Melatonin receptors expression (*Mt1* and *Mt2*) in the pituitary of European sea bass (*Dicentrarchus labrax*): arising explanations for daily and seasonal variations in pituitary hormones.

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INTRODUCTION

In fish as in other vertebrates, neuroendocrine functions display daily and annual variations. Most are under photoperiodic control. In an attempt to better understand the processes that regulate those variations, we investigated the daily and annual expression of the genes encoding POMC, GH, PRL, SL, TSHβ, FSHβ, and LHβ in European sea bass, *Dicentrarchus labrax*. Because the hormone melatonin translates environmental timing cues to the organisms, we also investigated the localization of the mRNA corresponding to the melatonin receptors MT1, MT2 and Mel1c, as well as the *in vitro* effects of physiological concentrations of melatonin on the expression of the pituitary genes.

MATERIAL AND METHODS

***In vivo*:** Sea bass were maintained under natural conditions of photoperiod and temperature for two successive years.

Daily sampling was done every 4 hours (n = 15/sampling point), and annual sampling was done at equinoxes and solstices.

***In vitro*:** One pituitary/well in HEPES buffered RPMI culture medium containing melatonin at different concentrations (n = 15/treatment).

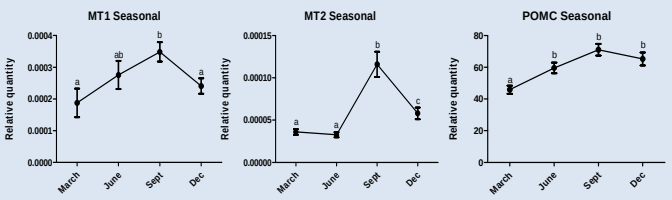
Analyses: qPCR (relative values; *L17* is the reference gene) and *in situ* hybridization (ISH).

Dicentrarchus labrax

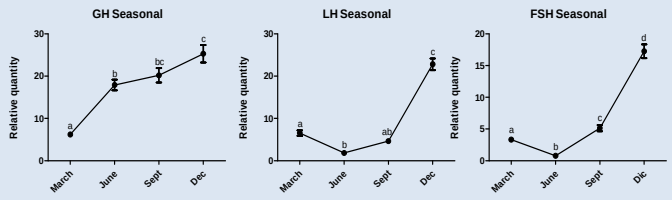
RESULTS

a) Annual variations of pituitary genes: *Mt1, Mt2, Pomc, Gh, β-Fsh* and *β-lh* showed consistent seasonal variations of expression in the sea bass pituitary during two successive years. Although *Tsh, Prl* and *Sl* variations were also observed, those were not consistent from one year to the other. No expression was detected for the *Mel1c* melatonin receptor.

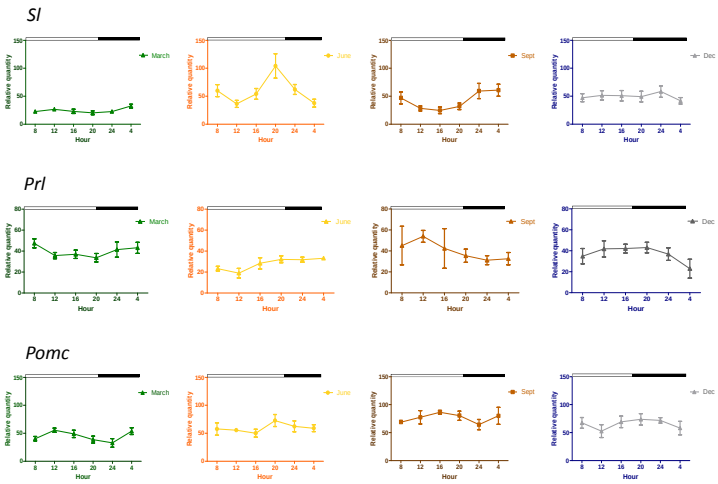
***Pomc*, *Mt1* and *Mt2* showed higher expression in September during both years**



***Gh, β-Fsh* and *β-Lh* mRNA peaked in December**

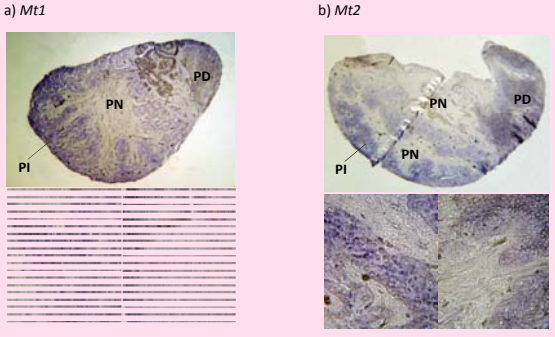


b) Daily variations of pituitary genes: Daily variations in the expression of pituitary genes were observed for *Prl* and *Sl* at some times of the year, not at others (*Prl* June p = 0.01, April and December p = 0.09; *Sl* June p=0.04 and September p = 0.002; Cosinor analyses).



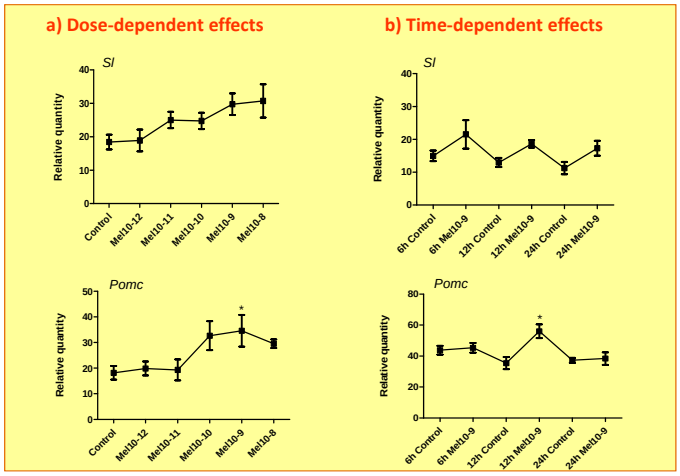
ISH localization of MT1 and MT2 melatonin receptors mRNA in the pituitary.

Mt1* and *Mt2* expression was widely distributed in the adenohypophysis, while no signal was observed in the *pars nervosa



***In vitro* effects of melatonin on the expression of pituitary hormones genes**

Melatonin modulated the expression of *Pomc* and *Sl* at the different concentrations tested from 10^{-12} M to 10^{-8} M. A 12h treatment was enough to stimulate gene expression at the concentration 10^{-9} M.



CONCLUSIONS

Daily and seasonal variations in the expression of some pituitary hormones suggests a photoperiodic control.

The presence of melatonin receptors in the pituitary suggests the time keeping hormone plays a role by acting directly in the gland (not excluding upstream hypothalamic control). This appears to be the case for *Sl* and *Pomc* gene expression.

The wide distribution of *Mt1* and *Mt2* receptors in the adenohypophyseal gland suggests that melatonin might also target the production and/or release of other hormones.

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