

# INTERACTIONS OF CORTISOL ON NITRIC OXIDE SYNTHESIS in *Oncorhynchus mykiss*



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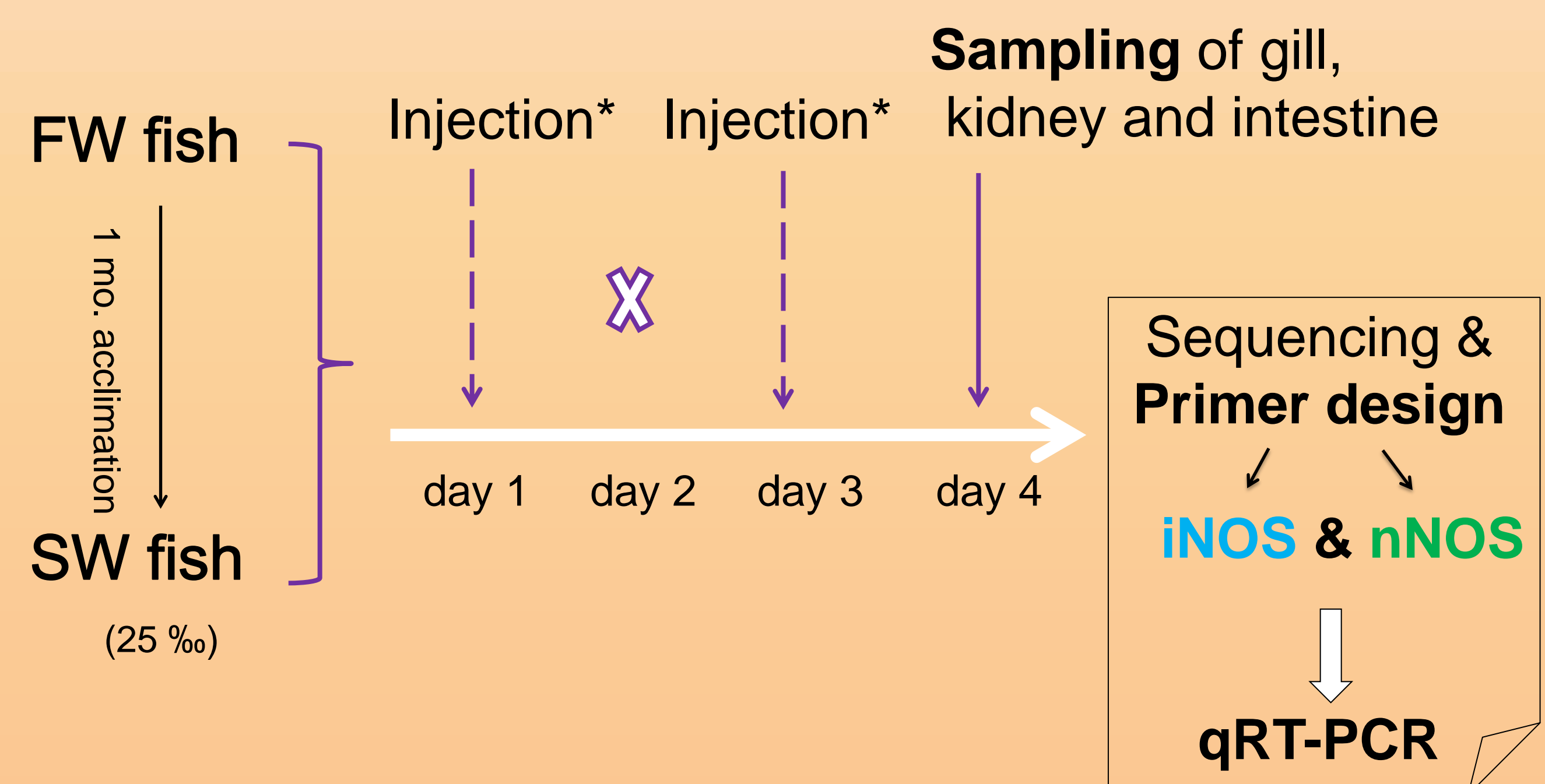
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## Background

