

**Prospects for selective breeding for stress tolerance in
aquacultured fish**

Svein-Erik Fevolden^{a1} & Knut H. Røed^b

^aNorwegian College of Fishery Science, University of Tromsø, N-9037 Tromsø,
Norway;

^bNorwegian School of Veterinary Science, P.O.Box 8146, Dep., N-0033 Oslo,
Norway

Abstract

Results from a closing EU-project "Selective breeding for stress tolerance in aquacultured fish" in which post-stress levels of cortisol and lysozyme stress responsiveness were used as selection criteria, are summarized. The study has been aimed at i) comparing heritabilities of cortisol and lysozyme and estimate phenotypic and genetic correlations between them; ii) producing selection lines based on high or low responsiveness for cortisol and lysozyme; iii) assessing the consistency of altered stress response in the progeny; and iv) testing progeny groups for various performance traits. The results have manifested that selection for both cortisol and lysozyme post-stress activity is indeed feasible, with cortisol slightly more promising than lysozyme. There is a tendency for negative correlations between the two traits following the applied confinement stress. Based on the performance of the progeny there is clear evidence that the selection has affected various performance characteristics, but the results are not straightforward as to which of the two selection criteria is the most promising in terms of practical aquaculture applications.

Key words: cortisol, lysozyme, rainbow trout, selective breeding, stress response

¹ sveinf@nfh.uit.no

Introduction

Given what is known about the adverse effects of stress, an enhanced tolerance of stressful procedures has been thought to improve e.g. food conversion and growth, and reduce the incidence of diseases under aquaculture conditions. Better tolerance of stress may also lead to improved fecundity, egg quality, post-spawning survival, and flesh quality. The goal of breeding for a low stress-responsive strain of fish, therefore, embraces several traits identified by the aquaculture industry as key candidates for improvement.

Cortisol is highly recognized as a stress indicator of fish (c.f. review by Wendelaar Bonga, 1997). In both Atlantic salmon and rainbow trout the genetic components of cortisol stress-response have been established (Fevolden et al. 1991, 1993, 1994, 1999; Pottinger & Garrick, 1999a). Lysozyme, which is known as a nonspecific lytic immune parameter, has also been shown to alter blood concentrations of fish following stressful stimuli (Moeck & Peters 1990; Fevolden & Røed 1993, Demers & Bayne 1997), and to be under a certain genetic control (Røed et al. 1993a, 1993b). A summary of results from a recently closed EU-project "Selective breeding for stress tolerance in aquacultured fish" (FAIR-CT95-0152), in which both cortisol and lysozyme were used as selection criteria, will be presented herein.

The aims of the Norwegian partners in this joint project (other participating countries were England, Spain, and Hungary) can be specified as follows:

- revisit the genetic components of cortisol and lysozyme responsiveness to stress in aquacultured rainbow trout
- compare heritabilities of the two traits and estimate phenotypic and genetic correlations between them
- using a family selection regime, produce selection lines based on high or low responsiveness for cortisol and lysozyme and, thus
- assess the consistency of altered stress response in the progeny, and finally
- test progeny groups for various performance traits

Material & Methods

Detailed information about the fish that were used for the study (F-O generation), and the stress procedures employed are presented in Fevolden et al. (1999). The following gives a brief summary of activities during the course of the study:

F-O generation (parental group)

In April 1996 ten fish were randomly selected from each of 50 full-sib families of the 1994 year group of rainbow trout at Norske Fiskeoppdretteres Avlslag AS (Norwegian Salmon Breeding Inc.). At approximately 6 - 8 week intervals the 500 fish were subjected to a standardized confinement stress. Fish were netted from the sea cage and transferred to a tank with low water depth for a period of 30 minutes. The fish were thereafter anaesthetized, weighed and length measured, and blood were collected for analyses of lysozyme, cortisol and a series of immune parameters.

