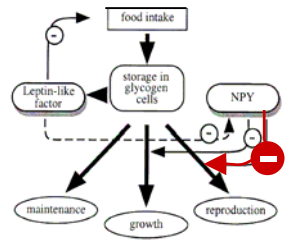
# NPY signalling in the gonad of the oyster *Crassostrea gigas*: involvement in summer mortality?

Charlotte Corporeau[a], Elodie Fleury[a,b], Jeanne Moal[a], Pierre Boudry[a], Pascal Favrel[b], and Arnaud Huvet[a*]

[a] Ifremer, UMR M100 PE2M, Centre de Brest, B.P. 70, 29280 Plouzané; * **ahuvet@ifremer.fr** / [b] Université de Caen, UMR M100 PE2M, 14032 Caen.

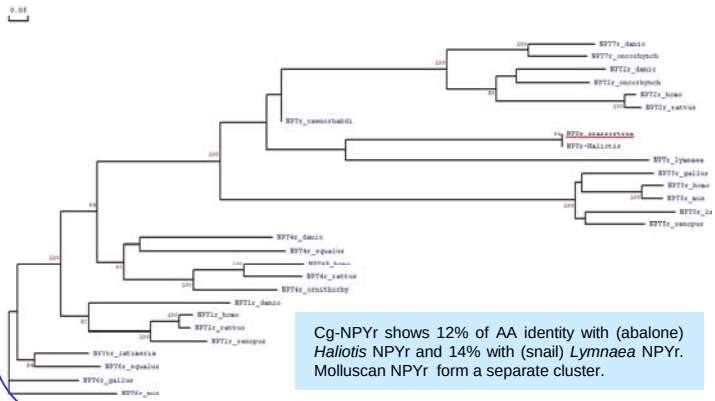
In summer, oysters suffer mortality that decimates this year about 60% of the population in France. We previously demonstrated a genetic basis for summer survival that allowed us to produce resistant (R) and susceptible (S) oyster families by divergent selection (Samain et al., 2007). At the physiological level, R families can survive summer mortality because they are reproductively less active than S families (Fleury et al., 2008).

In order to identify the molecular basis of the summer survival, a transcriptomic approach on R and S families was performed using microarray analysis and 34 differentially-expressed genes were identified between R and S in the gonad (Fleury *et al*., in prep). Among these genes, we found an EST encoding a neuropeptide Y related receptor (Cg-NPYr). Due to NPY major role in the coordination of energy balance and reproduction in other species, we studied Cg-NPYr expression and regulation in the gonad to gain insight into its function and its putative role in summer mortality.



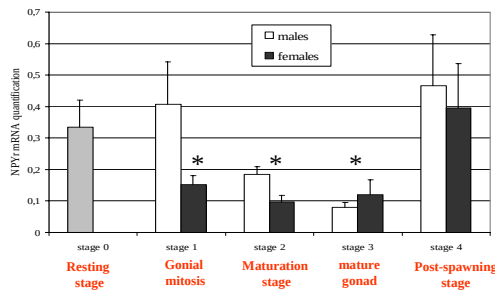

Schematic representation of the role of NPY in Lymnea (De Jong-Brink *et al.* 2001)

## • Phylogenetic analysis of Cg-NPYr related proteins.



Cg-NPYr shows 12% of AA identity with (abalone) *Haliotis* NPYr and 14% with (snail) *Lymnaea* NPYr. Molluscan NPYr form a separate cluster.

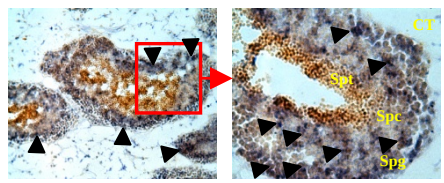
## • Expression of Cg-NPYr in the gonad by real time PCR




*Test kruskall wallis p<0.001 , as compared to stage 0

NPYr expression decreases as gametogenesis proceeds.

