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Induced Hatching to Avoid Infectious Egg Disease in Whitefish

Claus Wedekind[1]
Swiss Federal Institute for Environmental Science and Technology (EAWAG - ETH)
6047 Kastanienbaum
Switzerland
Institute of Cell, Animal and Population Biology
University of Edinburgh
West Mains Road
Edinburgh EH9 3JT
Scotland

Summary

Reacting to a threat before physical contact, e.g., induced by air- or water-borne substances [1], appears to be an elegant way of defense. The reaction may be behavioral [2–5], developmental, morphological, or physiological [5–7], and it can involve a shift in niche or life history [8, 9]. Hatching from eggs is a shift in niche and in life history. From niche shift and life history models [10–14], one would predict that the timing of hatching is, to some degree, phenotypically plastic, i.e., early or delayed hatching is likely to be inducible [15–19]. Temporary increased larval mortality (e.g., increased predation on larvae) would favor delayed hatching, while relatively high egg mortality would favor early hatching. Here, I show experimentally that eggs of the whitefish (*Coregonus* sp.) hatch earlier in the presence of a virulent egg parasite and that this early hatching is induced by water-borne cues emitted from infected eggs.

Results and Discussion

During the course of another study [20], I observed that embryonic duration covaries with the presence or absence of an infectious egg disease. In this other study, about 55,000 whitefish eggs (*Coregonus* sp., the parents had been collected during their breeding season in December 1998 from Lake Hallwil in Switzerland, see [21] for the yet unsolved taxonomy) had been distributed to 400 petri dishes and reared in a climate chamber at 8°C (see details about the methods in [20]). Shortly after the 30th day of egg development, an uncontrolled outbreak by *Pseudomonas fluorescens* occurred. I determined mortality and hatching rate in a sample of 300 petri dishes between day 51 and day 57 of egg development. High egg mortalities were associated with high hatching rates (Spearman's rank order correlation coefficient $r_s = 0.18$, $p = 0.002$). Differences in egg density could not explain this correlation (number of eggs at day 51 versus hatching rate: $r_s = -0.02$, $p = 0.79$; number of eggs versus mortality: $r_s = -0.03$, $p = 0.58$).

I performed a series of experiments to confirm whether the fish hatch more frequently in response to the egg disease and whether hatching rates correlate negatively with mortality rates in the presence of the disease. An additional 100 batches of eggs that were reared in petri dishes in the same way as the 400 batches above, each originating from a different pair of parents, were available for these experiments.

Nine batches with low previous mortality were chosen for a first experiment at day 69, when hatching had already started (as expected [22]). The eggs of each batch were about equally distributed to two new petri dishes. In one of these two petri dishes, I added 30 eggs with the typical phenotype of a fresh infection (i.e., being slightly cloudy). Nothing was added to the other one. I determined hatching rate and mortality 12, 36, and 60 hr later. At these times, I also collected the freshly hatched larvae with a pipette and transferred them to another petri dish. The overall mortality of these larvae was determined at the end of the experiment.

Exposure to infected eggs leads to new infections: after 60 hr, the median mortality in the exposed group was 4.7% (range 0%–39%), while in the nonexposed groups, there was no mortality at all (Wilcoxon signed rank test, $p = 0.016$). Exposure to the egg parasites also induced increased hatching (Figure 1A). This increased early hatching correlated with a reduced mortality (Figure 2A), and there was no mortality among the hatchlings that had been displaced from either experimental group. Differences in egg densities did not seem to affect mortality rates (Figure 2B).

To test whether early hatching is triggered by water-borne cues, or, for example, is just a consequence of an effect the parasite may have on the egg membrane, I chose 36 new batches of eggs (mean number of eggs: 88.5, SE = 6.2), with low previous mortality. The eggs of each batch were again about equally distributed to two new petri dishes. In one of these two petri dishes, I added 10 ml of "stimulus" water that had been in contact with freshly infected eggs (300 infected eggs in 600 ml for 4 hr at 8°C) and was sterile filtered (Nalgene 0.45-μm syringe filter) directly before use. The nonexposed groups received sterile filtered water from a beaker that did not contain any infected eggs. The hatching rates and the mortality were determined 12 hr later.