F-1 generation (progeny group)

In March/April 1997 four selection lines were made by crossing F-O families with consistently high or low cortisol and lysozyme post-stress responses producing high cortisol responders, low cortisol responders, high lysozyme responders, and low lysozyme responders. Controls comprised a random sample of families of the actual year class. The fishes were transferred to sea water in April 1998. Over the last two years (1998, 1999) these lines have been tested for the magnitude of inherited stress responsiveness by being subjected to repeated stress exposures, and for correlations between stress and various production traits. The lysozyme lines have also been compared for their performance in pathogen challenge tests (cf. Fevolden *et al.*, 1998)

Analytical work

Procedures for analyses of cortisol and lysozyme are in Fevolden *et al.* (1999). Growth performance was considered by means of specific growth rates defined as $100[\ln(WT2) - \ln(WT1)]/(T2 - T1)$ where WT2 and WT1 are the current and previous body weight measurements and T2 - T1 are days between measurements. Coefficient of condition (K-factor) was measured as $(100Weight)/length^3$. Flesh quality was measured with a texture analyser and expressed as the resistance force needed for a plunger to break through the fillet and until it had reached down to 60 % of the fillet height. These measurements were taken after 5 and 12 days storage at 4 °C. Details about these methods and about the bacteriological assays will be presented in upcoming papers.

Results

The following paragraphs outline some of the main findings of the project.

- Estimates of heritability of lysozyme and cortisol in the F-O generation were moderate to high, higher for cortisol (~0.5) than for lysozyme (≈ 0.3). Both traits displayed positive and highly significant phenotypic and genetic correlations in pair wise comparisons of stress exposures, (Fevolden *et al.* 1999).
- The post-stress cortisol responsiveness in the selected progeny lines (F-1) revealed highly significant differences between the two cortisol lines with the consistently higher mean values displayed in the line derived from high-cortisol parents (Fig. 1). Phenotypic correlations (comparing individual for individual) of cortisol response between the different stress samplings are very strong ($P \ll 0.001$).
- Following vaccination (triple vaccine against *Vibrio anguillarum*, *V. salmoninarum*, and *Aeromonas salmonicida*), fish of the high lysozyme responding line displayed higher post-stress lysozyme response than fish of the low lysozyme line (Fig. 2; two latter columns). Phenotypic correlations of post-stress lysozyme levels between stress samplings were in general significant at $P < 0.001$.
- There seems in general to be a higher cortisol responsiveness in the low lysozyme line and *vice versa*. Phenotypic correlations between the two traits were significantly negative in two of four cases.

- Mortality following confinement stress of fish recently transferred to high salinity water, was greatest among the high cortisol progeny. Osmolality and chloridity were significantly elevated in fish that died following the combined stress of transfer to salt water and confinement. Moreover, osmolality was significantly higher in the high cortisol line than in the low cortisol line.
- Progeny from low cortisol responders tended to display significantly higher growth rates than progeny from high responders. The coefficient factor was in general higher in low cortisol responders than in high responders.
- Progeny from high lysozyme responders exhibit consistently but insignificantly higher growth rates than progeny from low lysozyme responders. The coefficient of condition, however, was always significantly higher in low lysozyme responders than in high lysozyme responders.
- In two challenge experiments, following infection with *Aeromonas salmonicida*, progeny of high lysozyme responders displayed significantly higher mortality than low lysozyme progeny. However, accumulated mortality following infection with *Vibrio anguillarum*, was higher in low lysozyme progeny (cf. Fevolden et al., 1998).
- Before vaccination, levels of total IgM was significant higher in the low cortisol line than in the high cortisol line whereas no difference was detected between the two lysozyme lines. After vaccination, the levels of total IgM were significantly higher in the high lysozyme line than in the low lysozyme line whereas no difference was detected between the cortisol lines. Levels of specific antibodies against both *A. salmonicidae* and *V. anguillarum* were higher in the high lysozyme as compared to the low lysozyme line. The differences between high and low cortisol lines for these traits were less clearcut.
- Measurements of texture in fish that was stressed prior to slaughter gave evidence of significantly lower flesh quality than in flesh of non-stressed fish. In stressed fish there was also a trend towards better quality in flesh of low-cortisol responders than high cortisol responders. Bacterial counts were higher in stressed fish than in non-stressed fish.

Discussion & concluding remarks

The results herein (details will be presented in various papers *in prep.*), those of Fevolden et al. (1998, 1999), and of Pottinger & Carrick (1999a, 1999b) with reference to the same EU-project) have manifested that selective breeding of fish for altered stress tolerance is indeed feasible. Moreover, we have demonstrated that selection for stress responsiveness definitely results in altered performance of aquacultured fish.