**Acclimation** to various types of stress in fish often involves **stress hormones** such as **cortisol**. Recently the potential involvement of **nitric oxide (NO)** has also been emphasized. Both cortisol and NO have effects that influence metabolism as well as epithelial ion transport. Often these effects are antagonistic. Cortisol supports an increased metabolism and stimulates branchial Na<sup>+</sup>/K<sup>+</sup> ATPase activity, while NO inhibits mitochondrial respiration and Na<sup>+</sup>/K<sup>+</sup> ATPase activity [1, 2, 3]. Reports on mammals have shown an interaction between glucocorticoids and the NO system, including altered expression of nitric oxide synthase (NOS) in the kidney [4]. No studies have looked at the potential effect of cortisol on NO synthesis in fish. **We hypothesized** an interaction between cortisol and the NO system in fish, and **examined** the influence of cortisol on inducible (iNOS) and neuronal (nNOS) NOS expression in kidney, gill and intestine of rainbow trout acclimated to freshwater (FW) and seawater (SW).

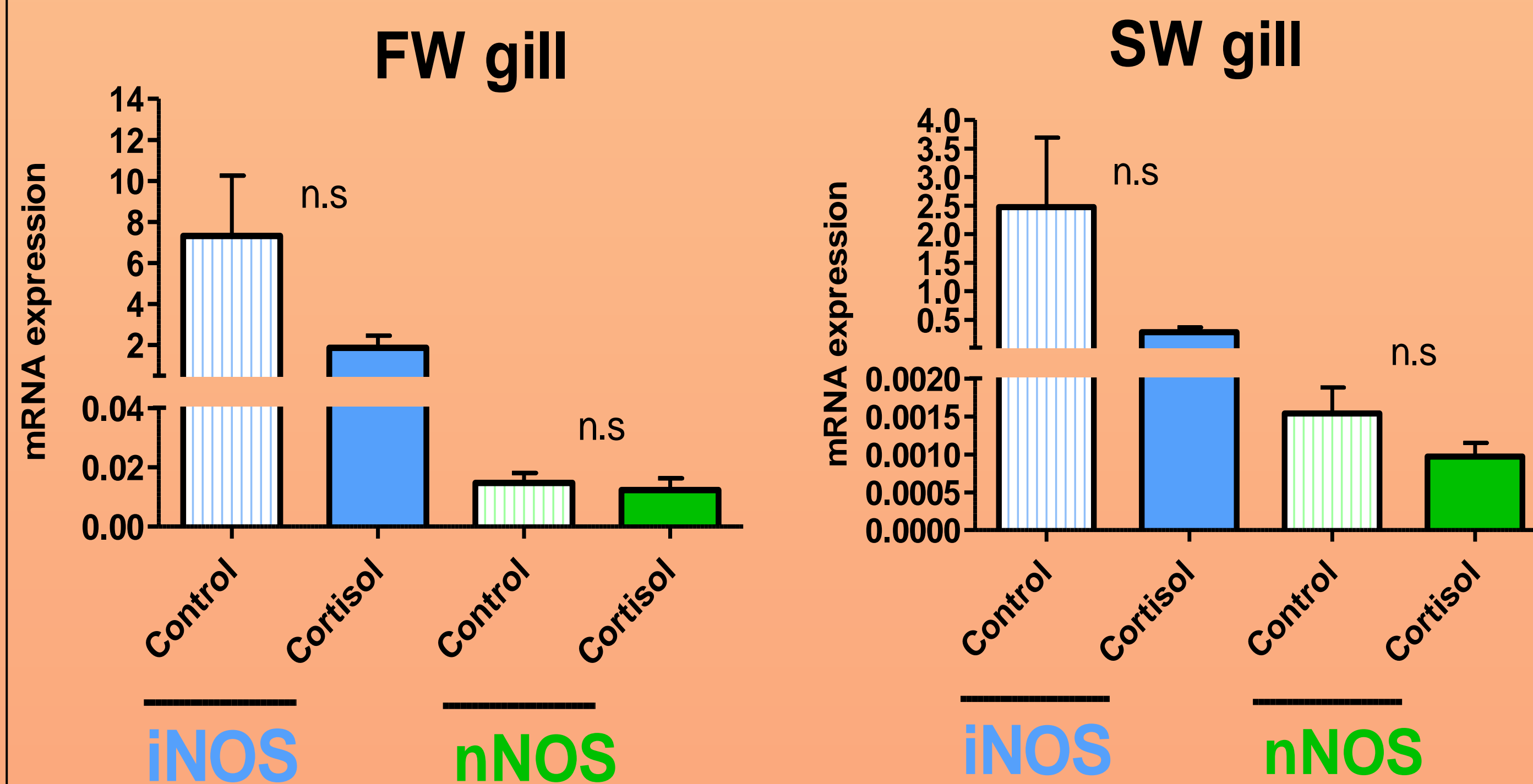
## Experimental design



\* Fish ( $\approx 45g$ ) were injected intraperitoneally with a hydro-cortisone hemi succinate solution ( $2\mu g/g$  of fish) - Control fish were injected with a saline solution

**GOAL** To evaluate the expression of iNOS and nNOS in osmoregulatory tissues in both FW and SW, and to examine the effect of cortisol injection on NOS isoforms.

