk density | -0.007 | 0.049 |
| | Mudsnail density | -0.014 | 0.052 |
| | Prevalence in whelks/site | 1.316 | 0.375 |
| | Prevalence in mudsnails/site | -0.052 | 0.367 |
| Cockle density GLMM (poisson) | (Intercept) | 3.934 | 0.151 |
| | Latitude | 0.511 | 0.151 |
| Whelk density GLMM (poisson) | (Intercept) | 0.741 | 0.153 |
| | Latitude | 0.521 | 0.154 |
| Mudsnail density GLMM (poisson) | (Intercept) | -0.538 | 0.7577 |
| | Latitude | 1.795 | 0.7577 |
| Prevalence in whelks GLMM (binomial) | (Intercept) | -4.227 | 0.274 |
| | Latitude | -0.556 | 0.255 |
| | Shell length | 0.715 | 0.238 |

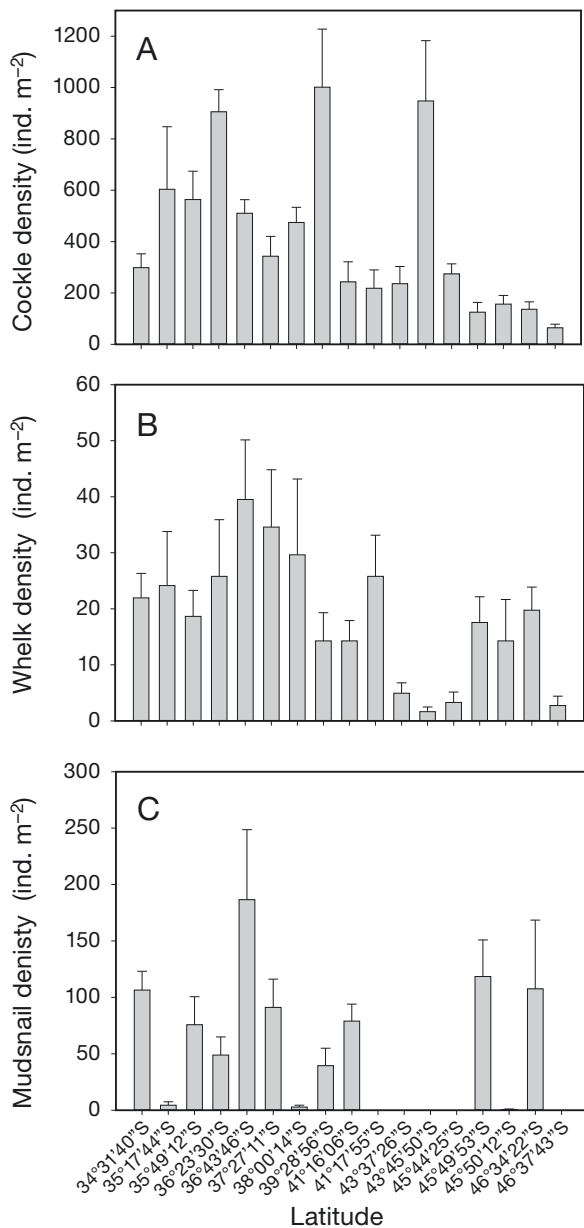


Fig. 4. Density (mean ± SE) of cockles, whelks and mudsnails in relation to latitude. (A) Density of cockles m⁻²; (B) density of whelks m⁻²; (C) density of mudsnails m⁻² at each site/latitude

Echinostome prevalence of first intermediate snail hosts

The infection status of whelks was significantly affected by latitude and shell length (Table 2), with whelks having higher infection levels towards higher latitudes and with increasing shell length. For mudsnails, the model including latitude and shell length was not significantly different from the null model ($\lambda^2 = 2.549$, $p = 0.636$).

DISCUSSION

To explore whether the temperature sensitivity of parasite transmission translates into a large scale biogeographic pattern, the present study assessed the relationship between climate (i.e. latitude as a proxy for temperature) and levels of trematode parasitism in the New Zealand cockle *Austrovenus stutchburyi*. No latitudinal gradient was found for metacercarial infection levels in cockles collected from the mid-upper intertidal zone, for which shell size did not vary along the latitudinal gradient covered. Instead, prevalence in first intermediate whelk hosts and cockle foot size were identified as the main predictors of metacercariae abundance in cockles. Echinostome prevalence in whelks increased towards higher (i.e. colder) latitudes, while the density of whelks increased towards lower (i.e. warmer) latitudes. These opposite trends resulted in the density of infected whelks not showing a latitudinal pattern. On the other hand, neither echinostome prevalence in mudsnails, nor density of infected mudsnails showed a latitudinal pattern. Latitudinal trends were, however, found for the density of cockles, whelks and mudsnails, with densities for all hosts increasing towards warmer latitudes.

