Light effect on the trematode *Himasthla elongata* – From cercarial behaviour to infection success

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ABSTRACT

Cerastoderma edule (cockle), a socio-economically important bivalve of the northeast Atlantic, is host to several trematodes, including *Himasthla elongata*. In the complex life cycle of this trematode, cercariae (free-living stages) emerge from the first intermediate host, a snail, to infect cockles as second intermediate host. During their short lifespan (i.e.less than one day), cercariae have to ensure a successful host-to-host transmission using the surrounding water as transference medium and therefore, they are exposed and impacted by different environmental conditions, including abiotic factors. Being light:dark cycle one of the major drivers of aquatic life behaviour, this study aimed to determine light influence on cercaria and their second host behaviour based on two hypotheses. By having a benthic second intermediate host, cercariae will display a photonegative orientation and, on the other hand, the host behaviour will not be influenced by light conditions. Results showed cercariae display a photopositive orientation (first hypothesis rejected), displaying movements towards light. The second host behaviour (evaluated by oxygen consumption) was similar among conditions, i.e. dark vs. light (hypothesis accepted), but acquired more parasites when experimentally infested in the dark treatment. This host light-dependent infection can be explained by a change on cercarial behaviour when exposed to a light stimulus that increased their infection success. This study highlights trematode responses to external conditions may be focused on the life cycle successful completion rather than altered by the host habitat. It was emphasized the light influence on cercarial behaviour resulting in an increased infection success that may be decisive on trematodes population dynamics and distributional range.

KEYWORDS: *Cerastoderma edule*; Parasitism; photosensitivity; dark: light cycle; oxygen consumption;

1. INTRODUCTION

Trematodes are prevalent macroparasites in coastal waters (Lauckner 1983) that display a heteroxenous life cycle (infecting up to three hosts) with alternation between parasitic and free-living stages (Bartoli & Gibson 2007). Cercariae, one of these free-living stages, develop by asexual multiplication inside the first intermediate host, usually a mollusc, from which they are emitted and display a short lifespan (< 48 hours) to infect the following host (de Montaudouin et al. 2016). These larvae form an essential component of the ecosystem, representing a high fraction of marine biomass (Lambden & Johnson 2013) and production (Thieltges et al. 2008). Cercariae can exert a strong influence on the structure, dynamics and function of food webs, altering their topology or lineage density (Dunne et al. 2013). Cercarial activity is strongly influenced by environmental abiotic conditions, among which light can be determinant. While studying cercariae from different trematode species, authors have been demonstrating their photosensitivity, either moving towards or away from light. Cercariae that are photosensitive, have developed photoreceptors (or proteins with analogous functions) to facilitate light perception (Haas 1992). The development of such responses might be related to the trematode life cycle and the habit of the hosts involved. Indeed, when the trematode downstream host is a benthic organism, cercariae usually remain near the sediment (positive geotaxis) and usually respond negatively to a light stimulus, moving away from it (Combes et al. 1994). Additionally, cercarial behaviour can display a synchronized activity with the daily active period of its host (Combes et al. 1994, Esch et al. 2001, Combes et al. 2002). Nonetheless, there are several behavioural patterns that can be combined to influence the cercarial performance (Morley 2020). For example, there are other external factors, such as the force of gravity, that can act together with light on defining cercarial swimming preferences (Kennedy 1979, Combes et al. 1994).

Cercariae of trematode species presenting three-host life cycles transform into metacercariae after penetrating the second intermediate host. The pathological effects of this trematode parasitic stage on the host depend on the number of accumulated cysts (Desclaux et al. 2004) as well on environmental abiotic factors such as pollutants (de Montaudouin et al. 2010, Paul-Pont et al. 2010). This complex interaction between host-metacercariae dynamics and environmental conditions have already been reported to cause host mortality (Desclaux et al. 2004, Thieltges 2006).

