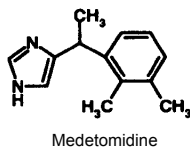


# The mRNA expression of the octopamine receptor in *Balanus improvisus*

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## Introduction

Medetomidine strongly inhibits settlement of *Balanus improvisus* both in the laboratory and in field studies. Our hypothesis is that medetomidine binds to the octopamine receptor, which belongs to the family of G-protein coupled receptors.



## Aims

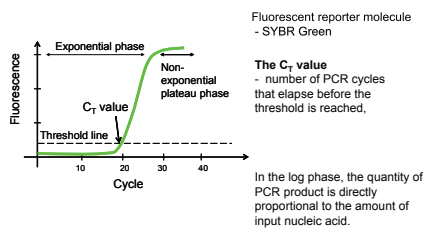
To compare the mRNA expression of the octopamine receptor in *B. improvisus* during different life stages and medetomidine exposures.

To investigate if medetomidine may cause tolerance development in barnacles, this due to down-regulation in the mRNA expression of the octopamine receptor.

## Method

We have developed a protocol for a quantitative PCR (Q-PCR) assay measuring mRNA expression of the octopamine receptor in *B. improvisus*. We use the DNA-binding dye SYBR Green, which binds nonspecifically to double-stranded DNA. The fluorescent signal from a reaction is proportional to the amount of double stranded DNA present, and will increase as the target is amplified.

### Amplification plot



### Relative quantification

The  $C_T$  value of the target gene is divided by the  $C_T$  value of a reference gene to give a normalized value.

The normalized value can then be compared against other samples to determine differential gene expression in different samples.

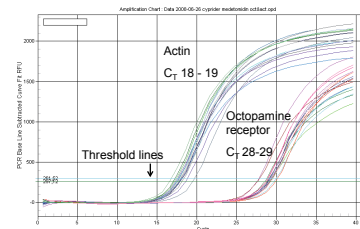
The Pfaffl Method – calculation of the relative gene expression

$$\text{Ratio} = \frac{(E_{\text{octR}})^{\Delta C_T \text{ octR (control-sample)}}}{(E_{\text{actin}})^{\Delta C_T \text{ actin (control-sample)}}} \quad E = \text{Efficiency}$$

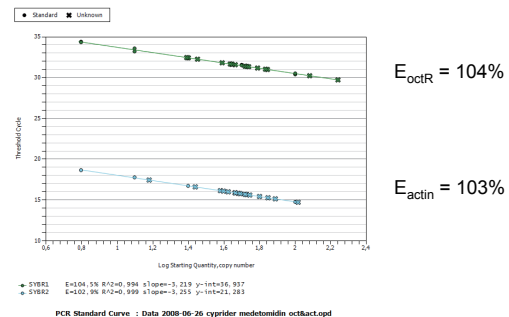
## Results

Cyprid larvae of *B. improvisus* were exposed to medetomidine or copper oxide (CuO) for 24 hours. Total RNA was isolated and converted to cDNA using the enzyme reverse transcriptase. The mRNA expression of the octopamine receptor and actin were measured.

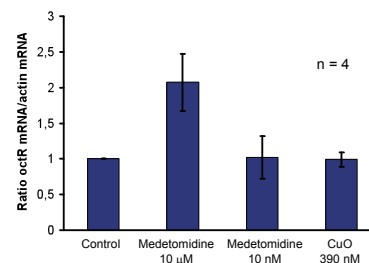
### The mRNA expression of the octopamine receptor vs. actin



To test the efficiency (E) of our Q-PCR assay we used serial dilutions of our templates and generated a standard curve.



The  $C_T$  values and the efficiencies were used to calculate the relative gene expression according to the Pfaffl Method.



## Conclusions

Medetomidine seems to up-regulate the mRNA expression of the octopamine receptor in cyprid larvae after 24 hours exposure.

We now have an assay for relative quantification of the mRNA expression of the octopamine receptor in *B. improvisus* with the reference gene, actin, as the normalizer.