Based on heritability estimates and consistency in performance of the progeny lines, selecting for post-stress cortisol levels seems more promising than selecting for post-stress lysozyme levels. The correlation between the two traits is in fact weak, and in cases of significant correlations among the progeny they are negative, which contradicts earlier suggestions (e.g. Fevolden & Roed, 1993).

The data obtained provide qualified support for better growth performance in low cortisol responders than in high cortisol responders. As to fish selected for post-stress lysozyme

response, there is a trend towards better growth performance in the high responding line (in vaccinated fish). Mortality following multiple stressors (high salinity plus confinement) was higher in high cortisol responders than in low cortisol responders. Although highly significant differences were seen between lines in susceptibility to bacterial challenge and immune response after vaccination, the response was not straightforward with respect to different pathogens and immune parameters, which reflects the complexity of processes involved in the immunological performance of the fish.

As to flesh quality low-stress fish tended to perform better than high-stress fish. The imposition of stress itself was the main determinant of changes in flesh quality although there was a trend towards better performance among fish of the high cortisol selected line than those of the low cortisol line.

Despite some promising results, we are not yet in a position to state whether selection for high- or low-stress responsiveness is advantageous or of negligible significance under aquacultural conditions. The altered performance that is reported is only on the first generation of progeny from selected parents. Application of selective pressure to a second generation should, hopefully, provide even greater divergence and offer a better opportunity to evaluate stress response as a useful selection criterion.

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Cortisol in cortisol lines

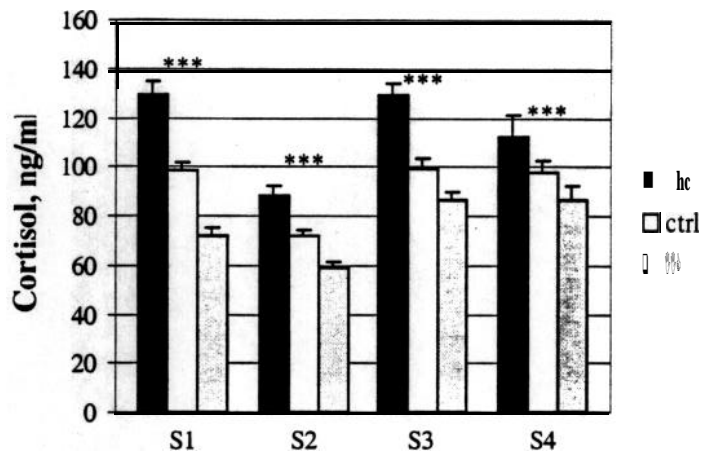


Figure 1. Mean post-stress levels of plasma cortisol following four stress exposures of the selected progeny groups: S1, May 1998; S2, August 1998; S3, October 1998; S4, February 1999. Vertical bars: SEM. *** $P < 0.001$ in comparison between high cortisol (hc) and low cortisol (lc) lines.

Lysozyme in lysozyme lines

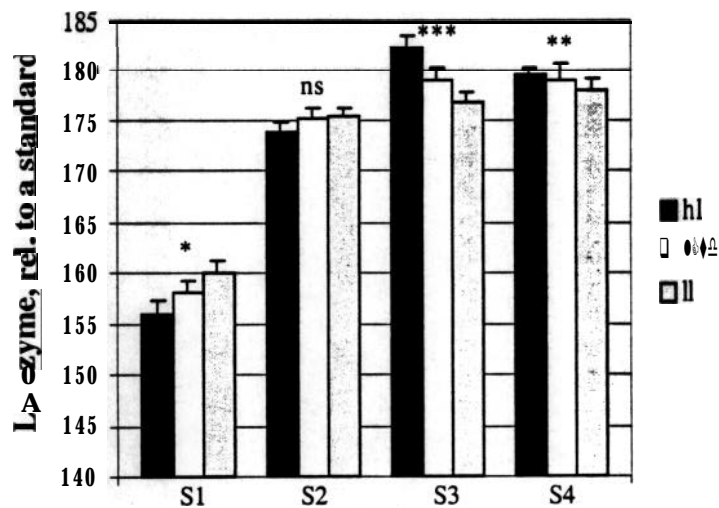


Figure 2. Mean post-stress levels of plasma lysozyme following four stress exposures (S1 - S4; cf. Fig. 1) of the selected progeny groups. Vertical bars: SEM. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ in comparison between high lysozyme (hl) and low lysozyme (ll) lines.