If temperature was a main driver of infection levels in cockles, then based on the fact that the density of infected first intermediate snail hosts did not differ across latitude, cockles should have been more infected at lower, warmer latitudes. This was clearly not observed in the present study, where sites with the highest infection levels in cockles were found at higher, colder latitudes (see also below). This highlights the importance of other local ecological factors for determining infection levels in *Austrovenus stutchburyi* (see also Mouritsen et al. 2003, Poulin & Mouritsen 2004 for small-scale, within mudflat patterns). Our results are in agreement with previous studies showing a relationship between abundance and infection levels in source hosts and infection levels in target hosts (Bustnes & Galaktionov 1999, Smith 2001, Hechinger & Lafferty 2005, Fredensborg et al. 2006, Byers et al. 2008), and hence the input of infective stages into a particular system. These studies mainly focussed on the abundance of definitive hosts (usually birds) and infection levels in first intermediate snail hosts. Although the link between bird abundance and infection levels in intermediate hosts is not directly considered here, this interrelation could be strong. Our results provide evidence for this link extending to a second intermediate host (see also Thieltges 2007, Thieltges & Reise 2007, de

Montaudouin & Lanceleur 2011). As the complex life cycle of trematode parasites requires all host types to be present in order to complete the cycle, the link between the abundance of definitive hosts, and first and second intermediate hosts is fundamental.

Our results therefore depart from the general expectation solely based on laboratory-based temperature effects on transmission processes (e.g. Poulin 2006, Studer et al. 2010) and also from the latitudinal trend described in a meta-analysis for amphipod hosts (across $\sim 20^\circ$ latitude) (Thieltges et al. 2009d). However, these contrasting patterns may be reconciled by reconsidering the spatial scale at which the temperature-sensitivity of trematode transmission affects infection levels. Almost all studies of the effects of temperature on the production, survival and infectivity of cercarial transmission stages involve experiments on single populations performed under simplified conditions (e.g. Thieltges & Rick 2006, Studer et al. 2010). They generally show that all else being equal, higher temperatures (up to some maximum) lead to higher net transmission. In natural conditions, however, all else is not equal: at higher temperatures, the feeding activity of non-host cercarial predators may increase, the target hosts may shift their microhabitat choice to seek cooler temperatures and coincidentally avoid cercariae, etc. Although the net effect of warming may still be that trematode transmission is enhanced, this cannot apply equally to different geographic localities, which will vary in terms of community composition (e.g. abundance of cercarial predators, alternative hosts or non-hosts) and physical features (e.g. availability of microhabitat refuges, sediment composition, mudflat topography). In addition, geographical variation, possibly due to genetic differences, has been observed in how temperature affects cercarial production under controlled conditions (Koprivnikar & Poulin 2009), adding yet another layer of complexity to the search for latitudinal gradients in infection levels in second intermediate hosts. Thus, the temperature-sensitivity of trematode transmission may result in local increases in infection risk as a consequence of climate warming, but may not result in a latitudinal gradient in infection levels; such effects may be offset by other important ecological factors that are not related with latitude.

The contrasting patterns may also be reconciled by considering the temperature range of laboratory experiments compared to that experienced by organisms in nature. Although temperature levels chosen in experiments are usually within the range of temperatures experienced in nature for a given host-par-

