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Effect of photoperiod change on chronobiology of cercarial emergence of *Schistosoma japonicum* derived from hilly and marshy regions of China

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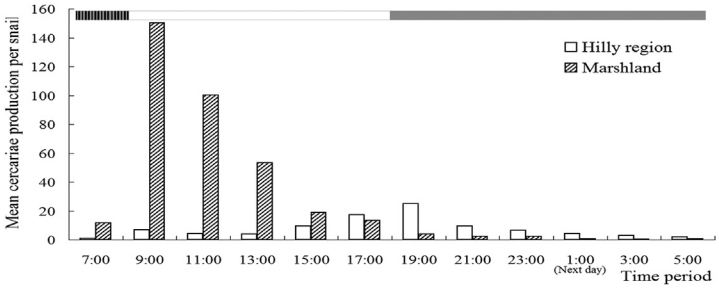
HIGHLIGHTS

- The pattern of *Schistosoma japonicum* cercarial emergence was not constant.
- Under a reversed photoperiod, the regular emergence pattern was reversed or disappeared.
- An input of 2 h darkness from 7am to 9am caused a delay of the emergence peak.
- An input of 2 h darkness from 5pm to 7pm did not affect the emergence pattern.

GRAPHICAL ABSTRACT



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ABSTRACT

The chronobiology of cercarial emergence appeared to be a genetically controlled behavior, adapted to definitive host species, for schistosome. However, a few physiological and ecological factors, for example the change of photoperiod, were reported to affect the rhythmic emergence of cercariae. Therefore, the effect of photoperiod change on cercarial emergence of two *Schistosoma japonicum* isolates, the hilly and the marshland, was investigated. Four shedding experiments each under a different photoperiod were conducted. Under a natural photoperiod, two distinct shedding modes, one from the hilly region and one from the marshland, were observed. Under a reversed photoperiod, the regular pattern (i.e. under a natural photoperiod) of *S. japonicum* cercarial emergence was reversed for the marshland isolate and disappeared for the hilly isolate. With an input of a 2 h darkness from 7am to 9am, the cercarial emergence peak were delayed for the two isolates; whereas with an input of a 2 h darkness from 5pm to 7pm, neither effect on the cercarial emergence rhythm was observed. The total cercariae emerged for both parasite isolates varied with a different photoperiod. The results indicate that the change of photoperiod could affect the chronobiology of *S japonicum* cercarial emergence.

1. Introduction

Schistosomiasis, a snail-borne neglected tropical disease, is a

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major parasitic disease ranking only second to malaria of medical and economic significance in the tropical and subtropical regions (Steinmann et al., 2006). Generally, an estimated 207 million people are infected with this disease, with a further 800 million at risk of infection (Steinmann et al., 2006). Over 200 thousand people die from schistosomiasis-related disorders each year worldwide, and the disease is estimated to cause annual loss of over 70 million disability adjusted life years (DALYs) in the world (King et al., 2005). *Schistosoma mansoni*, *Schistosoma japonicum* and *Schistosoma haematobium* are three major schistosome species infecting humans. In China, although great achievements have been gained in the control of *Schistosomiasis japonica*, the disease remains a public health problem and it is defined as one of the four major communicable diseases that have been given a high priority by the central government (Zhao et al., 2005; Liang et al., 2006). Data from the national epidemiology survey showed that *S. japonica* remains endemic in the middle and lower reaches of the Yangtze River and some mountainous regions, and over 0.29million people living in China are thought to have the disease (Zheng et al., 2012). Moreover, transmission of *S. japonicum* is more complicated due to the fact that 46 kinds of mammals are able to infect with the parasite and are suspected as reservoir hosts (He et al., 2001; Rudge et al., 2009; Lu et al., 2010).