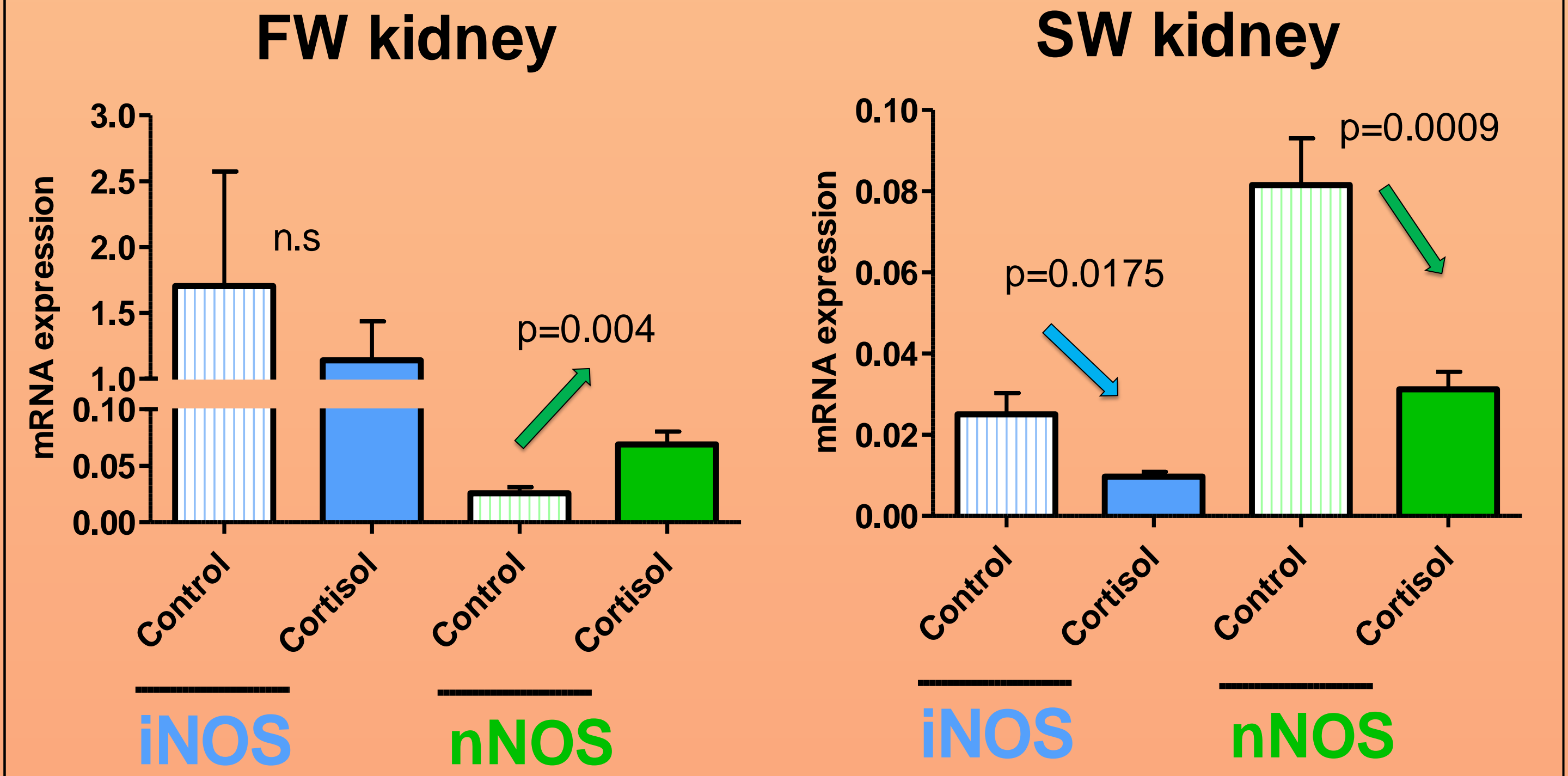
### Gill NOSs expression



Gill: **iNOS** > **nNOS** in both salinities  
**iNOS**: FW > SW; **nNOS**: FW > SW

No significant effect of cortisol on **iNOS** and **nNOS** expression in gill of FW and SW fish