asite system (e.g. Mouritsen 2002, Thieltges & Rick 2006), they often do not include upper temperature levels beyond the optimum range. Temperatures above the optimum are, however, important when considering the full thermal environment experienced by the organisms in their actual habitat (Studer et al. 2010, Studer & Poulin 2012), as an overall negative net effect on transmission can be expected. Mean annual sea surface water temperatures across New Zealand range from about 11°C to 18°C ; our sampling sites on the North Island have an annual mean $>15^\circ\text{C}$, whereas those on the South island are $<15^\circ\text{C}$. Temperatures on the mudflats where transmission occurs are more variable and extreme than in nearby water bodies (Studer & Poulin 2012) (e.g. Lower Portobello Bay mudflat [Site 14; $45^\circ 49' \text{S}$] mean 11.6°C , max. 26.5°C , min. 3.0°C compared to the adjacent main water body [Otago harbour] mean 11.6°C , max. 18.1°C , min. 6.5°C). For example, Allison (1979) assessed the emergence of *Curtuteria australis* cercariae from infected whelk hosts at 15°C and 23 to 25°C , finding an optimum around 25°C , but noted that temperatures on the mudflat of origin (Avon-Heathcote estuary, South Island, New Zealand; $43^\circ 33' \text{S}$) ranged from 3 to 33°C . Hence, the high inherent temperature variability of intertidal systems coupled with our limited understanding of the thermal biology of the species involved—especially in terms of their actual optimum temperature ranges—in addition to potential effects of local adaptations, further complicate our understanding of large scale patterns in trematode infection levels. Additionally, it is possible that due to this variability, the 12° latitude covered and the $\sim 7^\circ\text{C}$ difference in mean annual sea surface temperature may simply not be large enough to result in notable differences across the latitudinal gradient investigated here.

The present results are also not in full agreement with Thieltges & Reise (2007), in which the density of first intermediate snail hosts was the strongest determinant of infection levels in the European cockle *Cerastoderma edule*. The authors argued that density of first intermediate hosts should roughly correlate with the number actually infected. In our study, the density of whelks and mudsnails increased towards warmer latitudes, while echinostome infection levels only showed a latitudinal pattern for whelks, which offset the effect of whelk density. Hence, our results indicate that density of hosts and their infection levels should be considered separately. The 2 studies are, however, in agreement with regards to infection levels increasing with cockle size (but see

de Montaudouin et al. 2005, de Montaudouin et al. 2012), a pattern related to age and hence time of exposure to parasites and accumulation of metacercariae over time, as well as a greater filtration rate of larger cockles (Allison 1979). However, the size–age relationship in *Austrovenus stutchburyi* (which may live up to ~11 yr; Marsden & Pilkington 1995), is not straightforward as growth reflects a combination of environmental factors including temperature, salinity, food resources, as well as shore height (Dobbinson et al. 1989, Marsden & Adkins 2010). In terms of shore height, our results have to be understood in the context of only sampling mid-upper intertidal cockles, as their conspecifics at low shore sites tend to be larger (and less heavily infected; Mouritsen et al. 2003), but may not necessarily be older. In terms of temperature, it is expected that cockles in the North grow faster than in the South (Marsden & Adkins 2010). This would indicate that, due to the lack of a size difference in the cockles dissected in this study along the latitudinal gradient covered, there might indeed be an age difference, with cockles in the South being older. This may help explain why some of the highest metacercariae levels were found in the South. Indeed, exposure time rather than size has been shown to be of importance in determining infection levels in *C. edule*, in which mean infection levels strongly increased with age suggesting temporal exposure rather than size to be the main determinant of infection levels (Thieltges 2008). In addition, the fact that metacercariae density (density of cockles times average number of metacercariae in cockles in a particular quadrat) did not show a latitudinal pattern (see 'Results') despite the density of cockles increasing towards North (Fig. 4A), indicates that fewer cockles tend to harbour more metacercariae in mid-upper shore areas in the South (Fig. 3A). Overall, these patterns may therefore be best explained by a combination of prevalence of infection in whelks as well as the age of cockles (between sites) and size of cockles (within sites).

Cerastoderma edule along the Northeast Atlantic coast were found to be infected with trematodes at all sites (de Montaudouin et al. 2009), similar to the omnipresence of trematodes found in the present study. Different cockle populations, however, harboured different parasite communities; with some species having a restricted range and others showing a large latitudinal distribution (de Montaudouin et al. 2009, Thieltges et al. 2009a). In particular, the latitudinal distribution of first intermediate hosts was suggested to be important for understanding these patterns (de Montaudouin et al. 2009). In the present study, no

species differentiation was possible based on the morphology of the metacercarial cysts in cockle feet. However, future molecular analysis of the metacercariae collected may elucidate the species composition of the trematodes affecting *Austrovenus stutchburyi* and uncover the presence of other species occurring along the latitudinal gradient of the New Zealand coastline (see Leung et al. 2009a). Moreover, only foot tissue was assessed in our study, and hence it remains unknown to what extent other trematode (or parasite) species were present in the cockle tissues (e.g. *Gymnophallus* sp., Leung et al. 2009b). Latitudinal patterns may exist for individual trematode species, even though none were detected for all echinostome species combined infecting cockle feet.

