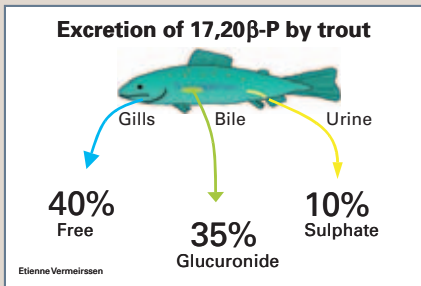


# Non-invasive measurement of fish steroids in water

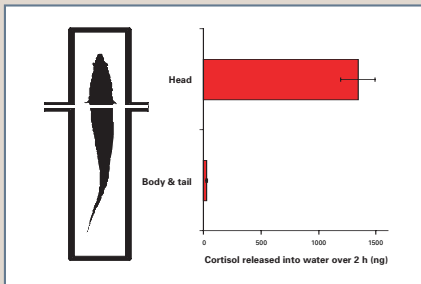
## The principle:

Free steroids circulating in the blood 'escape' into the water via the gills

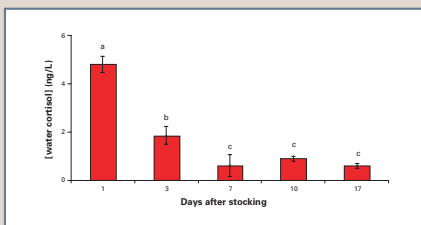


## Examples:

Free cortisol is released into the water via the gills (freshwater rainbow trout):



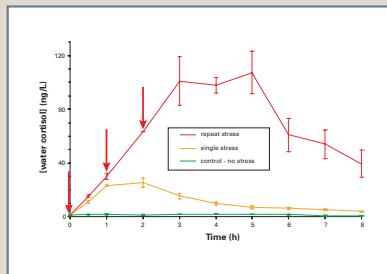
Cortisol levels take one week to recover when Atlantic salmon are transferred to a new tank:



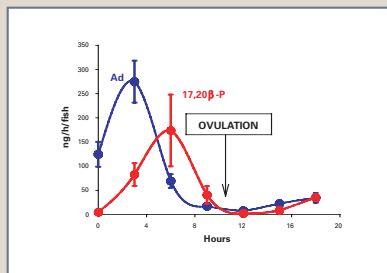
## The advantages:

- **Zero or minimal intervention (i.e. no anaesthetic, bleeding or handling stress)**
- **Repeat measurements on the same fish**
- **Results not biased by sampling stress**
- **Non-lethal measurements on small and/or rare fish**
- **Integration of response of many fish ... or single fish if required**
- **Measurements represent metabolically available (unbound) fraction**
- **Allows concurrent behavioural monitoring**
- **Gives early warning of disease outbreaks**

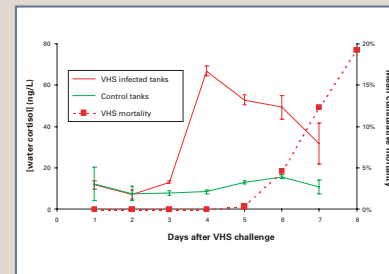
Water cortisol response to handling stress in freshwater rainbow trout:



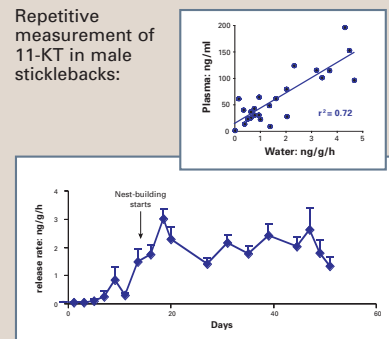
Release of 17,20β-P and Ad into water by HCG-induced female goldfish:



Water cortisol response to Viral Haemorrhagic Septicaemia infection in freshwater rainbow trout:



Repetitive measurement of 11-KT in male sticklebacks:



## How the steroids are measured:

By 'solid phase extraction' followed by immunoassay.