### Kidney NOSs expression

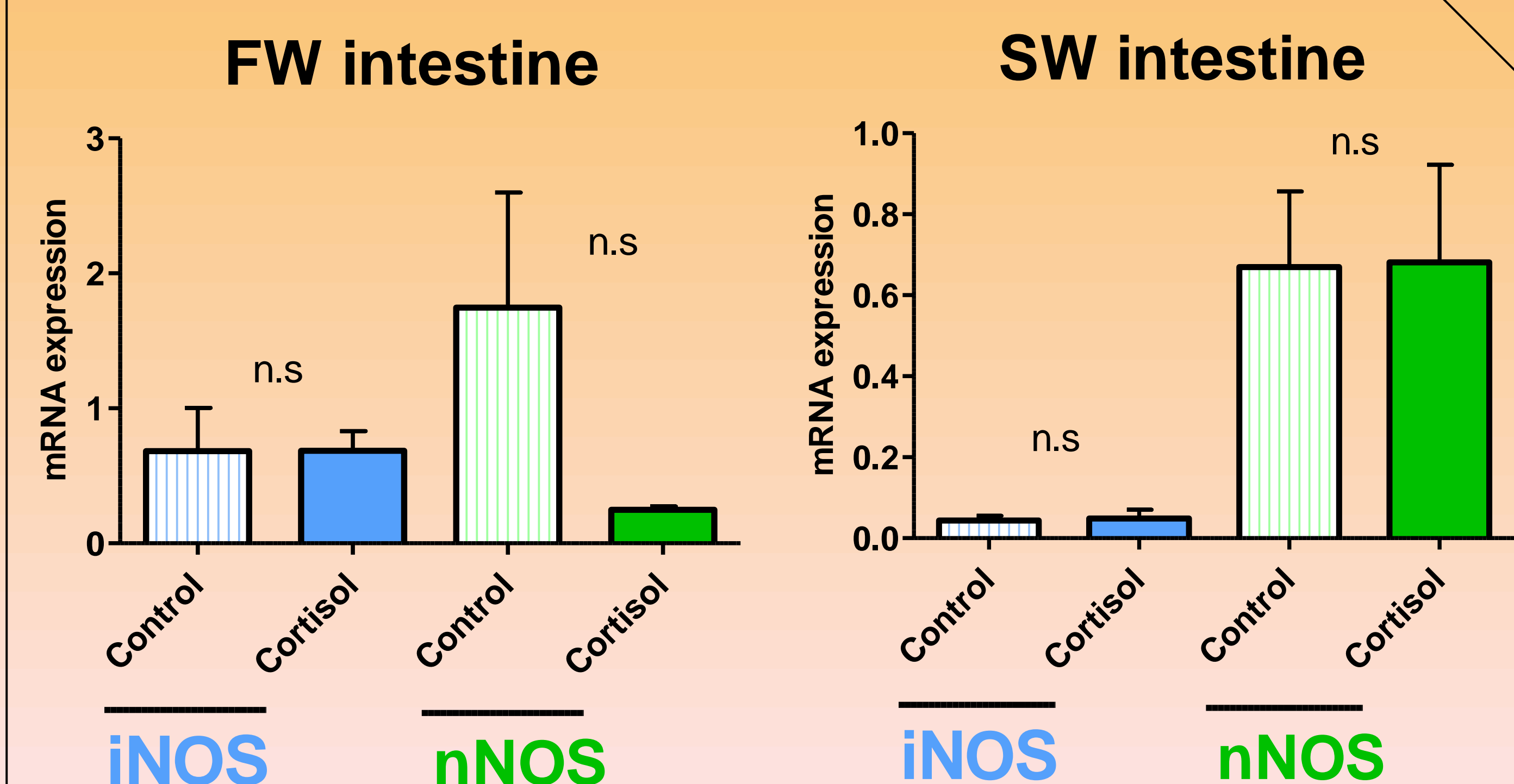


Kidney: **iNOS** > **nNOS** in FW whereas **iNOS** < **nNOS** in SW  
**iNOS**: FW > SW; **nNOS**: FW < SW

Up-regulation of **nNOS** expression in kidney of FW fish

Down-regulation of **iNOS** and **nNOS** expression in kidney of SW fish

### Intestine NOSs expression



Intestine: **iNOS** < **nNOS** in both salinities  
**iNOS**: FW > SW; **nNOS**: FW > SW

No significant effect of cortisol on **iNOS** and **nNOS** expression in intestine of FW and SW fish

## Conclusion

The iNOS and nNOS isoforms are **expressed in all three tissues** (gill, kidney and intestine), but expression levels varies between tissues. iNOS expression is higher in gill than in kidney and intestine, while nNOS is higher in intestine than kidney and gill. The expression of both iNOS and nNOS also **depends on salinity**. iNOS shows higher expression in FW than SW in all three tissues. The same apply to nNOS except for kidney, where nNOS is mostly expressed in SW.

A **significant effect of cortisol** on NOS expression was found in kidney, where cortisol induced down regulation on both nNOS and iNOS in SW, while cortisol increased nNOS in FW. The data suggest crosstalk between cortisol and NO mainly in the kidney, and that the effects of cortisol differ between FW and SW.

