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Effects of salinity on an intertidal host–parasite system: Is the parasite more sensitive than its host?

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ABSTRACT

Intertidal habitats are characterised by highly fluctuating environmental conditions including varying salinity regimes. Changes in salinity may be gradual or abrupt; for example, heavy rainfall or evaporation during warm periods can either decrease or increase salinity. Trematodes are the most common parasites in intertidal ecosystems and their transmission is known to be highly influenced by environmental conditions. However, effects of salinity on the transmission of intertidal trematodes are not well studied. Here, we investigated the effects of long-term (i.e. several weeks) exposure to different salinities (25, 30, 35 and 40 psu) on the transmission of *Maritrema novaezealandensis* from its first intermediate snail host (*Zeacumantus subcarinatus*) to a second intermediate amphipod host (*Paracalliope novizealandiae*), in order to evaluate overall net effects. The following steps were assessed: output of parasite transmission stages (cercariae) from infected snail hosts, survival and infectivity of cercariae, susceptibility of amphipod hosts to infection and survival of amphipod hosts including parasite development within amphipod hosts. Output and survival of cercariae increased with increasing salinity whilst infectivity of cercariae and susceptibility of amphipods to infection were not clearly affected. Survival of amphipods was significantly longer at lower salinities and parasite development in infected amphipods was concomitantly more advanced. Overall, the results suggest that the parasite and the amphipods are differentially affected, and that under normal to increased salinities conditions are more favourable for the parasite than for the amphipod host.

1. Introduction

In light of the large-scale environmental changes that are occurring and predicted to occur, understanding the effects of environmental factors on marine species and species interactions, such as those between hosts and parasites, seem not only crucial but urgent. Climate change is expected to have negative consequences particularly for the biota of intertidal and shallow marine areas (e.g. Brierley and Kingsford, 2009; Harley et al., 2006). These ecosystems are naturally subject to extreme environmental fluctuations, including changes in salinity, and therefore impose substantial physiological challenges for many of their inhabitants (e.g. Przeslawski et al., 2005). As an integral part of intertidal ecosystems, trematodes are not only the most common parasite group (e.g. Lauckner, 1984; Mouritsen and Poulin, 2002; Sousa, 1991), but are also of high ecological importance (e.g. Kuris et al., 2008; Mouritsen and Poulin, 2005, 2010). Despite the fact that parasite transmission is known to be strongly influenced by environmental conditions (e.g. Pietrock and Marcogliese, 2003) little is known about the effects of salinity on the transmission of intertidal trematodes and hence the potential effects on their host organisms.

Salinity is considered one of the most important environmental factors in marine ecosystems, influencing small and large-scale biotic interactions (Berger and Kharazova, 1997; Ingole and Parulekar, 1998). Whilst most marine systems have on average a relatively stable salinity of approximately 35 practical salinity units (psu), salinity in intertidal zones and especially estuaries can fluctuate gradually (e.g. seasonally) as well as rapidly. Tides, rainfall, freshwater inflow or runoff can cause relatively abrupt decreases in salinity, whereas evaporation in tide pools can raise salinities well above normal levels (e.g. Adam, 1990; Brierley and Kingsford, 2009; Wheatly, 1988). Salinity can affect the distribution (Crain et al., 2004; Kneib, 1984), physiology (Hylleberg, 1975; Pequeux, 1995; Shock et al., 2009) as well as the reproduction (Deschaseaux et al., 2010) of intertidal species, which also possess tolerance mechanisms to cope with changing osmotic conditions (for crustaceans, see Pequeux, 1995).

Salinity has also been recognised as an important environmental factor for parasitism and disease dynamics in estuarine or brackish environments (e.g. Haskin and Ford, 1982; Kesting et al., 1996; Koie, 1999; Messick et al., 1999; Reisser and Forward, 1991; Zander, 1998). However, little is known about the effects of salinity on intertidal trematodes, especially with regards to long-term effects (but see

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Lei and Poulin, 2011). Trematode parasites usually have complex life cycles involving several members of a community and transmission processes involve stages of the parasite being directly exposed to environmental conditions. For instance, transmission between first and second intermediate hosts is typically via a free-living, short-lived (<24 h) transmission stage (cercaria), which is asexually produced within gastropod first intermediate hosts. Like most larval endohelminths, cercariae are strongly affected by environmental factors (e.g. Pietrock and Marcogliese, 2003).

