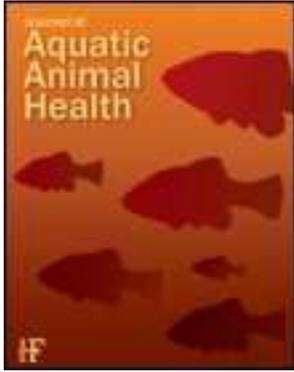


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COMMUNICATIONS

Culture Method Influences Degree of Growth Rate Reduction in Rainbow Trout Following Exposure to Hydrogen Peroxide

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Abstract.—We used hydrogen peroxide to reproduce gill lesions typical of a broad assemblage of gill diseases encountered in aquaculture and examined the degree of growth rate depression that it caused. Additionally, we compared growth rates of gill-damaged fish when they were either kept separate from or cohoused with healthy untreated fish. In contrast to expectations, treated fish reared separately from controls exhibited a much more dramatic decline in growth rate (30% less than controls) compared with those reared with controls (13% less than controls). Although the effect was transient and persisted for only 2 weeks, it suggests that when considering the bioenergetic costs of disease and when designing studies to quantify the costs, the interaction of diseased fish and healthy fish needs to be considered.

Although gill diseases are a common problem affecting the intensive culture of salmonids, their economic effects are incompletely understood, other than being estimated through mortality rate data (Speare and Arsenaault 1997). This approach can potentially grossly underestimate the true bioeconomic effects because it fails to consider the impact of depressed bioenergetic efficiency of survivors (Speare et al. 1998). Although such data would be relevant, they are elusive because, as noted by Beamish et al. (1996), research to elucidate the effects of fish diseases (in general) on bioenergetics is scant, and research approaches have not been refined. Secondly, with specific relevance to gill disease, there are only isolated functional experimental gill disease models that mimic aquaculture scenarios.

With respect to the problem of lack of gill disease study models, we have developed an experimental branchitis model that creates gill lesions that are typical of those arising during a broad assemblage of gill diseases encountered in aquaculture (Speare et al. 1999). In addition to being a generic gill disease model, the specific protocol of short-duration–high-dose exposure to hydrogen

peroxide (20 min exposure to 1,250 mg hydrogen peroxide/L) is identical to a treatment regime used to treat Atlantic salmon *Salmo salar* that were reared in a marine net-pen for the caligid copepod *Lepeophtheirus salmonis* (Speare et al. 1999). The gill lesions affecting juvenile rainbow trout *Oncorhynchus mykiss* created by this model are the same as those that arise in salmon following treatment for lice, and fish demonstrate marked growth rate depression that correlates with gill pathology (Speare et al. 1999).

In the present study, we used the hydrogen peroxide gill disease model to examine whether the degree of growth rate depression changes between gill-damaged fish kept with or kept separate from control fish. This question is relevant to developing appropriate methodologies for studying the bioenergetic costs, and hence the economic costs, of gill diseases.

Methods

Juvenile age-matched rainbow trout (mean weight 34.7 g) were obtained from a federal fish hatchery certified as specific pathogen-free. From a batch of 2,500 fish, 120 were arbitrarily removed, anaesthetized in an aqueous solution of benzocaine (60 mg of benzocaine/L of water), weighed, individually marked via implanted tags (Speare et al. 1999), and randomly assigned in equal numbers (20 fish) to each of 6 identical 70-L tanks supplied with freshwater at 10°C from a pumped well. For the next 3 weeks, fish were acclimated to tank and feeding conditions (Speare et al. 1999).

Two tanks of fish (tanks 1 and 2) were treated with 1,250 mg hydrogen peroxide/L for 20 min (Speare et al. 1999), and two other location-matched paired tanks of fish (tanks 3 and 4) received an identical sham-treatment only. To establish cohabitation, another tank of fish (tank 5) was also treated, and at the end of treatment, 10 fish from it were moved to another tank (tank 6) with untreated fish, and 10 untreated fish were moved reciprocally from tank 6 to tank 5 to replace them.

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The concentrations of hydrogen peroxide applied were verified by use of a potassium permanganate (KMNO₄) titration (Douglas and Donald 1982: 366–371). Peroxide was removed by lowering water levels and refilling with fresh water.

Fish were hand fed to satiation twice daily according to an ad libitum without wastage method (Speare and Arsenaault 1997) for 6 d/week with a commercially prepared trout ration (High Pro Grower, pellet size number 3, Corey Feed Mills, Ltd, Fredericton, NB). The trial was “single blind,” in that the person feeding the fish was unaware of treatment allocations. The sequence of tank feedings was altered daily to avoid anticipatory stress and possible artifacts created by feeding sequence on appetite. Fish were individually weighed every week after 1 d of starvation. The trial continued for 5 weeks after the time of exposure to peroxide.

