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The Effects of pH and Salinity on the Behavior of *Philophthalmus gralli* (Trematoda) Miracidia

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ABSTRACT

The responses of *Philophthalmus gralli* miracidia in pond water controls and pond water at pH 7.5 adjusted to 1.25% saline (percent NaCl), 1.75% saline, pH 5, pH 9 and pH 11 to solutions of 1.0 mM and 10.0 mM glutamic and aspartic acids were tested in phi-chambers. Reductions in response from the pond water control were found for all solutions tested but were most pronounced at pH 5 and pH 11. The swimming behavior of miracidia at pH 5, pH 11, and 1.75% saline were observed on videotape recordings. Miracidia in 1.75% saline showed normal behavior while those at pH 5 and pH 11 showed a significantly increased activity when compared to pond water controls. The swimming speed of miracidia in 1.75% saline was significantly slower than those in the pond water control.

INTRODUCTION

Miracidia of digenetic trematodes hatch from eggs and use various behavioral mechanisms to locate their snail hosts. Reactions to light, chemicals, and gravity place miracidia in the general vicinity of the specific snail host. Thus the behavioral responses of miracidia vary according to species and mimic the behavioral responses of the target snail. In some cases responses of miracidia may change with variations in environmental conditions. These could result from natural variations in pH and salinity or from man-made changes resulting from acid precipitation and salinization of aquatic systems.

Investigations with eyefluke miracidia (*Philophthalmus gralli*) indicated that positive geotactic and phototactic behavior were diminished somewhat by extreme deviations from room temperature (Keshavarz-Valian and Nollen, 1980). *Philophthalmus gralli* miracidia also show strong chemokinetic responses to certain chemicals including glutamic, aspartic, sialic, sulfuric, acetic, and hydrochloric acids (Keshavarz-Valian et al., 1981). These eyefluke miracidia are quite resistant to various environmental conditions such as abnormal salinities and pH, since *P. gralli* miracidia have a half life of 155 min at 2.0% NaCl, 88 min at pH 5, and 242 min at pH 11 (Nollen et al., 1979).

This study was undertaken to see if variations in environmental conditions, such as changes in pH and salinity, would cause a change in the chemotactic responses of *P. gralli* miracidia to chemicals or affect their swimming speed. *Philophthalmus gralli* miracidia react strongly to glutamic and aspartic acids under normal environmental conditions, These chemicals were used to test taxes in conditions not normally found in a fresh water environment, yet in which *P. gralli* miracidia survive. Swimming variations were determined by video recordings.

MATERIALS AND METHODS

Miracidia of *P. gralli* were obtained from adults grown in the eyes of chickens. Gravid adults, removed to test solutions, immediately laid eggs from which miracidia hatched within minutes. Saline solutions were prepared with NaCl by weight/volume in filtered pond water. Solutions of varying pH were prepared by addition of 0.1N HCl or 0.1N NaOH to filtered pond water until the desired pH was reached as determined by pH meter.

The responses of miracidia to known stimulants in various environmental conditions were tested using the phi-chamber technique of Roberts et al. (1978). The solutions used were 1.25 or 1.75% saline, pH 5, 9, or 11, and a filtered pond water control (pH 7.5). Three replicates were performed for each test chemical in each environmental condition.

Swimming speed and behavior were determined for miracidia in 1.75% saline, at pH 5 or 11, and in filtered pond water. Miracidia hatched in each of the specific environments were observed using a dissecting microscope and recorded with a JVC (model s-100CH) video camera. A 5-mm grid was placed under the dish containing the miracidia. Swimming behavior was determined by comparing the number of miracidia displaying normal swimming patterns to those showing modified swimming behavior (increased turning rate or erratic movements). Swimming speed was determined by timing individual miracidia as they swam across a specific 5-mm square. All miracidia used were less than 3 hr old.

RESULTS

Responses to stimulating chemicals by *P. gralli* miracidia in different environmental conditions are given in Table 1. A change in environment resulted in a reduction of response by miracidia to glutamic and aspartic acids; in some cases this reduction was significant. Altering the pH from the control value generally had a greater effect on the response than did a change in the salinity.

The effect of 1.25% saline was only significantly different from controls when higher concentrations of chemicals were used, A very significant reduction in response (85% to 57%) was observed when miracidia in 1.25% saline were exposed to 10.0 mM glutamic acid. In 1.75% saline, a significant reduction in response was only observed when glutamic acid served as the stimulant. The greatest reduction in response was observed when 1.0 mM glutamic acid was inoculated into 1.75% saline. This instance plus exposure of miracidia to 10.0 mM glutamic acid in 1.25% saline were the only experiments with increased salinities where no significant chemoresponses to either glutamic or aspartic acid were detected.