The few studies available on the effects of salinity on marine trematodes are focussed on the emergence and/or the survival of these cercariae as short-term responses and these studies have produced inconsistent results. Whilst some (Lei and Poulin, 2011; Rees, 1948; Sindermann, 1960; Sindermann and Farrin, 1962) reported a general increase in emergence of cercariae from first intermediate hosts with increasing salinity, Mouritsen (2002) found a greater cercarial emergence at higher salinity only at elevated temperatures. In a study by Koprivnikar and Poulin (2009) on two intertidal trematode species (*Maritrema novaezealandensis*, also used in the present study, and *Philophthalmus* sp.) using the same first intermediate snail host (i.e. *Zeacumantus subcarinatus*; same host as used for this study; see below), cercarial emergence was reported to increase with decreasing salinity. With regards to the survival of cercariae, several studies have reported that survival was generally not affected over a range of salinities (Mouritsen, 2002; Prokofiev, 1999; Rees, 1948; Stunkard and Shaw, 1931), but responses can be species-specific (Koprivnikar et al., 2010) (see also Lei and Poulin).

However, studying just one out of the many steps involved in the transmission process in short-term experiments can provide only limited information about the overall transmission success of a parasite species under certain conditions. To achieve a more comprehensive understanding, several steps, including those relating to the hosts, need to be considered. Here, we used the intertidal microphallid trematode *Maritrema novaezealandensis* Martorelli et al. (2004), a common and, due to its negative effects on intermediate hosts, important parasite in soft-sediment intertidal areas in New Zealand. The adult worms have been described from red-billed gulls, *Chroicocephalus scopulinus* (Fredensborg et al., 2004b; Martorelli et al., 2004), but probably occur in a range of other definitive bird hosts visiting intertidal mudflats. Eggs produced by adult worms are expelled with the bird's faeces and are ingested accidentally by first intermediate snail hosts, *Zeacumantus subcarinatus* Sowerby 1855. Within the snails, the parasite multiplies asexually, producing large numbers of the parasite's free-living cercarial transmission stage. These cercariae (mean body length including tail approx. 170 μm; Martorelli et al., 2004) emerge mostly at low tide when water in tide pools warms up (e.g. Fredensborg et al., 2004a) to infect (i.e. penetrate the cuticle or enter via joints or gills) and subsequently encyst within second intermediate hosts, consisting of a wide range of crustaceans (Koehler and Poulin, 2010), including the amphipod *Paracalliope novizealandiae* Dana 1853 used in this study. Definitive bird hosts acquire infections, thereby completing the life cycle, when feeding on crustaceans harbouring fully developed, mature metacercariae.

The main objective of this study was to assess the effects of salinity on the different steps of the transmission process of the intertidal trematode *M. novaezealandensis* from its first intermediate snail host to its second intermediate amphipod host, in order to evaluate the overall net effects of salinity on this host–parasite system. We investigated: (1) cercarial production and emergence from infected first intermediate snail hosts, (2) cercarial survival, (3) cercarial infectivity, (4) susceptibility of second intermediate amphipod hosts to infection, (5) survival of infected and uninfected amphipods, and (6) development of the parasite within the amphipods (see Studer et al., 2010 for a conceptual figure). The completion of a trematode life cycle is a multi-step process which may be differentially affected by environmental factors at various steps of the transmission process. Environmental effects on the parasite, on its hosts, as well as on their interactions can occur at any step. Therefore, the goal of the present study was to include the entire transmission process from first to second intermediate host, as well as long-term responses to salinity, in order to develop a better understanding of this environmental component on trematode transmission and hence affected host organisms in intertidal ecosystems.

2. Materials and methods

2.1. General remarks

Live material was collected from the Otago Harbour, South Island, New Zealand. Parasites and hosts were obtained and kept as described in Studer et al. (2010) (infected snails were collected from Lower Portobello Bay (45°50′S, 170°40′E) in July 2009; uninfected amphipods (all >2.25 mm in body length) from Hooper's Inlet (45°51′S, 170°40′E), Otago Peninsula, a few days before the start of an experiment). On the local mudflats of the harbour there are marked salinity gradients resulting from freshwater inputs (range 0 to ~34 psu). During heavy rainfall at low tide, salinity decreases due to direct mixing with seawater as well as increased run-off, whereas during warm, sunny days, salinity slightly increases due to evaporation (max. ~36 psu; A. Studer, pers. observation). The salinity levels used in the experiments were 25, 30, 35 and 40 psu (salinities ±1 psu; 20 °C). These were chosen to cover a wide range of naturally occurring salinities, including one level (40 psu) beyond what is currently experienced on local mudflats but towards which conditions change during exceptionally warm periods such as heat waves in summer, conditions predicted to occur more frequently with on-going global climate change (IPCC, 2007). For each salinity level a solution was prepared using artificial sea salt (Red Sea salt®). Solutions were stored at 20 °C in 20 l containers and kept aerated.