The knowledge about behavioural responses of trematode cercariae towards abiotic conditions (such as light) is still scarce, especially in what concerns the consequences of such responses on infection success and subsequent modulation of host population dynamics. In the present study, the trematode species *Himasthla elongata* and its second intermediate host, the bivalve *Cerastoderma edule* (edible cockle) were used as host-parasite model. This trematode uses the common periwinkle, *Littorina littorea*, an intertidal gastropod of the medio- and supralittoral zone (Eschweiler et al. 2009), as first intermediate host, and infects the second intermediate host, the infaunal intertidal bivalve *Cerastoderma edule,* by inhalation (Wegeberg et al. 1999). The present study aimed to experimentally assess the influence of light (dark vs. light exposure) on the cercarial behaviour, cercariae infection success and cockle susceptibility to infection. The hypotheses were: (1) due to the second intermediate host endobenthic habit, cercariae of *H. elongata* will display a photonegative orientation response; (2) cockles filtering activity will not be influenced by light and (3) cercarial performance and infection success will be influenced by light.

2. MATERIAL AND METHODS

2.1. Organisms collection and maintenance

Littorina littorea snails were previously collected in Texel, The Netherlands, and exposed to a temperature boost to incite cercariae emergence. Afterwards, the trematode metacercariae was identified to species level through morphological analysis by infecting cockles and following de Montaudouin et al. (2009). Infected snails were kept in laboratory in an aquarium with artificial seawater (salinity 35), at a temperature of 14 ± 1 °C and with a natural photoperiod (12:12 hours light/dark). During the maintenance period, snails were fed with *Ulva* sp. *ad libitum*. To obtain cercariae, infected snails were transferred to individual containers with artificial seawater (salinity 35) and exposed to a temperature boost (approximately 24 °C) and constant illumination (approximately 6 hours). Cercariae were then counted under a stereomicroscope and collected with a micropipette.

Individuals of *C. edule*, ranging from 13 to 17 mm shell length, were collected in the Mira channel of the Ria de Aveiro coastal lagoon, Portugal. Cockles were transported to the laboratory and acclimated for one week at controlled conditions of salinity (30), temperature (18 °C) and photoperiod (12:12 hours light/dark) and were fed with Algamac Protein Plus ® by Aquafauna, a heterotrophic and phototrophic species mixture of macroalgae, at a concentration of 730 cells μL^{-1} day^{-1} (Pronker et al. 2015).

2.2. Cercarial behaviour experiment

To study cercarial photosensitivity, a pre-designed oval shape aquarium (50 x 30 x 5 cm, 10 cm channel width) was used to create a light gradient (Appendix 1). The aquarium was built with half of transparent acrylic and half of black opaque acrylic. The aquarium was filled with artificial seawater at salinity 35, maintained at 18 °C and kept in a dark room exposed to a horizontal light source while covered by a black opaque lid so the only light input was horizontally. Light was supplied by a 7-volt, 1.5 watts LED bulb during the whole experiment. Maximum light intensity obtained was 50 μmol m^{-2} s^{-1}, similar to light intensities observed in clear shallow waters (dos Santos et al. 2015). Light intensity was measured with an Apogee handheld MQ-200 Quantum meter with a separate sensor.

In each experiment (same experiment was performed in duplicate), a total of 1000 cercariae (maximum: 6 hours old) were released in two different releasing areas (areas D and E, corresponding to mid position and intermediate light intensity). After 4 hours, cercariae were assigned to 8 different areas of similar volume and counted through image analysis. Three digital pictures per area were obtained using a high resolution photographic camera and cercariae were counted manually and using OpenCFU software. The percentage of cercariae present in each area was then calculated. Area A was exposed to maximum light intensity (50 μmol m^{-2} s^{-1}), while area H was exposed to minimum light intensity (dark – 0 μmol m^{-2} s^{-1}). The remaining six areas were exposed to equal light intensity in a two-by-two pattern. Areas B and C were exposed to a light intensity of 35 μmol m^{-2} s^{-1}, areas D and E to 23 μmol m^{-2} s^{-1} of light intensity and areas F and G to a total light intensity of 6 μmol m^{-2} s^{-1}.

A previous study performed with a trematode parasite of the same family revealed that cercariae mobility tend to decrease severely after 12 hours (de Montaudouin et al. 2016). Therefore, in the present study, a total 10 hours of lifespan (6 hours after emission + 4-hour exposure) was selected to ensure cercariae mobility until the end of the experiment.