The life cycle of *S. japonicum* involves a sexual reproduction phase in a mammalian host and an asexual phase in a molluscan host. Transmission from host to host occurs via two free-swimming larval stages, miracidia (hatched from eggs passed in host's feces and are infective to a mollusc) and cercariae (shed from a mollusc and are infective to a mammal). Therefore, cercarial emergence is considered as an essential part in the transmission of *S. japonicum* (Liu and Li, 2013). The distribution of the amount of cercariae emerged from one snail within each period (i.e. 1 h or 2 h) of the entire diurnal/nocturnal pattern over a 24 h is called the chronobiology of cercarial emergence, and Lu et al. had previously reported there were two distinct shedding patterns, under a natural photoperiod, for *S. japonicum* cercariae in two ecological endemic regions-the hilly and the marshland areas (Lu et al., 2009). Research on *S. mansoni* and *S. haematobium* has shown that few factors, for example change of photoperiod, may affect the schistosome cercarial emergence (Asch, 1972). However, there is no research on the effect of the change of the photoperiod on the emergence of *S. japonicum* carcaria. Therefore, in the current study four experiments, each under a different photoperiod, were conducted to evaluate the impact of photoperiod change on the chronobiology of *S. japonicum* cercarial emergence for the parasite from the hilly area, a post-transmission control area where this parasite has re-emerged. The aims were to investigate 1) whether the chronobiology of cercarial emergence is reversed when under a reversed photoperiod; and 2) whether and/or how the trait is impacted when an input of 2 h darkness is put in the early morning or the late afternoon. This seems of importance in using the associated biological traits to track the spread or transmission of the field parasite isolate within and from the hilly areas.

2. Materials and methods

2.1. Snails and parasite

Oncomelania hupensis hupensis snails were collected from the hilly region of Shitai county, a schistosomiasis-endemic area along the middle and lower reaches of the Yangtze River, Anhui Province, China in April of 2013, using an individual picking method with forceps (Zhou, 2005). All field-collected snails were checked for *S. japonicum* infection with cercarial shedding method, and a total of 67 infected snails were identified, out of which 37 were randomly chosen for the subsequent experiments. As we could not obtain infected snails directly from a marshland region, we then purchased 24 laboratory-infected snails from Jiangsu Institute of Parasitic Diseases (Wuxi, China). These snails were artificially infected with a marshland-isolate *S. japonicum* and here served as controls. As snails used for the two parasite isolates were not from the same area, and moreover, infection process (laboratory-infected VS field-infected) with *S. japonicum* were different, these could have potential influences on our results. However, our previous work have shown that, under the same condition, the hilly parasite, either in a hilly or marshland snail, presents the same cercarial emergence pattern (Wang et al., 2014). All infected snails were individually raised in 24-well culture plates for two weeks before experiments, with one snail per well. Each well had a small piece of damp sponge and a piece of wet culture paper. The plate was covered with a mesh screen to prevent snails escape. The ambient temperature was constantly maintained at 25 °C.

2.2. Cercarial shedding from infected snails

Four shedding experiments, at an interval of 2–3 weeks, were performed on the above two batches of the snails. The number of infected snails used in each experiment was shown in Table 1. All shedding experiments each started at 7am and last over a 24 h. The first experiment was performed under a natural and normal photoperiod. In the second experiment, snails were put in darkness for cercarial shedding from 7am to 7pm (i.e. 12 h in darkness), followed by exposure to continuous artificial illumination (500 lux) for further cercarial shedding from 7pm to 7am (the next day) (i.e. 12 h in light). In the third experiment, snails were placed in darkness for cercarial shedding from 7am to 9am (i.e. 2 h in darkness), and then transferred to the environment under a natural photoperiod for further cercarial shedding until 7am of the next day. The fourth experiment is slightly different from the third, in which cercarial shedding period in darkness was set from 5pm to 7pm. See Table 2.

The procedure for cercarial shedding is as follows. Snails were put into 24-well culture plates with one snail in a well filled with water. After shedding for 2 h, the snails were transferred to another plate for the next 2 h period. Before being transferred to the next well, each snail was washed with dechlorinated tap water twice to remove the cercariae, which possibly attached onto a snail's shell. Such procedures were repeated until the end of the experiment. After experiment, each well was added with Lugol's iodine solution and then the number of cercariae was counted under a dissecting microscope. All experiments were performed at 25 °C, and artificial illumination (500 Lux) was derived from a 25 W incandescent lamp. Light intensity was measured at the beginning of each 2 h period with a TES-1330A digital light meter (Taipei, Taiwan), as seen in Table 2.

2.3. Statistical analysis

Analysis of chronobiology of cercarial emergence was performed by counting the number of cercariae released from one snail in each 2 h period. All data were entered into Microsoft Excel, and a histogram was plotted to show the chronobiology of *S. japonicum* cercarial emergence. To investigate the variation in cercarial emergence among the four experiments, we calculated the total number of cercariae emerged over a 24 h for each snail and the percentage of the number of cercariae emerged in the diurnal (07.00–19.00) phase as related to the total number of cercariae emerged from that particular snail. As the data based on measuring cercarial production were not in normality, statistical comparisons among experiments under different photoperiods were conducted