Another potential issue with our data stems from the fact that the same sampling protocol was applied for mudflats differing substantially in size, topography and sediment composition. Moreover, as cockle density is not uniform across a mudflat, and infections in hosts are usually patchy, it is possible that our sampling design (e.g. number of replicates, quadrat size) was not adequate in some places to provide representative data. That cockles are an important target for commercial and recreational fisheries should, on the other hand, not be of major concern here, as cockles in the mid-upper intertidal areas are usually not targeted.

In conclusion, despite the known temperature sensitivity of trematode transmission, no latitudinal or temperature-related pattern was identified for metacercariae abundance in second intermediate cockle hosts. Instead, infection levels in first intermediate whelk hosts and cockle size were found to be the main predictors. This probably stems from the fundamental relationship between infection levels in first intermediate (source) hosts and second intermediate (target) hosts, and supports the importance of other local ecological factors in determining levels of parasitism as opposed to large scale climatic factors. In the context of climate change, how host species respond to altered conditions in terms of their abundance and distribution will therefore be of particular relevance. This may have important repercussions for parasite species and their ability to complete their life cycle, and hence infection levels in hosts. Due to the complexity of ecological systems, anticipating how marine host-parasite systems may respond to climate change still remains a major challenge. This study, however, provides baseline data on key species in intertidal systems, and hence should be useful for assessing potential temporal and/or spatial changes.

Acknowledgements. We thank the reviewers for their constructive comments on an earlier version of this manuscript. We also thank staff at the Department of Zoology and the Portobello Marine Laboratory, University of Otago, in particular N. McHugh, for technical support. Sincere thanks also to K. Mouritsen for providing unpublished data on infection levels in cockles for the Otago Harbour. Thanks also to all the people encountered during the sampling trip for their helpfulness. This research was funded by a University of Otago Research Grant to M.K.