Exposure to the stimulus water lead to increased hatching rates (Figure 1B). The total mortality during this second experiment was 0.002 and was not significantly different between the exposed and the nonexposed groups (stimulated: in total, 4 of 1608 eggs; controls: 3 of 1579 eggs; paired t test, $p = 0.74$).

These results show that whitefish have evolved a mechanism to sense and respond to the presence of a virulent egg parasite before physical contact. This supports analogous findings with bullfrog tadpoles (*Rana catesbeiana*) [2]. In the present study, however, the individuals that react to the disease are still embryos within their egg membrane. The reaction, earlier hatching, enables them to drift or swim away from infected eggs, i.e., it enables them to physically escape infection. Indeed, I did not observe any mortality among the larvae

[1]Correspondence: c.wedekind@ed.ac.uk

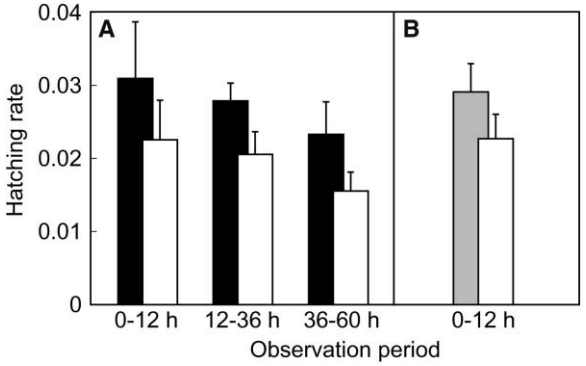

Figure 1. Hatching Rates per Hour, Means + SE, of Whitefish Eggs during Different Observational Periods

The hatching rates are given here as the number of hatchlings at the end of an observational period/number of eggs at the beginning of the observational period/number of hours.

(A) Batches exposed to freshly infected eggs (filled boxes) and nonexposed controls (open boxes; comparison between the total hatching rates over 60 hr: paired t test, $t = 3.21$, $n = 9$, $p = 0.01$; within each observational period, t is always ≥ 2.7, and p is always < 0.05).

(B) Batches exposed to water-borne cues emitted from freshly infected eggs (shaded box) and nonexposed control (open box; $t = 3.03$, $n = 36$, $p = 0.0045$).

that had hatched in the experimentally infected petri dishes and that had been removed after some time.

The exact origin and biochemical nature of the cues that induce early hatching is presently unknown. It could, in principle, stem from the pathogen itself (a cue that the pathogen may not be able to avoid [23, 24]), or it could stem from damaged eggs [25]. In the latter case, the cues could be substances that are simply released by the eggs because of the damage the pathogen causes, or it could be evolved alarm substances that are emitted to warn nearby eggs. Female whitefish normally spawn several thousands of eggs per breeding season. Although this species is a group spawner, nearby eggs may often be relatives. Therefore, it seems possible that such alarm substances could evolve through kin selection [26].

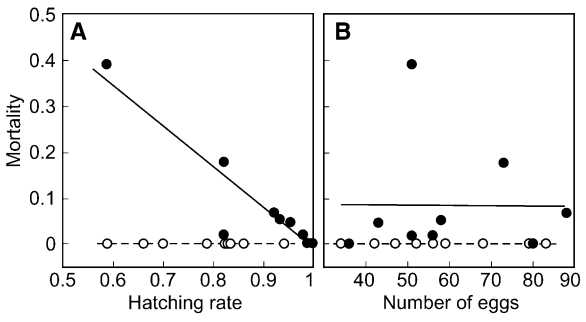

Figure 2. Egg Mortality during 60 hr of Exposure to Infected Eggs

(A) Regression between total mortality and total hatching rate for exposed batches (filled points, nonhatched line; Spearman's correlation coefficient $r_s = -0.74$, $p = 0.021$) and for nonexposed controls (open points, hatched line; $r_s = 0.0$). These two correlations are significantly different from each other (Spearman's correlation coefficient between hatching rates and the differences in mortality of exposed and nonexposed batches: $r_s = -0.74$, $p = 0.021$).

(B) Regression between total mortality and the number of eggs at the start of the experiment (exposed: $r_s = 0.24$, $p = 0.54$; nonexposed: $r_s = 0.0$). Nonparametric statistics was used in order to avoid any bias from extreme values.

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