In all experiments (except the cercarial output time series; see details later), the cercariae used were pooled from 25 or 40 snails (either snails randomly selected from stock aquaria kept in natural seawater or from infected snails acclimatised to different salinities for several weeks, depending on the experiment; see later). To obtain cercariae, snails were incubated in five or eight replicate Petri dishes (depending on the number of snails used) containing 7 ml of aerated water of the given salinity or natural seawater, respectively, for 1 h at 25 °C under constant illumination. After removal of the snails, water from the different dishes containing the emerged cercariae was pooled to ensure a genetic mixture of parasites was used. To assess the number of cercariae added per volume of each mixture, cercariae in 10 aliquots were counted. For the cercarial survival and all experimental infections of amphipods, 96-well plates (wells 7×10 mm; total volume 320 μl) were used. All amphipods used in the experiments were grouped into size classes (2.5, 3.0, 3.5, 4.0, 4.5±0.25 and ≥4.75 mm) and sexed prior to their dissection under a dissecting microscope. Statistical analyses will be discussed in each of the following subsections. We checked for differences between replicates and assumptions of parametric tests, and data were transformed or other tests used where necessary.

2.2. Output of *M. novaezealandensis* cercariae from first intermediate snail hosts

Long-term cercarial output was assessed by counting the number of cercariae emerging from individual *Z. subcarinatus* snails (marked with plastic tags; The Bee Works) during weekly incubations over six weeks. At the beginning of the experiment, 112 infected snails were incubated at 25 °C for 24 h under constant illumination in order to induce emergence of fully developed cercariae. Snails were then distributed to two replicate aquaria at each of 25, 30, 35 and 40 psu (at 20 °C). After one week of acclimatisation, half of the snails were incubated for 6 h at 25 °C under constant illumination at the

same salinity, whilst the other half were incubated at one salinity level higher (25 at 30, 30 at 35, 35 at 40 psu), to simulate a salinity increase that would be experienced in tide pools at low tide during hot sunny days due to evaporation. Snails kept at 40 psu were incubated only at 40 psu (n = 16 for each treatment; seven treatments in total). For the incubations, snails were individually placed in 1.5 ml Eppendorf tubes filled with 1 ml of water of the respective salinity. Emerged cercariae were preserved and counted under a dissecting microscope (see details in Studer et al., 2010). After testing with a General Linear Model (GLM) for the effect of acclimatisation salinity and the salinity increase during incubation in the 25, 30 and 35 psu treatments only, the latter factor was omitted from a subsequent analysis with a GLM assessing the effect of the acclimatisation salinity only on the average number of cercariae per snail per week (square root transformed) including all salinity levels. Due to snail mortality, only snails that survived the entire duration of the experiment were included in the analyses.

2.3. Survival of cercariae

Functional activity of cercariae at different salinities was compared between cercariae from infected snails that were acclimatised to these salinities for approx. four weeks prior to the experiment. A cercarial mixture was obtained for the different salinities using 25 snails per salinity. Approximately 25 cercariae were then transferred into individual wells of 96-well plates and incubated under constant illumination at 25 °C (12 wells in two replicate well plates per salinity; volume of water in wells was standardised to 150 µl). Activity of the cercariae was checked at 3, 5, 7, 9, 12 and 14 h post-emergence. Cercariae were classified by visual assessment as fully active, sluggishly motile or immotile/dead. The data were analysed using a repeated measures ANOVA to determine the effect of salinity on the proportions of fully active cercariae (category with the most ecological relevance in terms of infectivity) 3, 5, 7, and 9 h post-emergence (arcsine-square root transformed). Due to the assumption of sphericity being violated (Mauchley's test), multivariate results for within-subjects are reported.