For all qRT-PCR: **N=10** for FW and **N=14** for SW. Data are expressed as the **mean  $\pm$  s.e.m.** A **p-value= 0.05** was retained as threshold value. (n.s. = no significant); **EF1- $\alpha$**  was used as Reference gene.

**Inducible (i)** and constitutive **neuronal (n)** NOS isoforms are expressed in gill, kidney and intestine of rainbow trout.  
**iNOS**: gill > kidney > intestine in FW ; gill > intestine > kidney in SW.  
**nNOS**: intestine > kidney > gill in both salinities.

- [1] Madsen SS. (1990). Effect of repetitive cortisol and thyroxine injections on chloride cell number and Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in gills of freshwater acclimated rainbow trout, *Salmo gairdneri*. *Comp. Biochem. Physiol.* 95A: 171-175.
- [2] Tipsmark CK. and Madsen SS. (2003). Regulation of Na<sup>+</sup>/K<sup>+</sup>-ATPase activity by nitric oxide in the kidney and gill of the brown trout (*Salmo trutta*). *J. Exp. Biol.* 206: 1503-1510.
- [3] Jensen FB. (2009). The role of nitrite in nitric oxide homeostasis: a comparative perspective. *Biochim. Biophys. Acta* 1787: 841-848.
- [4] Lou YK., Wen C., Li M., Adams DJ., Wang MX., Yang F., Morris BJ., Whitworth JA. (2001). Decreased renal expression of nitric oxide synthase isoforms in adrenocorticotropin-induced and corticosterone-induced hypertension. *Hypertension* 37:1164-1170.