Trychophrya intermedia on the gills of rainbow trout acclimating to low ambient pH

P. H. M. Balm*, T. R. Carrick†, A. J. M. Coenen* and T. G. Pottinger†

*Department of Animal Physiology, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands and †Institute of Freshwater Ecology, Windermere Laboratory, Far Sawrey, Ambleside, Cumbria LA22 0LP, U.K.

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Rainbow trout under low pH conditions acquire heavy infections with Trychophrya intermedia, not secondary to gill damage, which suggests that the parasite may have a primary effect on gill function in fish under acid conditions.

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Although the impact of low environmental pH on fish is still debated, it is generally assumed that fish under such conditions become sensitized to additional challenges. Among these, abiotic factors such as aluminium have been the subject of many studies, demonstrating a toxic synergy between this metal and low pH (Wood, 1989). Whether acidic depositions increase susceptibility of fish to pathogenic microorganisms at low pH, has not been tested experimentally.

Rainbow trout Oncorhynchus mykiss (Walbaum) could acclimate well to soft water of pH 4.0, in the absence of aluminium (Balm & Pottinger, 1993). Although their gills showed no morphological differences between control and acid treated fish by light microscopy, immune reactions were evident by electron microscopy (Balm & Pottinger, 1993). The present communication records the number of protozoan ectoparasites on the gills of these fish.

Gill tissue samples (first arch, middle region) were examined from control (pH 7.1) and low pH (4.0) treated fish, taken 10 and 17 days after the onset of acidification during the 1989 experiments described in detail by Balm & Pottinger (1993). All fish were sampled on the same day, exposure of the 17-day group commencing 7 days prior to the 10-day exposed group. Parasites were quantified in paraffin sections (10 µm thickness) of the gills from 18 fish (six controls and six low pH fish exposed for 10, or 17 days respectively). Longitudinal sections of gill filaments were examined over 200 µm, covering about 10 secondary lamellae, in four different sections of the material, and the results were averaged to yield one value per fish. The experimental groups (n=6) consisted of animals from duplicate tanks (Balm & Pottinger, 1993; three fish from each tank). Both juvenile and adult stages were counted. To confirm the identity of the parasites, tissue samples collected in August 1993 using identical procedures, were examined under a scanning electron micrograph. A section of gill arch supporting four filaments was fixed in 3.5% glutaraldehyde in 0.1 M cacodylate buffer for 24 h. The sections were rinsed three times in 15-2% sucrose in 0.1 M cacodylate buffer and post-fixed in 2% osmium tetroxide in 0.1 M cacodylate buffer. After rinsing another three times with buffered sucrose the samples were dehydrated through acetone, critical point dried and sputter-coated with gold-palladium, before being examined with a JEOL 25S SEM.

The only microorganism found was the ectoparasitic ciliate Trychophrya intermedia (Prost, 1952; Protozoa, Ciliophora, class Suctoria; Figs 1–3).
Figs. 1–3. *T. intermedia* on the gills of *O. mykiss*. Fig. 1, scanning electron micrograph, bar represents 10 μm; Figs 2 and 3, light microscopical photographs of juvenile (Fig. 2) and adult (Fig. 3) specimen (Shul'man & Shtei, 1964), ×425. All photographs are from low pH-treated fish (17 days).

Fig. 4. The effect of pH 4.0 on the number of *T. intermedia* specimens on the gills of *O. mykiss*; averages ± S.E.M; n=6 for each group. Significance was tested by means of the Mann–Whitney U-test. Fish were infected heavier with *T. intermedia* after 17 days (P<0.05), but not after 10 days, at low pH.

may be synonymous with *Capriniana piscium* (Buetschli, 1889; Lom, 1971)]. After 17 days, there were significantly more ectoparasites on the gills of the low pH-treated fish than of control fish (Fig. 4). There were no differences between the experimental groups in condition factor, plasma cortisol, chloride, or glucose.
It is not clear why branchial ectoparasites were commoner on rainbow trout at low pH. However, the effect was not secondary to tissue damage, which previously was shown to be absent from animals in this study (Balm & Pottinger, 1993); nor was it due to cortisol-induced immunosuppression, because plasma cortisol levels were not elevated at 10 and 17 days, nor in other fish at 4 h, 2 d, 7 d, and 14 d after the onset of acidification (Balm & Pottinger, 1993). The low pH treated fish showed no other signs of stress, such as a reduced feeding response or lower K-factor (Balm & Pottinger, 1993). Tank conditions may have favoured the parasites through increased food supply inducing the differential parasite loading and tissue response. However, the incoming 30 l. min⁻¹ of lake water was acidified just before it entered the tanks. Although rapid turnover of the water would not preclude the developments of biofilms, the acid-exposed tanks appeared markedly cleaner than control tanks, suggesting that they actually supported less algal/bacterial growth than the control tanks. Hence the low pH treated fish may have become more susceptible to the ectoparasite, or the acidity may have been more conducive to parasite reproduction in the gills.

Many parasitic organisms evoke immunological responses in fish (Woo, 1992). We therefore suggest that the present parasitic infection contributed to the branchial immune reactions observed at the EM level in companion samples (Balm & Pottinger, 1993). Although Trychophrya species have been suggested to be ectocommensal and to feed on mucus (Rogers & Gaines, 1975), bacteria, or on other ciliates (they are ideally positioned with tentacles extended into the branchial chamber to capture such prey; Figs 1–3), they also have been considered injurious to fish (Meyer, 1964, cited in Hoffman, 1967). Destruction of gill epithelium (Shul'man & Shtein, 1964), and mortalities of both brood fish and fingerlings (Rogers & Gaines, 1975) have been described as consequences of infection with Trychophrya species.

The present data suggest that the gill pathology, characteristic of fish in acidified waters, might be caused in part by ectoparasites. The parasites might even complicate the fishes ionoregulation, since pathogenic microorganisms affect these mechanisms in epithelial cells (Wick et al., 1991). By occluding the respiratory surface of the branchial epithelium, the parasites will impair gaseous and ion exchange, processes also threatened by the low pH conditions (Wood, 1989) (Figs 1–3). Also, the increased mucification reported for fish under low pH conditions might have been triggered in part by ectoparasites. Recently, Urawa (1992) described a dramatic increase in epidermal mucous cells of chum salmon Oncorhynchus keta Walbaum experimentally infected with the ectoparasite Ichthyobodo necator, particularly in AB-negative, PAS-positive mucous cells, which were suggested to contain anti-parasitic substances. Balm et al. (1995) have described similar qualitative changes in the epidermis of these trout resembling also those reported by Pickering & Fletcher (1987) for Salmo trutta L. and Salvelinus alpinus L. infected by ectoparasites.

Increased ectoparasitic infections have occurred in fish exposed to toxicants (Koskiivaara, 1992; Poulin, 1992), but the underlying mechanisms and their relative contribution to toxic effects are largely unknown. Our data show that branchial infection by parasites might occur in fish exposed to acid environments before major integumental dysfunctions, and may even be responsible for such pathology.

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