2.4. Infectivity of cercariae

Infectivity was assessed by comparing the success of cercariae from snails acclimatised to different salinities for approx. six weeks at infecting second intermediate amphipod hosts (*Paracalliope novizealandiae*). For these experimental infections, uninfected amphipods (n = 48 per salinity) were first transferred into plastic containers with water of the respective salinity (approx. 300 ml of the 25, 30, 35 or 40 psu solution), and then individually put in wells of two replicate well plates filled with 75 µl of water of the respective salinity (i.e. 25, 30, 35 or 40 psu). A cercarial mixture was obtained from snails kept at the different salinities which was added to each well (at 25 psu, 50 µl, at 30, 35 and 40 psu, 75 µl were added corresponding to approx. 20 cercariae; all added volumes standardised to 75 µl). Amphipods and cercariae were then incubated for 2 h at 25 °C under constant illumination, ensuring optimal conditions for infections. Subsequently, amphipods were placed in two replicate plastic containers (approx. 300 ml natural aerated seawater) per treatment and left for 2 d at 16 °C until dissected. The number of parasites infecting the amphipods was assessed. Effects of salinity, sex and size of amphipods on the proportion of parasites successfully infecting amphipods was analysed with a Generalised Linear Model (GLM) fitted with a quasi-binomial error structure.

2.5. Susceptibility of amphipods to infection

Susceptibility of amphipod hosts to infection was investigated by exposing amphipods to different salinities (25, 30, 35 and 40 psu) for 24 h before adding untreated cercariae, i.e. cercariae derived from stock snails kept in natural seawater, and then comparing the infection success of these cercariae. After the 24 h exposure of amphipods to different salinities in plastic containers filled with 300 ml, amphipods (n = 37 per treatment in two replicate groups per salinity) were transferred into wells containing 75 µl of water of the respective salinity. From a cercarial mixture (generated from 40 snails kept in stock aquaria), 25 µl was added to each amphipod, which corresponded to an addition of approx. 20 cercariae per amphipod. Well plates were then incubated under constant illumination for 2 h at 25 °C and were subsequently processed and the data analysed as described for the infectivity experiment.

2.6. Survival of infected and uninfected amphipods and development of parasites within amphipod hosts

Infected and uninfected amphipods were exposed to different salinities (25, 30, 35 and 40 psu) and their survival under these conditions was monitored. For this, uninfected amphipods were placed individually in wells filled with 75 µl natural aerated seawater (n = 90 amphipods in two replicate well plates per salinity). A cercarial mixture was prepared using 40 snails kept in stock aquaria. Of this mixture, 75 µl was added to half of the amphipods (corresponding to an addition of approx. 15 cercariae per amphipod) whilst the same volume of pure seawater was added to the controls. Well plates were incubated for 4 h at 20 °C under constant illumination. Amphipods were then transferred into plastic containers filled with aerated seawater (approx. 300 ml) and stored overnight at 16 °C to allow the cercariae to fully penetrate the hosts whilst ensuring optimal survival of amphipods. The following day, amphipods were distributed to two replicate aquaria per salinity (volume 6.5 l, half filled; aerated; at 19 ± 1 °C). Survival of the amphipods was subsequently checked 2–3 times a day for 30 d. Dead amphipods were recovered and dissected to assess their infection status, infection intensity (number of metacercariae per amphipod) and the developmental stage of the parasites (early immature metacercariae, late immature metacercariae, early cyst, mature cyst, according to Keeney et al., 2007). After 30 d, all remaining amphipods were dissected. The risk of dying during the experiment was analysed with a Cox proportional hazard regression model with salinity, sex, size and infection status of amphipods as predictor variables. A Spearman's Rank correlation was used to assess the effect of infection intensity on the survival time of infected amphipods in the experiment.

3. Results

3.1. Cercarial output from snails

Salinity had a significant effect on the average number of cercariae that emerged from infected snails (square root transformed; GLM, $F_{3, 38} = 7.99$, $p < 0.001$), with output of cercariae increasing with increasing salinity (Fig. 1). The simulated short-term salinity increase that half of the snails were exposed to in the 25, 30 and 35 psu treatments did not have a significant effect on cercarial output (square root transformed; GLM, $F_{1, 30} = 2.36$, $p = 0.135$). The overall average output per snail and per incubation ranged from 15.9 ± 4.6 at 25 psu to 111.6 ± 24.6 at 40 psu (mean ± standard error, as for all subsequent results) with similar levels at 30 and 35 psu (Fig. 1). Shell length of snails used was not significantly different between treatments (average shell length 12.8 ± 0.10 mm; ANOVA, $F_{6, 81} = 0.43$, $p = 0.86$).

