Why Measure Steroids in Fish Plasma When You Can Measure Them in Water?

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Summary

The measurement of steroid concentrations in water rather than in blood plasma, has potential in a number of situations: to study steroidal pheromones; to measure sequential hormonal changes in fish from which it is difficult to get a blood sample (because the fish are too valuable or too small); to carry out non-invasive studies on stress and reproductive status. The main site of release of 'free' (as opposed to conjugated) steroids is the gills. Steroid concentrations in the water are related to levels in the plasma. Some steroids are released more readily than others – probably due to their affinity for steroid binding proteins.

What Steroids Are Produced by Fish?

Fish gonads produce a range of estrogens (C_{18}), androgens (C_{19}) and progesterone-like (C_{21}) steroids. The main estrogen produced by female teleosts is, as in mammals, 17β-oestradiol. Also, as in mammals, the C_{18} steroids, testosterone (T) and androstenedione, are common products of teleost gonads. However, in teleosts, they can occur in the blood plasma of females as well as males. The male-specific androgen in teleosts is 11-ketotestoterone. Teleost gonads produce

many different types of C_{21} steroids. However, most female teleosts produce, at the time of oocyte final maturation, the 'maturation-inducing hormone' (MIH) 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P). As in humans, the main corticosteroid produced by fish when they are stressed is cortisol. Conjugation plays an important role during both the synthesis and disposal of steroids in fish. The two main types of conjugated steroids in fish are sulphates and glucuronides. They can be formed directly in the gonads or by peripheral metabolism.

How Do Steroids Get Into the Water?

There are several potential routes of excretion of steroids into the water: gills; urine; faeces (via the bile); skin; seminal or ovarian fluid; specialised organs (e.g. the seminal vesicles of the African catfish). In a study by Vermeirssen & Scott (1996) on the fate of injected radioactive 17,20β-P in rainbow trout, most of the steroid had disappeared from the circulation by 6 h. During that time, 40% had been released through the gills, 35% was present in the bile and 10% had been released in the urine. The material released from gills was essentially unmodified 'free' steroid; that in the bile was mostly glucuronidated; and that in the urine was mostly sulphated. A later study confirmed that the gills also appear to be the main source of free steroids which are released into water by goldfish (Sorensen *et al*., 2000). The measurement of intact steroids which have 'leaked' from the gills forms the basis of our procedure – rather than the measurement of urinary or faecal metabolites.

How Are Steroids Measured in Water?

The concentrations of steroids in water are normally too low to be assayed directly. There are two ways to overcome this problem:

Extraction with organic solvents. Samples of water are shaken with organic solvents such as diethyl ether, the solvent dried down and the residue redissolved in a small amount of assay buffer. The drawbacks to the method are: the large amount of solvent that needs to be used if one is extracting a large amount of water; only the 'free' (non-conjugated) steroid fraction is extracted.

Solid phase extraction cartridges. This involves pumping or drawing water through cartridges containing octadecylsilane. Free, sulphated

and glucuronidated steroids are all trapped by the matrix and can be eluted with a small amount of methanol. This is the method of choice for extracting steroids from water and is described in detail by Scott & Sorensen (1994). This same paper also describes how free, sulphated and glucuronidated steroids can be quantified separately.

Are Plasma and Water Steroid Concentrations Related?

A comparison between the results of Scott & Sorensen (1994) and Moriwaki et al. (1991) on water and plasma steroid concentrations, respectively, in HCG-injected female goldfish, shows that the rise and fall of 17,20β-P and T in water exactly matches that found in the plasma. However, the peak of 17,20β-P in water is higher than that of T, whereas the reverse is true in the plasma.

What Applications Are There?

The study of fish pheromones – several of which, in fish, have been shown to be steroids. The one which is best known is 17,20β-P in goldfish. In the hours leading up to spawning, this steroid is produced in the gonads in order to induce oocyte final maturation. From the gonads, it finds its way into the bloodstream and from there into the water, where it is detected by specific receptors on the olfactory epithelium of male goldfish. This causes an increase in gonadotropin concentrations in the blood and an increase in sperm production. The pattern of release of 17,20β-P by the females and the 'active space' of the pheromone have been determined by extraction and assay of not only 17,20β-P, but a range of other steroids, from the water (Scott & Sorensen, 1994).

Monitoring the reproductive status of fish but without having to disturb them. 17,20β-P is not used as a pheromone by all species. However, it is still released into the water at the time of oocyte final maturation (Vermerissen & Scott, 1996). This means that one has the potential to monitor the final maturation process without having to bleed or, in some cases, even handle the fish. The method is particularly useful when one is dealing with fish which are too small or too valuable to take blood samples.