A change from pond water (pH 7.5) to an acidic pH 5 or an alkaline pH 11 elicited a significant decrease in response by miracidia to all concentrations of chemicals used. Approximately a 50/50 distribution in the phi-chambers indicating a lack of response by miracidia to the chemicals was observed in several trials with solutions of pH 5 and 11. Changing the pH from the pond water control to pH 9 did not have a significant effect on the response except with 1.0 mM glutamic acid (77% to 51%) which is comparable to that found for pH 5.

Results of the swimming behavior experiments as determined by videotape analysis showed that a 1.75% saline solution has no apparent effect on the swimming behavior of *P. gralli* miracidia. Eighty-one percent of miracidia in pond water exhibited normal swimming behavior compared to 73% in 1.75% saline. Measurements of swimming speed from the video tapes showed that these miracidia had a mean speed of 1.84 mm/sec which was significantly slower than the 2.15 mm/sec calculated for miracidia in pond water. However, placing miracidia in pH 5 and pH 11 solutions did cause a significant deviation from normal straight-line swimming behavior. Only 37% of miracidia in pond water at pH 5 and 21% in pH 11 pond water were observed in straight-line movements. Swimming speed for these miracidia was impossible to determine because of their erratic movements through the grids.

DISCUSSION

The results of the phi-chamber experiments indicated that a change in pH or salinity from normal pond water caused a reduction in the response to stimulating chemicals by *P. gralli* miracidia. Changes in pH, either to a basic or acidic condition, proved to have a greater effect on response than did salinity. Nollen et al. (1979) found that the half-life of *P. gralli* miracidia was greatly reduced by changing the pH to either 5 or 11. Approximately a 7-fold reduction in half-life was observed for miracidia in pH 5 solution. In contrast, they found that an increase in salinity to 1.4% had little effect on the half-life of the miracidia; a drastic drop in longevity was not observed until miracidia were exposed to levels above 2.0% saline.

The effects of pH and salinity on chemoresponses of miracidia have not been tested for any other trematode species. Changes in response to gravity and light have been documented with variations in temperature and light (Takahashi et al., 1961; Shiff, 1974; Mason and Fripp, 1976; Keshavarz-Valian and Nollen, 1980). The only previous work to document a loss of chemoresponse was due to aging of the miracidia. This was reported by Prechel and Nollen (1979) who found that miracidia of *Megalodiscus temperatus* became less reactive to snail-conditioned water with increasing age.

The reduced response of *P. gralli* miracidia in saline might be explained by their slower swimming speed when exposed to saline (1.84 mm/sec in 1.75% NaCl vs. 2.15 mm/sec in pond water). Slower swimming speeds have been observed in other species as miracidia age. This may account for the reduced chemoresponse in this study as was reported for *M. temperatus* by Prechel and Nollen (1979).

Results of our study showed that either a basic or acidic solution caused a reduced response by *P. gralli* miracidia to stimulating chemicals. Videotape observations of miracidial behavior in these solutions without glutamic and aspartic acids present showed a significant increase in the numbers exhibiting a stimulated behavior. This behavior is undoubtedly the cause of the reduced response to stimulants in phi-chambers. This could result from a decrease in the area of the phi-chamber frequented by the miracidia thereby reducing the chance of encountering the inoculated stimulants or a reduced ability to react to added chemicals when already stimulated by the acidic or basic conditions.

The normal swimming speed of *P. gralli* (2.15 mm sec) is almost identical to that recorded for miracidia of *Schistosoma mansoni* (2.19 mm/sec) by Mason and Fripp (1976) and *M. temperatus* (2.19 mm/sec) by Prechel (1974).

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Table 1. The response to stimulating chemicals of *Philophthalmus gralli* miracidia in various test solutions.

Test solution		Percent miracidia in inoculated side of phi-chamber			
Salinity %	pH	1.0 mM Glutamic acid	10.0 mM Glutamic acid	1.0 mM Aspartic acid	10.0 mM Aspartic acid
0	7.5	77 (69/90)	85 (79/93)	66 (117/176)	75 (145/194)
1.25	7.5	73 (33/45)	57 (31/54)*+	64 (85/132)	62 (89/144)*
1.75	7.5	42 (40/96)*+	67 (39/58)*	62 (53/86)	67 (53/79)
0	5.0	52 (72/138)*+	60 (59/98)*	47 (34/72)*+	51 (39/76)*+
0	9.0	51 (82/161)*+	74 (50/68)	59 (123/208)	73 (179/247)
0	11.0	62 (55/89)*	63 (52/83)*	49 (131/266)*+	58 (120/206)*

(total number inoculated side/total number of miracidia used in 3 trials)
* Significantly different from pond water control (P = 0.05) (Chi Square, 2x2)
+ Not significantly different from 50/50 distribution (P = 0.05) (Chi Square)