3.2. Cercarial survival

The salinity at which the cercariae were incubated, time and their interaction all had a significant effect on the proportion of fully active cercariae (arcsine-square root transformed, Table 1). Full activity of

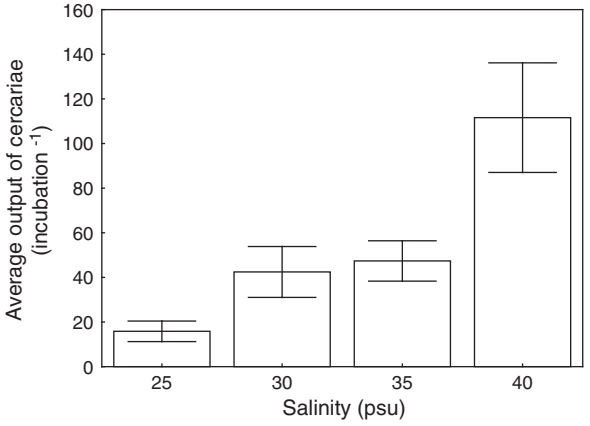

Fig. 1. Effect of salinity (25, 30, 35 and 40 psu) on the mean (±SE) output of cercarial transmission stages of *Maritrema novaezealandensis* from infected *Zeacumantus subcarinatus* snails per incubation (6 weekly incubations; n = 27, 22, 25 and 14 at 25, 30, 35 and 40 psu).

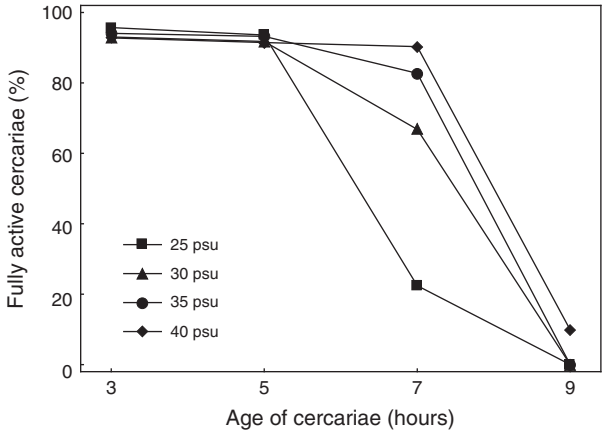

Fig. 2. Functional activity of the cercariae of *Maritrema novaezealandensis* at different salinities (25, 30, 35 and 40 psu) shown as the percentage of fully active cercariae at an average age of 3, 5, 7 and 9 h.

cercariae ceased within 9 h at all salinities except at 40 psu (incubation temperature: 25 °C). Overall, the lower the salinity, the faster full activity decreased (Fig. 2).

3.3. Infectivity of cercariae

Salinity had a significant effect on the proportion of parasites successfully infecting amphipod hosts (GLM, quasi-binomial; $F_{3, 184} = 3.47$, $p = 0.017$). The proportion of successful parasites was highest at 25 psu, but was due to the presence of outliers in one replicate (0.34 ± 0.06). The proportion in the other replicate (0.10 ± 0.02) was similar to those in all other treatments (0.14 ± 0.02 at 30 psu, 0.13 ± 0.02 at 35 psu and 0.13 ± 0.02 at 40 psu). When the analysis was repeated after the exclusion of three outliers with unusually high infection levels, there was no significant effect of salinity on the proportion of cercariae successfully infecting amphipods ($F_{3, 181} = 0.93$, $p = 0.426$). Overall, 81% of the amphipods were infected with a mean infection intensity of 2.8 ± 0.2 parasites per amphipod. Sex of amphipods did not significantly affect the proportion of successful parasites ($F_{1, 184} = 0.33$, $p = 0.564$), whereas size of amphipods did with smaller size classes being infected by a larger proportion than larger size classes ($F_{3, 184} = 3.02$, $p = 0.031$) (0.17 ± 0.03 (n = 53), 0.18 ± 0.02 (n = 84), 0.01 ± 0.01 (n = 41) and 0.06 ± 0.01 (n = 14) parasites per amphipod for the 3.0, 3.5, 4.0, 4.5 ± 0.25 mm size classes).