2.3. Cockles oxygen consumption

To test the light effect on cockles filtering activity, oxygen consumption used as a proxy of this activity (Wegeberg et al. 1999), was measured through simple static respirometry. After acclimation period, twelve cockles of similar shell length (between 15 and 17 mm) were individually placed in 1L respirometric chambers. All chambers were filled to their maximum capacity with artificial seawater at salinity 30, to avoid formation of bubbles. In order to allow cockles acclimation to the respirometric chamber conditions, individuals were left for 1 hour inside the respirometric chamber. Six of these chambers were covered in aluminium foil, preventing the entrance of light, and the other six were exposed to natural light condition (approximately 25 μmol m^{-2} s^{-1}). In addition, four other respirometric chambers (two covered in aluminium foil and two uncovered) were used as control, i.e. no organisms to account for background oxygen consumption. Each chamber was equipped with an oxygen sensor. Oxygen concentration was measured twice, in equal time intervals (1 hour), by contacting the oxygen sensor with a multi-channel optic fiber oxygen meter (Multi channel oxygen meter, PreSens, GmbH, Regensburg, Germany) and reading the concentration value after stabilization in the PreSens Measurement Studio software.

Oxygen consumption was calculated as the difference between 2 reading points (separated by a period of two hours) and expressed in ppmO_2 gram of cockle fresh weight^{-1} h^{-1}. Cockles fresh weight was calculated using the shell length: weight relationship previously determined for the area where cockles were sampled (S. Correia unpubl. data) and described as:

$$\log W = 3.1886 \log L + 3.7577, \; R^2 = 0.92$$

Where, *W* is cockles fresh weight, *L* is the shell length (in mm) and R^2 is the coefficient of determination.

2.4. Cercariae infection success

After cockles acclimation period, an experiment using twenty-four replicates was performed in duplicate. Previously, a subsample of 15 cockles were dissected and observed under a stereomicroscope to obtain natural infection intensities at the sample site. To minimize uncontrolled effects, similar sized cockles (ranging from 13 to 17 mm), were placed separately into 50 mL glass flasks at two different conditions (light vs. dark) in a dark room. Twelve cockles were exposed to a vertical light source supplied by a 7-volt, 1.5 watts LED bulb with a light intensity of 50 μmol m^{-2} s^{-1}. The flasks of the other 12 cockles were covered in aluminium foil and exposed to dark. During this experiment, water medium was maintained at 18 °C and salinity 30.

A total of 600 cercariae, 25 per flask, were collected and used to individually infect each cockle. Experiments lasted for 48 hours, the time required for cercariae to encyst (de Montaudouin et al. 2016). After, metacercariae of *H. elongata* were counted under a stereomicroscope by squeezing cockles' flesh between two glass slides.

2.5 Data analysis

Concerning cercarial behaviour experiment, two chi-squared tests were performed, one to check the homogeneity among experiment duplicates and the second to test the data adjustment between the expected and the observed frequencies of cercariae per area.

For cockles oxygen consumption, a Mann-Whitney U test was used to assess the differences in terms of oxygen consumption among conditions (light vs. dark), whereas for cercariae infection success experiment, a Kruskal-Wallis H test followed by Dunn-Bonferroni post hoc test were performed to compare cockles infection between light conditions and natural infection (light vs. dark vs. natural infection).

All non-parametric statistical analyses were performed using the IBM SPSS Statistics software v.25 after testing the parametric statistical assumptions (Levene's and Shapiro-Wilk tests for homoscedasticity and normality, respectively) and failing samples normality.