LITERATURE CITED

- Allison FR (1979) Life cycle of *Curtuteria australis* n. sp. (Digenea: Echinostomatidae: Himasthinae), intestinal parasite of the South Island pied oystercatcher. NZ J Zool 6:13–20
- Babirat C, Mouritsen KN, Poulin R (2004) Equal partnership: two trematode species, not one, manipulate the burrowing behaviour of the New Zealand cockle, *Austrovenus stutchburyi*. J Helminthol 78:195–199
- Bates, D, Maechler, M (2009) lme4: linear mixed-effects models using Eigen and Eigen++. R package version 0.999375-32
- Burnham KP, Anderson DR (2002) Model selection and multimodel inference. Springer, New York, NY
- Bustnes JO, Galaktionov K (1999) Anthropogenic influences on the infestation of intertidal gastropods by seabird trematode larvae on the southern Barents Sea coast. Mar Biol 133:449–453
- Byers JE, Blakeslee AMH, Linder E, Cooper AB, Maguire TJ (2008) Controls of spatial variation in the prevalence of trematode parasites infecting a marine snail. Ecology 89: 439–451
- Cattadori IM, Haydon DT, Hudson PJ (2005) Parasites and climate synchronize red grouse populations. Nature 433: 737–741
- Chiswell SM (1994) Variability of sea surface temperature around New Zealand from AVHRR images. NZ J Mar Freshw Res 28:179–192
- de Montaudouin X, Lancelot L (2011) Distribution of parasites in their second intermediate host, the cockle *Cerastoderma edule*: community heterogeneity and spatial scale. Mar Ecol Prog Ser 428:187–199
- de Montaudouin X, Jensen KT, Desclaux U, Wegeberg AM, Sajus MC (2005) Effect of intermediate host size (*Cerastoderma edule*) on infectivity of cercariae of *Himasthla quissetensis* (Echinostomatidae: Trematoda). J Mar Biol Assoc UK 85:809–812
- de Montaudouin X, Thielges DW, Gam M, Krakau M and others (2009) Digenean trematode species in the cockle *Cerastoderma edule*: identification key and distribution along the north-eastern Atlantic shoreline. J Mar Biol Assoc UK 89:543–556
- de Montaudouin X, Binias C, Lassalle G (2012) Assessing parasite community structure in cockles *Cerastoderma edule* at various spatio-temporal scales. Estuar Coast Shelf Sci 110:54–60
- Dobbinson SJ, Barker MF, Jillett JB (1989) Experimental shore level transplantation of the New Zealand cockle *Chione stutchburyi*. J Shellfish Res 9:197–212
- Fingerut JT, Zimmer CA, Zimmer RK (2003) Patterns and processes of larval emergence in an estuarine parasite system. Biol Bull 205:110–120
- Fredensborg BL, Mouritsen KN, 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 BL, Mouritsen KN, Poulin R (2006) Relating bird host distribution and spatial heterogeneity in trematode infections in an intertidal snail—from small to large scale. Mar Biol 149:275–283
- Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfeld RS, Samuel MD (2002) Climate warming and disease risks for terrestrial and marine biota. Science 296: 2158–2162
- Hechinger RF, Lafferty KD (2005) Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts. Proc R Soc B 272:1059–1066
- Koprivnikar J, Poulin R (2009) Interspecific and intraspecific variation in cercariae release. J Parasitol 95:14–19
- Kutz SJ, Hoberg EP, Polley L, Jenkins EJ (2005) Global warming is changing the dynamics of Arctic host-parasite systems. Proc R Soc B 272:2571–2576
- Lafferty KD, Allesina S, Arim M, Briggs CJ and others (2008) Parasites in food webs: the ultimate missing links. Ecol Lett 11:533–546
- Lauckner G (1984) Impact of trematode parasitism on the fauna of a North Sea tidal flat. Helgol Meeresunters 37: 185–199
- Leung TLF, Keeney DB, Poulin R (2009a) Cryptic species complexes in manipulative echinostomatid trematodes: when two become six. Parasitology 136:241–252
- Leung TLF, Poulin R, Keeney DB (2009b) Accumulation of diverse parasite genotypes within the bivalve second intermediate host of the digenean *Gymnophallus* sp. Int J Parasitol 39:327–331
- Marcogliese DJ (2001) Implications of climate change for parasitism of animals in the aquatic environment. Can J Zool 79:1331–1352
- Marcogliese DJ (2004) Parasites: small players with crucial roles in the ecological theater. EcoHealth 1:151–164
- Marsden ID, Adkins SC (2010) Current status of cockle bed restoration in New Zealand. Aquacult Int 18:83–97
- Marsden ID, Pilkington RM (1995) Spatial and temporal variations in the condition of *Austrovenus stutchburyi* Finlay, 1927 (Bivalvia: Veneridae) from the Avon-Heathcote estuary, Christchurch. New Zealand Natural Sciences 22:57–67
- Martorelli SR, Poulin R, Mouritsen KN (2006) A new cercaria and metacercaria of *Acanthoparyphium* (Echinostomatidae) found in an intertidal snail *Zeacumantus subcarinatus* (Batillariidae) from New Zealand. Parasitol Int 55: 163–167
- Morley NJ (2011) Thermodynamics of cercarial survival and metabolism in a changing climate. Parasitology 138: 1442–1452
- Mouritsen KN (2002) The *Hydrobia ulvae*-*Maritrema subdolum* association: influence of temperature, salinity, light, water-pressure and secondary host exudates on cercarial emergence and longevity. J Helminthol 76: 341–347
- Mouritsen KN, Jensen KT (1997) Parasite transmission between soft-bottom invertebrates: temperature mediated infection rates and mortality in *Corophium volutator*. Mar Ecol Prog Ser 151:123–134
- Mouritsen KN, Poulin R (2002a) Parasitism, climate oscillations and the structure of natural communities. Oikos 97: 462–468