Monitoring stress in fish – but without any effect of sampling stress This is a potentially very important application. To date, all

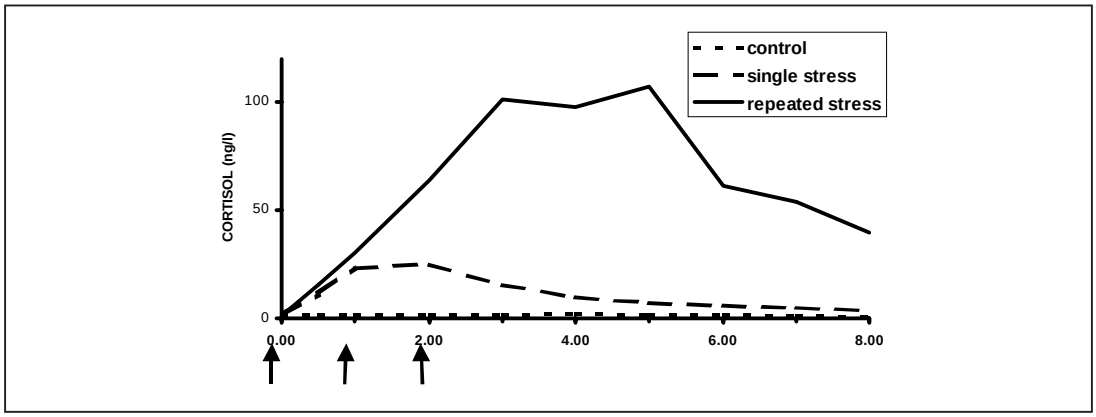

Fig. 1 Effect of a single or a repeated stress (lifting fish out of tank for 90 sec) on the amounts of cortisol released by rainbow trout. Tank volume = 150 L ; flow rate = 2 L·min^{-1}; fish density 30 kg·m^{-3}; temperature = 15 ^{o}C; control = no stress. Arrows indicate time of application of stress.

studies on the time course of the stress response in fish have had to take into account the fact that the very act of catching and bleeding a fish is a stress in itself.

The only way that researchers have been able to get around this problem is to employ a large number of replicate tanks and to use a different tank of fish at each time point. We have now shown (Fig. 1), by measurement of cortisol in the water outflow, that one can continuously follow the cortisol response to stress in single tanks of fish. The specificity of the cortisol immunoassay has been tested by running water extracts on TLC (Fig. 2; note >98% of the activity co-eluted with ^{3}H-cortisol).

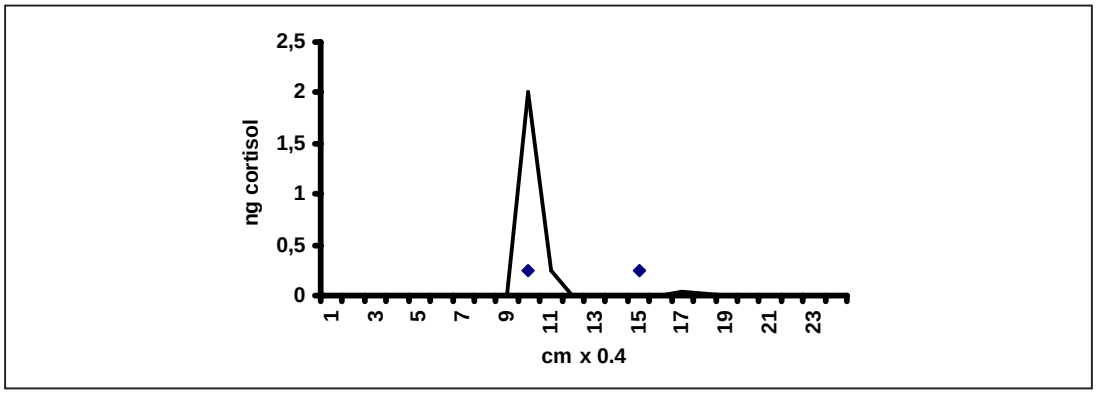

Fig. 2 Thin-layer chromatographic separation of material which cross-reacts with the cortisol antiserum in water extracts from rainbow trout tanks. The elution positions of cortisol and cortisone, respectively, are shown by diamonds.

Are There Any Interesting Implications?

The fact that unmodified steroids are released via the gills and are just as easily reabsorbed (Vermeirssen & Scott, 1996) might explain some of the reports of 11-KT being found in the blood of female fish and of E_2 in the blood of males. Also, the fact that some steroids, such as T, are released less readily than others, such as 17,20β-P, may be because they bind more avidly to plasma sex steroid binding proteins. The measurement of stress by the measurement of cortisol in water is a potentially powerful indicator for fish welfare studies. Research we have carried out to date have been very encouraging, although much remains to be done on the interactive effects of density, water flow rate, temperature and fish size on cortisol release.

The pros and cons of measurement of conjugated steroids and metabolites released in faeces and/or urine of fish are considered by Oliviera et al. (1999).

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