3.4. Susceptibility of amphipods

Salinity did not significantly affect the proportion of parasites that successfully infected amphipods that had previously been exposed to different salinities (GLM, quasi-binomial; $F_{3, 139} = 0.69$, $p = 0.562$). Overall, 92% (25 psu), 95% (30 psu), 78% (35 psu) and 87% (40 psu) of the amphipods got infected (mean infection intensity across all amphipods 2.6 ± 0.2; n = 148). Sex of amphipods did not significantly influence the proportion of successful parasites ($F_{1, 139} = 0.39$, $p = 0.534$), whereas size did ($F_{4, 139} = 2.54$, $p = 0.043$). The smallest amphipods (2.5 ± 0.25 mm) had the highest proportion (0.19 ± 0.05; n = 9) and the largest had the lowest (0.05 ± 0.02, n = 5), whilst intermediate size classes were infected by very similar proportions of successful parasites (0.13 ± 0.01 (n = 44), 0.13 ± 0.01 (n = 65), 0.11 ± 0.02 (n = 25), respectively for the 3.0, 3.5, and 4.0 ± 0.25 mm size classes).

3.5. Survival of infected and uninfected amphipods

The risk of amphipods dying was significantly affected by salinity, with a higher risk at 35 and 40 psu (model: $\chi^2 = 59.88$, df = 4, $p < 0.001$; Table 2). Mean survival times in the experiment ranged from 10.7 ± 0.8 d at 25 psu (n = 79) and 10.8 ± 0.7 d at 30 psu (n = 77), to 8.5 ± 0.5 d at 35 psu (n = 81) and 9.4 ± 0.5 d at 40 psu (n = 84). Whilst size of amphipods also significantly affected the risk of dying with larger ones having a higher proportional risk than smaller ones, sex and infection status of amphipods did not (Table 2). There was a weak positive correlation between the intensity of infection and survival time of infected amphipods (Spearman's $\rho = 0.15$, $p = 0.01$), indicating that more heavily infected amphipods survived slightly longer than lightly infected ones. In total, 345 amphipods were dissected (180 females, 158 males, 7 unsexed) of which 44% were infected with a mean infection intensity of 5.9 ± 0.4 parasites per infected amphipod.

3.6. Parasite development

The salinity at which the amphipods were kept affected the amphipod's survival and concomitantly the parasite's development. At 25 and 30 psu, amphipod survival was longer and parasite development

Table 1
Effects of salinity (25, 30, 35 and 40 psu) on the functional activity of cercariae of *Maritrema novaezealandensis* (proportion of fully active cercariae; arcsine-square root transformed; n = 12 per salinity). Results from a repeated measures ANOVA (with multivariate within-subjects results).

Factor	df	MS	F	p
Between subjects				
Salinity	3	0.48	11.74	<0.001
Error	44	0.04		
Within subjects				
Time	3	17.43	1120.86	<0.001
Time × salinity	9	0.42	16.66	<0.001
Error	132	0.01		

Table 2
Effects of salinity (25, 30, 35 and 40 psu), sex, size and infection status on the mortality risk of *Paracalliope novizealandiae* amphipod hosts. Results from a Cox proportional hazard model.

Factor	Cox's parameter	SE	P
Salinity	0.02	0.01	0.037
Amphipod sex	−0.13	0.15	0.391
Amphipod size	0.53	0.09	<0.001
Infection status	−0.01	0.11	0.939

progressed more. A substantial number of parasites were recovered as mature cysts at 30 psu (23%, n = 230) compared to 0% at 25 and 40 psu (n = 181 and 214, respectively) and 1% at 35 psu (n = 218). At 25 psu, 22% of the parasites at least reached the early cyst stage, whereas at 35 and 40 psu, over 90% of the metacercariae recovered were still immature. Overall, 863 metacercariae were counted (47% early immature metacercariae, 39% late immature, 8% early cysts and 7% mature cysts).