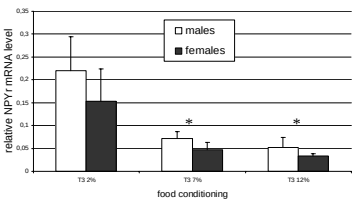
## • Localization of Cg-NPYr in the gonad by *in situ* hybridization




CT : conjunctive tissue
Spg : spermatogonia
Spc : spermatocyte
Spt : spermatid

NPYr expression is detected in early germ cells.

## • Effect of food availability on Cg-NPYr expression in the gonad

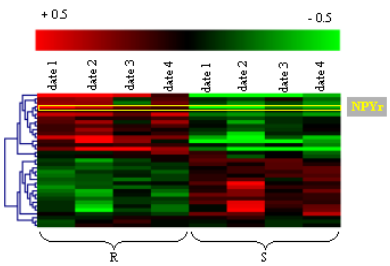



Stage 3 (T3) gonads (mature gonad) after 3 months (April - June) of low (2%), medium (7%) or high (12%) food level with a mixed diet of 3 algae (% of dry weight algae/ dry weight oyster).

*Test kruskall wallis p<0.05 , as compared to 2%

NPYr expression is down-regulated in high food level conditions known to increase gametogenesis investment (number of produced gametes).

## • Differential expression between R and S




Supervised average linkage analysis of 34 genes significantly differentially expressed between Resistant (R) and Susceptible (S) lines in the gonad (False Discovery Rate=2.6%) at 4 points from May to June corresponding to different reproductive stages. Each row represents a gene and each column a date for each line.

NPYr expression is down-regulated in oysters susceptible (S) to mortality.

## • What about a Cg-NPYr putative ligand? Cg-NPY…

***Crassostrea gigas* preproNPY sequence**.

Signal peptide

*M Q T S S L L A V L L V T L L S V W T V L G* N D S L L P P N R P S R F S S P G Q L **R Q**

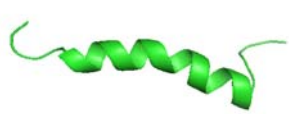
Cg-NPY — C-terminal peptide

**Y L K A L N D Y Y A I V G R P R F** G K R D S E F S S Y G Q E P R S D G I L S S S R D

R E D F A G P Q W W *

G: Glycine for amidation. KR: putative proteolytic processing sites.

**Swiss model:**



**Alignment of NPY peptides**

| | |
|---|---|
| *Crassostrea* (NPY) | --NDSLLPFNRFSRFSSFGQLRQYLKALNDYYAIVGRPRFa |
| *Lymnaea* (NPY) | -TEAMLTPPQRPEEFKNPNELRKYLKALNEYYAIVGRPRFa |
| *Alysia* (NPY) | DNSEMLAPPPRPEEFTSAQDLRGYLAALNEYYSIMGRPRFa |
| *Drosophila* (NPF) | ----SNSRPPRKNDVNTMADAYKFLQDLDTYYGDRARVRFa |
| *Rattus* (NPY) | ----YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRYa |

Molluscs species

We cloned a full-lenght NPY cDNA. Corresponding mature NPY protein (38 AA) has several PRO/TYR conserved between NPY/F families, and shares 71% AA-identity with NPY-Lymnea. 3D-structure of NPY shows 50% identity with pancreatic hormone domains.

An ancestral NPYr /NPY signalling could exist in oysters and exerts its role directly in the gonad via a specific receptor.
In oysters susceptible (S) to mortality, the investment in reproduction is higher and NPYr is down-regulated, suggesting that NPYr/NPY signalling might exert an inhibitory control of reproduction, as already documented in Vertebrates. The development of GPCR deorphanization technology will be done to ascertain CgNPYr/CgNPY pairing. Functional studies will be carried out *in vivo* using RNAi invalidation.

De Jonk-Brink et al, (2001) *Peptides* 22, 309-315. Fleury et al, (2008) *Gene* 410, 187-196. Samain et al, (2007) *Aquaculture* 268, 227-243.