1. Tank water sample
2. Peristaltic pump
3. Pre-filter - to remove suspended solids
4. Solid phase extraction cartridge
5. Flask for measuring processed water volume

## Compounds measured in water:

- Cortisol
- Cortisone
- Androstenedione (Ad)
- 11-Ketotestosterone (11-KT)
- 17,20β-Dihydroxypreg-4-en-3-one (17,20β-P)
- Melatonin

## Key Cefas publications relating to measurement of steroids (and melatonin) in water:

Scott, A. P. and Sorensen, P. W. 1994. Time course of release of pheromonally active gonadal steroids and their conjugates by ovulatory goldfish. *General and Comparative Endocrinology* 95, 309-323. (first comprehensive study of the release of free, sulphated and glucuronidated steroids into water)

Sorensen, P. W. and Scott, A. P. 1994. The evolution of hormonal sex pheromones in teleost fish: poor correlation between the pattern of steroid release by goldfish and olfactory sensitivity suggests that these cues evolved as a result of chemical spying rather than signal specialization. *Acta Physiol. Scand.* 152, 191-205. (first report that fish release large amounts of cortisol into the water)

Vermeirssen, E. L. M. and Scott, A. P. 1998. Excretion of free and conjugated steroids in rainbow trout (*Oncorhynchus mykiss*): evidence for branchial excretion of the maturation-inducing steroid, 17,20β-dihydroxy-4-pregnen-3-one. *General and Comparative Endocrinology* 101, 180-194. (first evidence that free steroids are released via gills)

Greenwood, L. N., Scott, A. P., Vermeirssen, E. L. M., Foster, A., Mylonas, C. and Pavlidis, M. 2001. Plasma steroids in mature common dentex (*Dentex dentex*) stimulated with a gonadotropin-releasing hormone agonist. *General and Comparative Endocrinology* 123, 1-12. (first report of a correlation between steroid concentrations in plasma and water)

Sorensen, P. W., Scott, A. P. and Kihlström, R. L. 2000. How common hormonal metabolites function as relatively specific pheromonal signals in the goldfish. In *Proceedings of the 6th International Symposium on Reproductive Physiology of Fish*, Bergen, Norway, July 4-9, 1999 (Norberg, B., Kjesbu, O. S., Taranger, G. L., Andersson, E. and Stefansson, S. O., Editors), pp. 125-128. Bergen 2000, Norway. (further evidence that free steroids are released via gills)

Scott, A. P., Pinillos, M. and Ellis, T. 2001. Why measure steroids in fish plasma when you can measure them in water? *The Proceedings of the 14th International Congress of Comparative Endocrinology*, Sorrento, Italy, 26-30 May, 2001 (Goos, H. J. Th., Rastogi, R. K., Vaudry, H. and Parantoni, ed.), 1291-1295. Monduzzi Editore S.p.A. -Medimond inc., Italy. (first claim for a non-invasive assay of stress based on measurement of free cortisol in water)

Ellis, T., James, J. D., Stewart, C. and Scott, A. P. 2004. A non-invasive stress assay based upon measurement of free cortisol released into the water by rainbow trout. *Journal of Fish Biology* 65, 1233-1252. (first comprehensive validation of the procedure for measuring cortisol in water)

James, J. D., Scott, A. P. and Ellis, T. 2004. Water based measurement of rainbow trout melatonin. *Journal of Fish Biology* 65, 1298-1304. (first suggestion for a 'housekeeping' compound for cortisol)

Sorensen, P. W., Pinillos, M. and Scott, A. P. 2005. Sexually mature male goldfish release large quantities of androstenedione to the water where it functions as a pheromone. *General and Comparative Endocrinology* 140, 164-175. (discovery of a fish that releases a strikingly large amount of a particular sex steroid into the water)

Ellis, T., James, J. D. and Scott, A. P. 2005. Branchial release of free cortisol and melatonin in rainbow trout. *Journal of Fish Biology* 67, 535-540. (first proof that the gills are the main route of release of cortisol into the water)

Scott, A. P., Pinillos, M. and Huertas, M. 2005. The rate of uptake of sex steroids from water by teleost *Trutta trutta* L. is influenced by their affinity for sex steroid binding protein in plasma. *Journal of Fish Biology* 67, 182-200. (first evidence of a mechanism that controls the rate of release of different steroids into the water)