4. Discussion

Salinity has been shown to be an influential environmental factor for parasitism and diseases in brackish and estuarine ecosystems (e.g. Haskin and Ford, 1982; Thieltges et al., 2010; Zander, 1998). Salinity tolerance of parasites and their hosts can vary greatly and can therefore influence the interaction between them. For example, the distribution of marine endoparasites can be limited by the salinity tolerance of hosts as certain marine parasites have been suggested to have a higher tolerance than their hosts (Möller, 1978). However, it has also been reported that the salinity tolerance of parasite larvae can constrain parasite incidence in hosts that tolerate a greater range of salinities than their parasites (e.g. Haskin and Ford, 1982; Reisser and Forward, 1991). In our study, salinity also differentially affected the parasite *M. novaezealandensis* and its second intermediate amphipod host. Whilst output of cercariae from snail hosts and their survival was highest at normal to increased salinities, survival of amphipod hosts was most prolonged at lower salinities.

4.1. Effects on the parasite

Output of cercariae from infected snails was significantly affected by salinity, with increasing numbers of cercariae emerging with increasing salinity. This finding is in accordance with most previous studies and also indicates a roughly two-fold increase in cercarial emergence with an increase of about 10 psu (Mouritsen, 2002; Rees, 1948; Sindermann, 1960; Sindermann and Farrin, 1962). The main transmission window of *M. novaezealandensis* is thought to be during low tides on warm sunny days when the water in tide pools warms up (Fredensborg et al., 2004a; Koprivnikar and Poulin, 2009; Studer et al., 2010), conditions implying normal to elevated salinity levels due to evaporation. Although the effect of the short-term salinity increase in our experiments (incubation salinity equal or greater than acclimatisation salinity) was not significant, the long-term response to the different salinities strongly indicated that cercarial production and emergence are highest at normal to increased salinities. As for temperature, this can be interpreted as an optimal transmission strategy, whereby the production and emergence of cercariae is highest under conditions which maximise the chance of successful transmission to the next host is (Mouritsen, 2002).

However, our finding differs from the results described by Koprivnikar and Poulin (2009) for the same parasite species (*M. novaezealandensis*) and snail host (*Z. subcarinatus*). One possible explanation for this discrepancy is that short-term responses to salinity are different from long-term responses (snails exposed to different salinity levels for days in Koprivnikar and Poulin (2009), and for weeks in the present study). In a preliminary experiment assessing cercarial emergence of *M. novaezaealandensis* at different salinities without previous acclimatisation, salinity also had a significant effect on cercarial emergence but, in accordance with Koprivnikar and Poulin (2009), the lowest number emerged at the highest salinity (A. Studer, unpubl. data). This clearly emphasises the need to consider and distinguish between short and long-term experimental responses. Long-term exposure to different salinities may lead to changes in snail physiology in turn affecting the response of the parasite once the host has acclimatised to new conditions, which may take a few days (Berger and Kharazova, 1997).

Survival of cercariae also increased with increasing salinity, suggesting that conditions considered to be optimal for the transmission of this parasite species are not only promoting larger numbers of cercariae being released into the environment, but also prolonging their survival. This would further increase the chance of successful transmission. Shorter survival at low salinities might be an issue during heavy rainfall at low tide during summer, or for infected snails located close to a freshwater inflow, and may be indicative of low salinity being a barrier for the distribution of this trematode. This finding contrasts with studies which did not report an effect of salinity on the longevity of *Maritrema subdolum* and other marine cercariae (Mouritsen, 2002; Prokofiev, 1999; Rees, 1948; Stunkard and Shaw, 1931) (but see Koprivnikar et al., 2010; Lei and Poulin, 2011).

Considering the main transmission window for *M. novaezealandensis*, an increase in infectivity with increasing salinity was expected. Excluding the unusual values from one replicate at 25 psu, our results suggested that long-term acclimatisation of infected snails to different salinities did not influence the functionality of the cercariae they release. Infectivity of cercariae has been shown to increase with increasing cercarial density up to a threshold before dropping off (Evans and Gordon, 1983). Based on the results described above for cercarial production and output, this should have translated into increasing infectivity with increasing salinity and it remains unclear why this was not observed.

