Journal of Experimental Marine Biology and Ecology 412 (2012) 110–116

Contents lists available at SciVerse ScienceDirect

Journal of Experimental Marine Biology and Ecology

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

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

A. Studer *, R. Poulin

Department of Zoology, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand

ARTICLE INFO

Article history:
Received 4 August 2011
Received in revised form 7 November 2011
Accepted 12 November 2011
Available online 6 December 2011

Keywords:
Amphipoda
Host–parasite interaction
Parasitism
Salinity
Transmission
Trematoda

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

* Corresponding author. Tel.: +64 3 479 7986; fax: +64 3 479 7584.
E-mail addresses: studeranja@gmail.com (A. Studer), Robert.Poulin@otago.ac.nz (R. Poulin).

0022-0981/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jembe.2011.11.008

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