Growth rates were presumed to be exponential and were calculated for each fish as the specific growth rate (SGR = logarithmic growth per unit time) (Jørgensen and Jobling 1993) expressed as percentage daily gain. Following assessment of tank effects within a treatment class and pooling of data, SGR values for treated and control fish were analyzed with a two-way analysis of variance (ANOVA; factors, treatment and time) followed by individual comparisons (where appropriate) between treatments with the Tukey–Kramer multiple-comparison test (Instat; GraphPad Software, Inc., San Diego, California). Differences were considered significant at $P = 0.05$.

Results and Discussion

Hydrogen peroxide treatment suppressed the growth of fish; however, the extent of this effect differed between the two rearing conditions (Table 1). When peroxide-treated fish were kept separate

from control fish, there was a significant marked effect of peroxide treatment on the SGR (Table 1). Specifically, compared with control fish, peroxide-treated fish had an SGR that was depressed by 31% during the first week after treatment and 16% during the second week after treatment. However, in contrast, when treated fish were kept as cohabitants with untreated fish, the relative depression in growth rate was not statistically significant (Table 1) and was only 13% during the first week after treatment and 7% during the second week after treatment when compared with cohabitating control fish. This can only partly be explained by growth suppression of control fish that were moved into the cohabitant tanks. By the third week after treatment, growth rates of all treated fish had returned to match those of untreated controls. There was no evidence of compensatory growth (Sumpster 1992) in the ensuing weeks (Table 1).

Results of this trial concur with an earlier report (Speare et al. 1999) in which the mechanism of hydrogen peroxide gill damage and impairment of growth rates through altered feed conversion ratios were described. The present study extends the original findings in that the relative degree of growth rate depression appears to have been moderated when treated fish were kept as cohabitants with untreated fish. This finding has application, relative to the choice of methodologies, for future studies aimed at addressing the effect of various diseases on growth. For example, to model the growth-inhibiting effects of a gill disease that may have low-to-moderate prevalence (for example: that induced by the microsporidian parasite *Loma salmonae*) within a fish population, a cohabitation experimental study may provide more realistic data. The reverse would apply for a disease in which all fish would be affected (for example, algal bloom branchitis).

TABLE 1.—Comparison of mean specific growth rates (SGR [% daily gain] \pm SD) of peroxide-treated rainbow trout when kept either separated ($N = 40$) or cohabiting ($N = 20$) with untreated controls. Week 4 was 1 week after peroxide treatment. Asterisks indicate significant differences ($P < 0.05$) between treated and control fish.

Week	Separate tanks				Co-habitated			
	Treated	Control	% Δ^a	P	Treated	Control	% Δ	P
1	1.35 \pm 0.48	1.28 \pm 0.48	-5.5	0.53	1.31 \pm 0.48	1.57 \pm 0.79	16.6	0.21
2	2.29 \pm 0.48	2.31 \pm 0.44	0.9	0.88	2.16 \pm 0.37	2.23 \pm 0.46	3.1	0.61
3	1.93 \pm 0.33	1.81 \pm 0.33	-6.7	0.12	1.71 \pm 0.54	1.79 \pm 0.38	4.5	0.59
4	1.20 \pm 0.48	1.74 \pm 0.61	31.0	0.00*	1.37 \pm 0.79	1.58 \pm 0.51	13.3	0.32
5	1.78 \pm 0.55	2.12 \pm 0.53	16.0	0.00*	1.64 \pm 0.40	1.76 \pm 0.50	6.8	0.40
6	1.44 \pm 0.51	1.45 \pm 0.57	0.7	0.92	1.47 \pm 0.68	1.52 \pm 0.61	3.3	0.81
7	1.69 \pm 0.29	1.87 \pm 0.33	9.6	0.06	1.70 \pm 0.32	1.76 \pm 0.24	3.4	0.53
8	1.49 \pm 0.40	1.51 \pm 0.40	1.3	0.80	1.41 \pm 0.45	1.50 \pm 0.53	6.0	0.57

^a $100 \times [\text{SGR}(\text{control}) - \text{SGR}(\text{treated})]/\text{SGR}(\text{control})$.

In designing this experiment we anticipated finding a greater growth rate depression in those fish kept as cohabitants with untreated fish. This was based on the assumption that fish with gill lesions would become subdominant fish and, therefore, have slower growth rates because of competitive interaction (Li and Brocksen 1977; Metcalfe 1994). This effect would theoretically not arise when all the fish in a tank had a similar degree of gill damage. However, our data shows an opposite effect to our expectation, and there are several possibilities for this. In our study, the fish were fed to satiation. Hence, subdominants would not have restricted access to feed; they may simply feed later than dominant fish. If a restricted amount of feed had been offered, the results may have been different. It must also be considered that social behavior may have benefited the treated group by stimulating feeding activity by association with their untreated cohorts. Further studies are therefore warranted to understand the relevant mechanisms and interactions that would explain the differences in growth suppression induced by gill disease that arise in different cultivation scenarios.

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