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3. RESULTS AND DISCUSSION

3.1. Cercarial behaviour experiment

After 4 hours exposure, 83.2 % and 81.8 % of total released cercariae were retrieved from each duplicate of the cercarial behaviour experiment. Cercariae of *Himasthla elongata* displayed a photopositive orientation behaviour. From the total cercariae found, 80.3 % ± 2.1 standard deviation (SD) were detected in area A, corresponding to the highest light intensity (50 μmol m^{-2} s^{-1}). Area H (0 μmol m^{-2} s^{-1}), was identified as the area with the lowest percentage of cercariae abundance (3.0 % ± 0.9 SD of total cercariae retrieved). The remaining cercariae were found in the areas located between maximum light intensity and dark. In detail, areas B and C presented 9.3 % ± 0.8 SD of cercariae final count, while 3.8 % ± 0.4 SD were found in the areas D and E. In the areas F and G, areas positioned closer to the dark condition, the number of cercariae found corresponded to 3.8 % ± 0.8 SD of the final count. The chi-squared test showed that the cercariae proportion retrieved by area did not follow a uniform distribution (χ^2 (7) = 8412.72; $p < 0.001$), i.e. there were significantly more cercariae in some areas compared to others. Cercarial behaviour showed to be the same among experiment duplicates (χ^2 (7) = 8.444; p = 0.295). Since *H. elongata* first host, *Littorina littorea*, is in an upstream (supralittoral) and therefore more lighted area compared to cockles, the second host, it was hypothesized a photonegative attraction by cercariae to optimize cockle infection. However, the behavioural pattern found, i.e. the cercariae movement towards light, allows to reject the postulated hypothesis. These results were the opposite found by other authors for *H. elongata* (Prokofiev 2006) and *H. rhigedana* (Fingerut et al. 2003), possibly due to a stronger geotactic response that supress cercarial photosensitivity or, in the case of *H. rhigedana*, due to its second host (*Pachygrapsus crassipes*) higher activity at night (Morgan et al. 2006). In fact, *H. elongata* cercarial orientation towards light may still be related to the host habit, in this case, its intertidal habitat. While being attracted by light, the cercariae of *H. elongata* can settle in a water column with higher light incidence and lower water column height, characteristics of an intertidal area. Coupled with the positive geotactic behaviour that this trematode species already demonstrated (Nikolaev et al. 2017), the orientation positively influenced by light can promote the chances to infect cockles. This behaviour has already been described for other cercariae species infecting intertidal hosts (e.g. Kennedy 1979). Accordingly, higher trematode infection levels of cockles from intertidal areas compared to subtidal ones have already been described in the literature (Correia et al. 2020). At the same time, *Himasthla* spp. are also natural parasites of mussels (Galaktionov et al. 2015), which inhabit in a higher position of the littoral zone, and therefore a photopositive attraction can benefit the infection of these hosts, i.e. *Himasthla* spp. could be more adapted to infect upstream positioned hosts.

3.2. Cockles oxygen consumption and cercariae infection success

Cockles oxygen consumption was not significantly different between light conditions (Z (16) = -1.435, p = 0.151). In fact, it is already described in the literature that if they are submersed, cockles can filter actively regardless light conditions (Newell & Bayne 1980).

Results of the present study showed that when cockles were not exposed to light, they displayed higher infection levels (mean infection = 14.29 metacercariae cockle^{-1} ± 4.14 SD) compared to cockles exposed to a vertical light source (mean infection = 10.16 metacercariae cockle^{-1} ± 4.34 SD) and the sampling site natural infection (mean infection = 2.07 metacercariae cockle^{-1} ± 2.15 SD) (H (2) = 33.751, p > 0.001). Differences between groups were confirmed by post hoc tests (Light (L) vs. Natural conditions (NC): Z = XXXX, p < 0.001; Dark (D) vs. NC: Z = XXXX, p < 0.001; L vs. D: Z = -2.598, p = 0.009). Cockles were experimentally infested in low volume containers to promote host-parasite contact. However, given that no differences were found in the oxygen consumption of cockles exposed to light compared to those maintained in dark conditions, and that cercariae of Himasthlidae family (family of the studied trematode species) are passive invaders, i.e. cercariae infect the second intermediate host by being ingested through their feeding activity (Wegeberg et al. 1999, Galaktionov & Dobrovolskij 2003), the results lead to infer that recorded infection differences are rather dependent on cercarial behaviour towards distinct light conditions than on cockles individual filtering activity. Indeed, when exposed to a light source, cercarial movements can be enhanced (Haas 1992) which, due to the absence of water current in the experimental containers used, could have led to a rise in the water column. Additionally, the low water column