- Mouritsen KN, Poulin R (2002b) Parasitism, community structure and biodiversity in intertidal ecosystems. *Parasitology* 124:S101–S117
- Mouritsen KN, Poulin R (2005) Parasites boosts biodiversity and changes animal community structure by trait-mediated indirect effects. *Oikos* 108:344–350
- Mouritsen KN, Poulin R (2006) A parasite indirectly impacts both abundance of primary producers and biomass of secondary producers in an intertidal benthic community. *J Mar Biol Assoc UK* 86:221–226
- Mouritsen KN, Poulin R (2010) Parasitism as a determinant of community structure on intertidal flats. *Mar Biol* 157: 201–213
- Mouritsen KN, McKechnie S, Meenken E, Toynbee JL, Poulin R (2003) Spatial heterogeneity in parasite loads in the New Zealand cockle: the importance of host condition and density. *J Mar Biol Assoc UK* 83:307–310
- Pietroock M, Marcogliese DJ (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
- Poulin R, Morand S (2004) Parasite biodiversity. Smithsonian, Washington, DC
- Poulin R, Mouritsen KN (2003) Large-scale determinants of trematode infections in intertidal gastropods. *Mar Ecol Prog Ser* 254:187–198
- Poulin R, Mouritsen KN (2004) Small-scale spatial variation in rates of metacercarial accumulation by a bivalve second intermediate host. *J Mar Biol Assoc UK* 84:1209–1212
- Poulin R, Mouritsen KN (2006) Climate change, parasitism and the structure of intertidal ecosystems. *J Helminthol* 80:183–191
- Poulin R, Steeper MJ, Miller AA (2000) Non-random patterns of host use by the different parasite species exploiting a cockle population. *Parasitology* 121:289–295
- Rohde K (1992) Latitudinal gradients in species diversity: the search for the primary cause. *Oikos* 65:514–527
- Rohde K (2002) Ecology and biogeography of marine parasites. *Adv Mar Biol* 43:1–86
- Rohde K, Heap M (1998) Latitudinal differences in species and community richness and in community structure of metazoan endo- and ectoparasites of marine teleost fish. *Int J Parasitol* 28:461–474
- Schell SC (1970) How to know the trematodes. WMC Brown Company, Dubuque, IA
- Skaug H, Fournier D, Nielsen A, Magnusson A, Bolker B (2012) Generalized linear mixed models using AD model builder. R package version 0.7.2.12
- Smith NF (2001) Spatial heterogeneity in recruitment of larval trematodes to snail intermediate hosts. *Oecologia* 127:115–122
- Studer A, Poulin R (2012) Seasonal dynamics in an intertidal mudflat: the case of a complex trematode life cycle. *Mar Ecol Prog Ser* 455:79–93
- Studer A, Thieltges DW, 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 DW (2007) Habitat and transmission—effect of tidal level and upstream host density on metacercarial load in an intertidal bivalve. *Parasitology* 134:599–605
- Thieltges DW (2008) Effect of host size and temporal exposure on metacercarial infection levels in the intertidal cockle *Cerastoderma edule*. *J Mar Biol Assoc UK* 88: 613–616
- Thieltges DW, Reise K (2007) Spatial heterogeneity in parasite infections at different spatial scales in an intertidal bivalve. *Oecologia* 150:569–581
- Thieltges DW, Rick J (2006) Effect of temperature on emergence, survival and infectivity of cercariae of the marine trematode *Renicola roscovita* (Digenea: Rencolidae). *Dis Aquat Org* 73:63–68
- Thieltges DW, Jensen KT, Poulin R (2008) The role of biotic factors in the transmission of free-living endohelminth stages. *Parasitology* 135:407–426
- Thieltges DW, Ferguson MAD, Jones CS, Krakau M and others (2009a) Distance decay of similarity among parasite communities of three marine invertebrate hosts. *Oecologia* 160:163–173
- Thieltges DW, Ferguson MAD, Jones CS, Noble LR, Poulin R (2009b) Biogeographical patterns of marine larval trematode parasites in two intermediate snail hosts in Europe. *J Biogeogr* 36:1493–1501
- Thieltges DW, Fredensborg BL, Poulin R (2009c) Geographical variation in metacercarial infection levels in marine invertebrate hosts: parasite species character versus local factors. *Mar Biol* 156:983–990
- Thieltges DW, Fredensborg BL, Studer A, Poulin R (2009d) Large-scale patterns in trematode richness and infection levels in marine crustacean hosts. *Mar Ecol Prog Ser* 389: 139–147
- Thomas F, Renaud F, de Meeus T, Poulin R (1998) Manipulation of host behaviour by parasites: ecosystem engineering in the intertidal zone? *Proc R Soc B* 265:1091–1096
- Uddstrom MJ, Oien NA (1999) On the use of high-resolution satellite data to describe the spatial and temporal variability of sea surface temperatures in the New Zealand region. *J Geophys Res* 104:20729–20751, doi:10.1029/1999JC900167
- Ward JR, Lafferty KD (2004) The elusive baseline of marine disease: Are diseases in ocean ecosystems increasing? *PLoS Biol* 2:e120
- Wood CL, Byers JE, Cottingham KL, Altman I, Donahue MJ, Blakeslee AMH (2007) Parasites alter community structure. *Proc Natl Acad Sci USA* 104:9335–9339

Editorial responsibility: Romuald Lipcius,
Gloucester Point, Virginia, USA

Submitted: March 1, 2013; Accepted: July 15, 2013
Proofs received from author(s): September 23, 2013