4.2. Effects on the host

Effects of salinity on amphipod hosts differed from the effects described for the parasite. Susceptibility of *P. novizealandiae* amphipods to infections was not significantly affected, presumably due to adaptive mechanisms allowing adjusting to short-term changes in osmotic conditions in a way not affecting the susceptibility to infections or alternatively, due to methodological reasons which did not allow amphipods to exploit their full behavioural repertoire to potentially avoid infections under certain conditions. On the other hand, the amphipod's survival was affected by salinity. In contrast to patterns observed for the parasite, amphipods survived longer at lower than at normal to increased salinities. This may be consistent to some extent with studies on adult littoral amphipods which have shown that these organisms tolerate a relatively wide range of salinities (e.g. Dorgelo, 1974, 1976; McLusky, 1971), and the fact that crustaceans living in habitats of changing salinities possess tolerance mechanisms to cope with varying osmotic conditions (Pequeux, 1995). This result may also suggest that these amphipods are also capable of living in more estuarine ecosystems. The differential responses of amphipods in terms of susceptibility and survival may be due to short versus long term effects, with possible mechanisms remaining to be further investigated.

However, the longer survival of amphipods at lower salinities also allowed parasite development to progress further. The difference in the percentages of mature cysts at low salinities clearly illustrated the importance of indirect effects of environmental factors. Such an effect was also observed for temperature (Studer et al., 2010). The presence of mature cysts in the ecosystem is crucial for transmission, as only mature cysts can successfully establish in a definitive bird host. In addition, the infectivity and susceptibility experiments also indicated that smaller amphipods were getting more infected than larger ones. Although such an effect of amphipod size has not been found in any previous study (Fredensborg et al., 2004a; Studer et al., 2010), this may indicate that differences in osmotic regulatory mechanisms exist in amphipods of different size classes, resulting in a lower resistance of smaller individuals to infections.

4.3. Net effects

Assuming no other major ecological factor influences the inferences made and acknowledging the complex issue of simultaneous

long-term versus short-term effects, the net effects of salinity on the transmission process of *M. novaezealandiae* from first to second intermediate host based on our findings can be summarised as follows. At low salinities (25 and 30 psu) relatively few cercariae emerge from infected snails and their survival is relatively short. Whilst susceptibility of amphipods to infection is equal across all salinities, the survival of the amphipods is comparatively long at low salinities and the development of the parasite within infected amphipods thus positively influenced. Such conditions would be encountered during periods of rain or in close proximity to freshwater inflows and would benefit the amphipod hosts, but less so the parasite. Incorporating predicted environmental changes due to climate change (IPCC, 2007), altered rainfall patterns (e.g. increased regional rainfall) as well as a long-term, large scale freshening of the oceans due to melting of ice, may in some areas negatively influence the transmission of *M. novaezealandensis* and consequently reduce the levels of parasite prevalence and infection intensity.

In contrast, at normal to elevated salinities (35 and 40 psu) which would be encountered during periods considered optimal for transmission, more cercariae are being produced and emerge from snail hosts and their survival is better compared to low salinities. Higher transmission to amphipod hosts under these conditions is likely. The survival of amphipods is also directly negatively affected and the increased transmission potential of the parasite may further elevate their risk of mortality. During more frequent and/or more extreme heat waves or in areas that are predicted to become more arid (IPCC, 2007), evaporation may become more pronounced in tide pools and high salinity levels and high temperatures may occur over longer low tide series. These conditions would be favourable for parasite transmission but not for the amphipods.

However, the differentiation between long-term and short-term effects on the various steps of the transmission process as well as on the overall net effects clearly require more in depth consideration. This is especially true in the context of consequences of climate change, where long-term as well as short-term changes may have important implications on the local level, where both processes occur simultaneously and may affect the hosts, the parasite as well as their interactions. Furthermore, it also needs to be stressed that fluctuations in intertidal habitats are highly complex as multiple environmental factors vary simultaneously on various spatial and temporal scales. For all results described (from experiments conducted at constant temperatures and salinities) and all inferences made, actual effects under natural conditions may be different. For example, interactive effects of salinity and temperature in particular are highly likely, and temperature may further enhance or reduce any effects of salinity on the parasite and/or the host.

In conclusion, salinity had differential effects on the various steps of the transmission process of *M. novaezealandensis*. Overall, high salinity levels benefited the transmission of the parasite in terms of output and survival of cercariae, whereas low salinities mostly benefited the amphipods but also had an indirect positive effect on parasite development within amphipod hosts. However, during the main transmission window of *M. novaezealandensis*, thermal and osmotic conditions seem optimal for the parasite but may entail direct and indirect negative effects on amphipods. Hence, not only temperature, but also salinity should be considered a potential key regulator of the transmission dynamics of *M. novaezealandensis* under natural conditions.

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