

USING MUCUS TO ESTIMATE CORTISOL LEVELS TO ASSESS STRESS RESPONSE

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Introduction & objectives

The most commonly used method to assess stress in fish is by assessing cortisol levels in blood. However, sampling blood from fish is not always a good option e.g. where you have to work very fast, or where the workplace makes it very impractical (figure 2). Also, blood sampling could harm the fish and cause unnecessary stress in itself.

Over the recent years more attention has been drawn to other and less invasive methods than from blood to assess stress in fish. One of these methods, is by assessing the cortisol levels in the mucus of fish. Using mucus to estimate cortisol concentrations is still new, and therefore not much literature exists on this method compared to using blood.

We therefore aimed to do a comparative assessment of cortisol concentration in blood and mucus in Atlantic salmon (*Salmo salar*), while the fish were exposed to stress, to examine if the same reaction pattern, regarding cortisol production, occurs in the mucus as in plasma.

Results

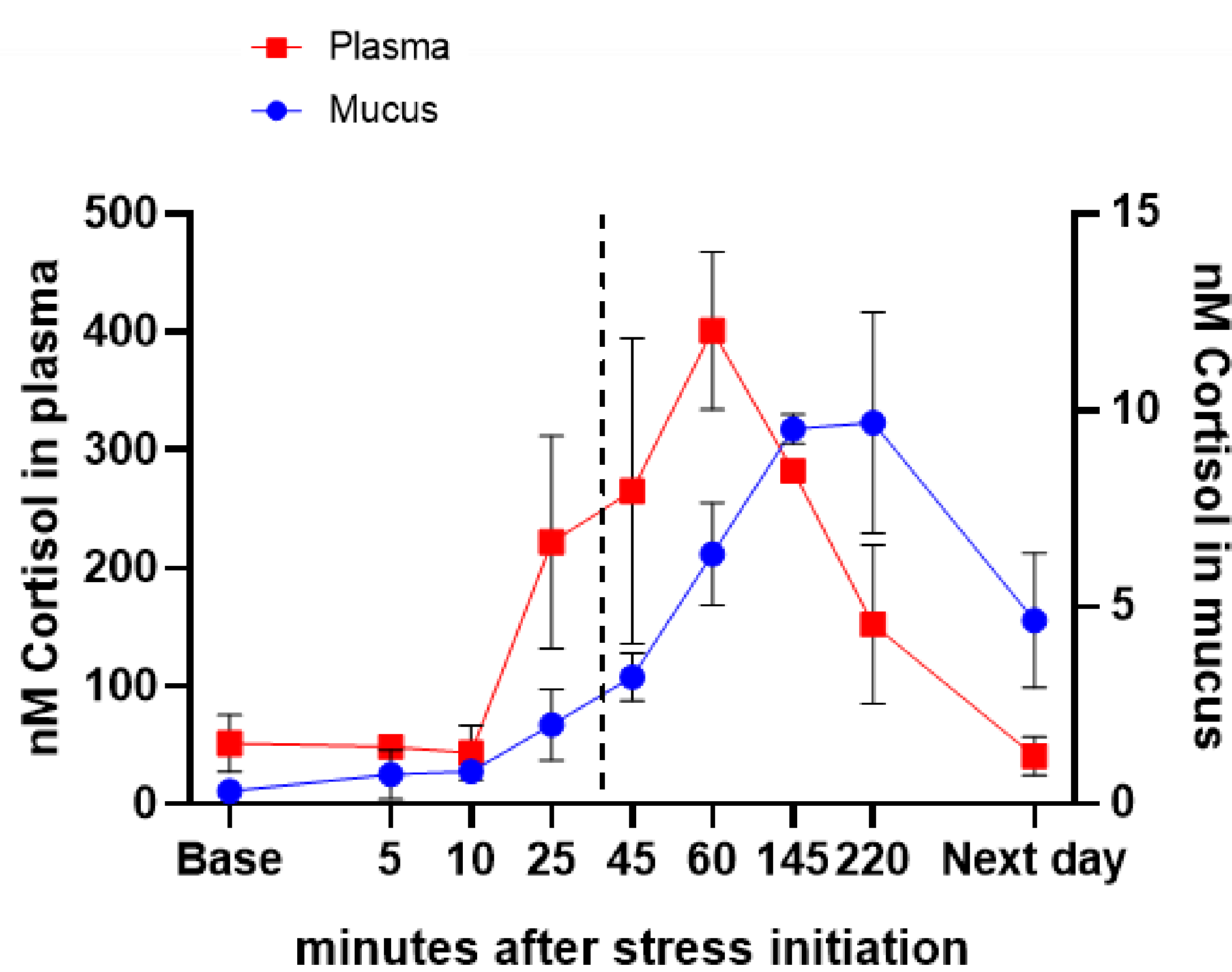


Figure 1. Cortisol levels in plasma and mucus. Dashed line at 30 min. represents the time when the water was raised to normal level again. Each point represents the average concentration of cortisol from 3 samples, and each sample is a pooled sample from two individuals.



Figure 2. Sampling mucus from Atlantic salmon on top of a transporting tank, a work-place where blood drawing is not a practical option.

Method

Atlantic salmon (~1 kg) were exposed to stress by lowering the water level in the tank, making it very shallow. Mucus and blood samples were taken 5, 10 and 25 minutes after the water level was lowered. After 30 minutes, the water was raised to normal level, and mucus and blood samples were taken 45, 60, 120 and 220 minutes after initial start. Samples were also taken the next day. Base cortisol levels were estimated 3 days prior to the experiment.

At each sampling point, a total of 6 fish were carefully captured and immediately anesthetized, that stops further cortisol production. Mucus was collected by gently scraping the lateral surface from cranial to caudal body. Blood was drawn from the caudal vein with a 21G Vacutainer needle, and spun and plasma was collected.



Mucus and plasma from two fish were pooled into one Eppendorf tube each, and immediately frozen at -80 °C until analysis. Plasma and mucus cortisol levels were measured using ELISA kit (Arbor Assay, Catalogue Number K003-H5) following the manufacturer's protocol.

Discussion & conclusion

Our results showed that the reaction pattern regarding cortisol production when the fish were exposed to stress, are very similar in the mucus and in plasma, even though the concentration of cortisol was substantially higher in plasma than in the mucus. Our results, however, showed that the cortisol concentration peaked earlier in plasma than in the mucus, and that it takes a longer time for the cortisol level to drop again in the mucus compared to plasma. This is something that you have to take in to consideration when results from mucus samples are being analysed.

This study shows that mucus is a good alternative to blood when it comes to estimating cortisol levels to assess stress response.

Example where mucus has been used to test stress response

A salmon fish farm in the Faroe Islands wanted to test how the fish responded to a new pumping system, that pumps fish from smolt tanks to transportation tanks. This required that we had to sample from the top of the transportation tank (figure 2), which is not a practical place to draw blood. Therefore, mucus was sampled instead.

The first time the pumps were used, our results showed that the fish got very stressed. The next day, after some adjustments to the pumping system, the fish did not get as stressed (figure 3.)

Figure 3. Stress response test to a new pumping system, using mucus instead of blood.

