A NON-INVASIVE STRESS ASSAY FOR RAINBOW TROUT

by T. Ellis, A.P. Scott and J. James

CEFAS
The Centre for Environment, Fisheries & Aquaculture Science

Introduction

Stress in farmed fish is of considerable significance to both welfare and productivity as it can reduce immune status, growth and reproductive success, and cause mortality. Assessment of stress level typically involves measurement of the concentration of the stress hormone, cortisol, in the blood. However, blood sampling necessitates capture, handling and bleeding and is therefore a harmful procedure and is inherently stressful to both the sampled fish and those remaining in the tank. Previous pheromone research has shown that fish release hormones into the water. Here we report on the development of methodology to measure free cortisol released into the water via the gills as a non-invasive stress assay for rainbow trout.

Methodology

Water samples are taken from fish tanks without disturbing the fish and pumped through solid-phase extraction cartridges (Figure 1). The cartridges are eluted with an appropriate solvent to retrieve the cortisol, which is then quantified by radioimmunoassay (Figure 2).

Figure 1: Pumping water samples through solid-phase extraction cartridges

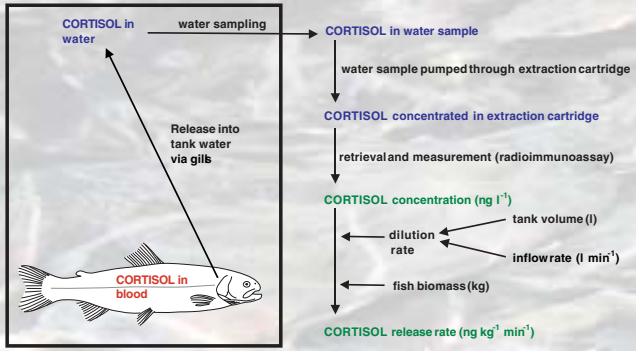

Figure 2: Basis of non-invasive cortisol stress assay.

Demonstration that cortisol is released into the water at levels reflecting the severity of the stress

Replicate tanks containing rainbow trout were exposed to standardised handling stresses and water samples were collected at regular intervals. Levels of cortisol in the control (unstressed) tanks remained constant (2 ng l^{-1}) over the 8 h duration of the study. In the stressed tanks, cortisol levels increased markedly and reflected the level of the stress, peaking at 20 & 100 ng l^{-1} in the single and repeat stress treatments respectively (Figure 3).

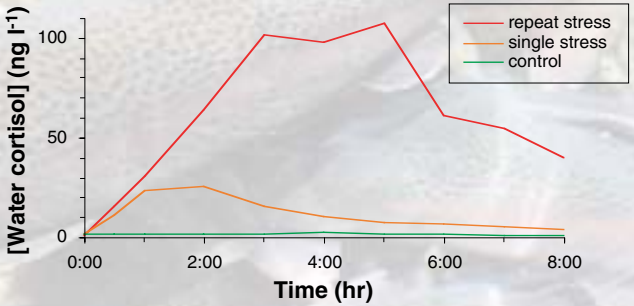

Figure 3: Release of cortisol by rainbow trout after exposure to no stress (control), a single handling stress (t = 0 h) or a repeated handling stress (t = 0, 1 & 2 h).

Validation of the methodology

We have done extensive validation experiments to verify that

- plasma and water cortisol levels are correlated (r_s=0.93, n=9, p=0.001)
- the recovery efficiency of the entire methodology is ≈ 87%, and the variability is acceptably low: CoV ≈ 10%
- the radioimmunoassay meets the required standards of intra- and inter-assay variation, specificity and parallelism
- the volume of water processed does not affect the concentration of cortisol measured
- freeze storage of water samples and extraction cartridges does not cause loss of cortisol
- water cortisol levels change as expected with temperature (Figure 4), dilution (Figure 5), fish size and biomass loading.

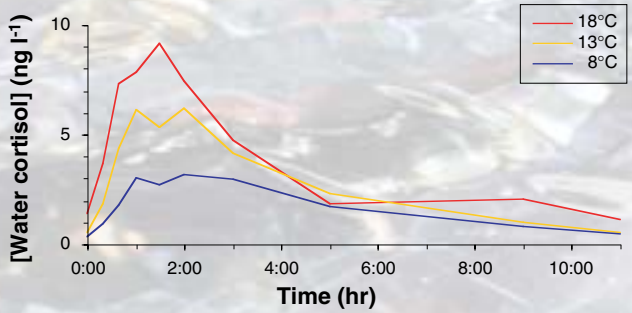

Figure 4: Release of cortisol by rainbow trout held at three different temperatures and exposed to a single handling stress at t = 0 h.

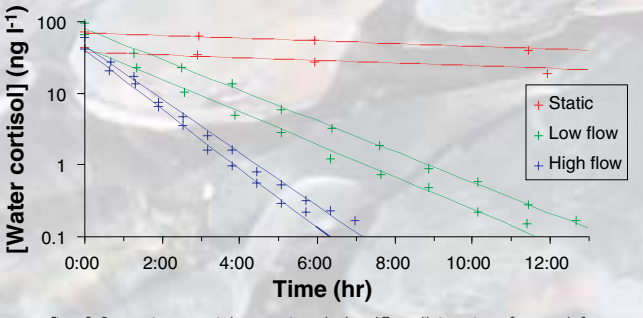

Figure 5: Decrease in water cortisol concentration under three different dilution regimes, after removal of previously handled rainbow trout. The exponential rates of decrease conform to a dilution model.

Discussion

We believe that this novel methodology has great potential for use in welfare research into husbandry and transportation practices. Water cortisol concentration (ng l^{-1}) can be used as a relative measure of stress level - over time or between replicate systems. Cortisol release rate (ng kg^{-1} min^{-1}) can be used as an absolute measure, but requires accurate information on biomass and dilution rates. The advantages of this technique for assessing fish stress level are that

- sampling does not disturb the fish or interfere with normal function
  - enabling repeated sampling thereby reducing the numbers of fish required for time-course experiments
  - allowing replacement of the stressful and harmful procedure of blood sampling which is lethal to small fish
- it is suitable for all fish sizes, including those too small to obtain a blood sample
- it is theoretically suitable for quantifying both acute and chronic stress
- it is (probably) transferable to other finfish species

We would be keen to apply our methodology to other systems and species.

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The Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Weymouth Laboratory, Barrack Road, The Nothe, Weymouth, Dorset DT4 8UB, UK. http://www.cefas.co.uk