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height could have neglected the geotactic effect. Consequently, these vertical movement decreased the chance of contact, departing cercariae from the feeding area of the second intermediate host. On the other hand, in the absence of a light stimulus, cercariae swim without orientation, slowly sinking in the water column (Feiler & Haas 1988, Haas et al. 1990). In this way, the cercariae tended to approach the feeding area of its host, increasing the chance of contact and consequent infection. When in close contact with the host, the previously described passive behaviour of cercariae shifts to an inciting active infection (Galaktionov & Dobrovolskij 2003), increasing the probability of infection, thus explaining the results obtained. Nonetheless, further studies should be taken into account to fully understand the effect of depth.

The cercarial behaviour reported in this study allows *H. elongata* to use light as a guidance mechanism to disperse throughout the aquatic system and seek for a suitable habitat for its life cycle completion, promoting the infection of cockles more susceptible to be predated by the final host (shorebirds). Meanwhile, during night, with lower light incidence, cercariae would save their energies, sinking to its second host active area, waiting to be inhaled by cockles.

4. CONCLUSION

In conclusion, the present study showed that by displaying a photopositive sensitivity, cercariae of *Himasthla elongata* can increase their dispersion ability, invading the most suitable habitats where the second intermediate host lives. It is important to highlight that this study clearly demonstrated a higher infection success in cockles exposed to dark compared to cockles maintained in the light. This higher infection can only be ascribed to cercarial behaviour since host filtering activity is not affected by light. These results support the theory that trematode responses besides being dependent on the second intermediate host type of habitat (benthic vs. pelagic habitat) are also subordinated by the will to complete the life cycle. In this particular case, as the second intermediate host is widely distributed along the whole tidal gradient (from lighter to darker areas) cercariae can use external stimuli (such as light) to relocate themselves towards areas where the second intermediate host is more likely to be predated by its final host (shorebirds in case of the species under study) and, therefore be able to proceed the life cycle.

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Figure 1. Schematic representation of cercariae light behaviour aquarium subdivided in the 8 areas of light gradient. A: Side View; B: Top View; C: Front View. Cercariae were released in areas 4 and 5.

Figure 2. Experimental design of the cercariae infection success assay. Each condition contained 12 replicates with 25 cercariae released per replicate.

Figure 3. Percentage of cercariae found in each experimental area of the behaviour aquarium. Area 1 presented the maximum luminosity, while area 8 was exposed to dark. The remaining areas had an equal luminosity two-by-two.

Figure 1

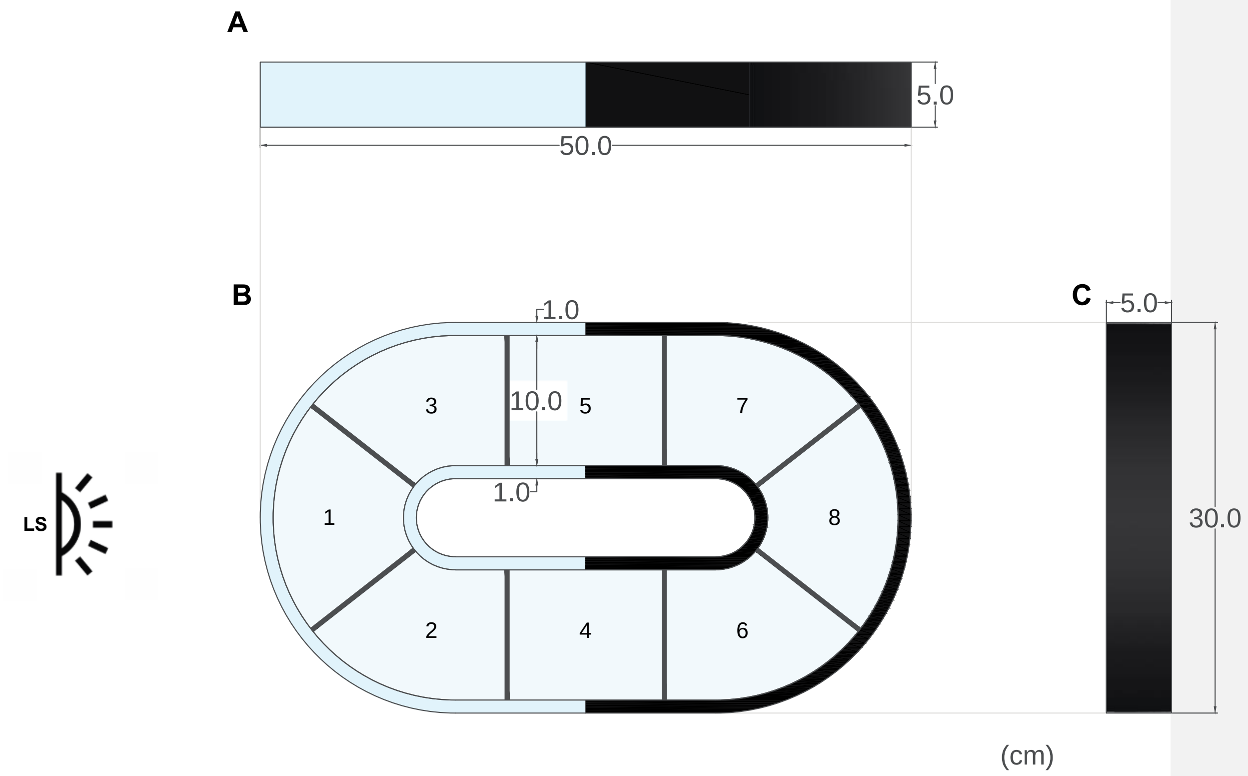

Figure 2

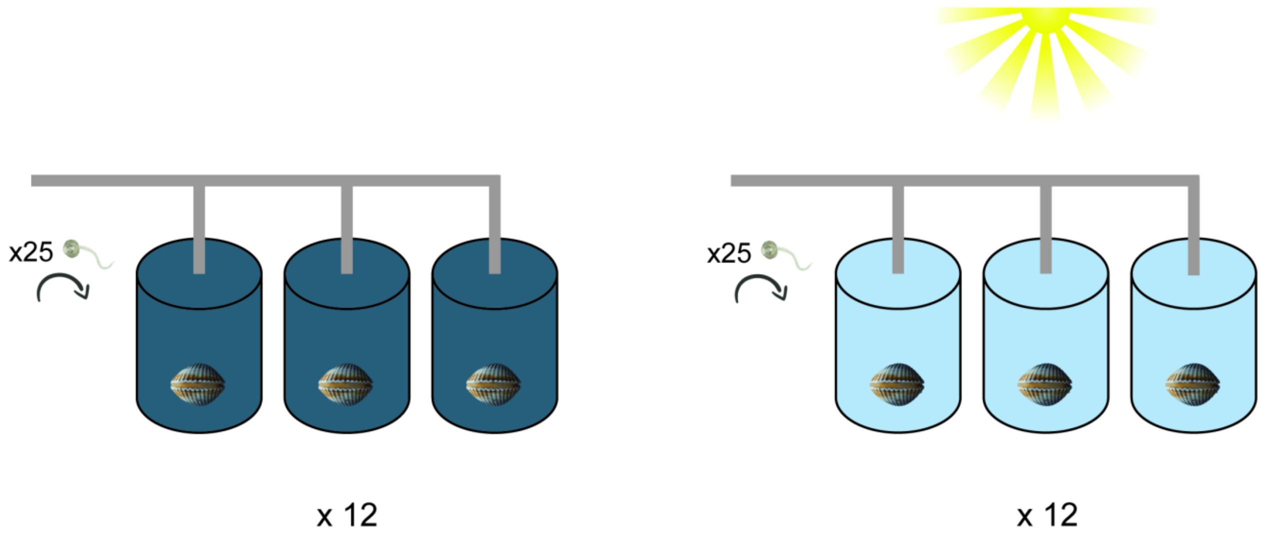

Figure 3