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Physiological and molecular basis of gametogenesis in triploid Pacific oysters, *Crassostrea gigas*.

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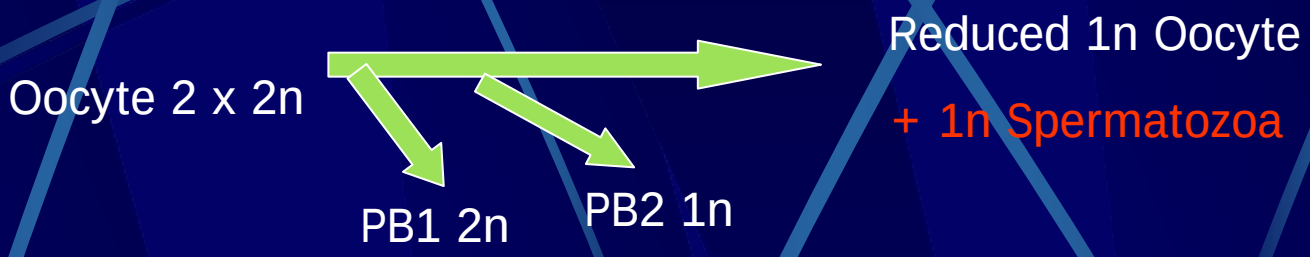
Introduction:

Genetic improvement of *C. gigas* through triploidization:

- reduced (but variable) gonad development
- better growth (Goulletquer et al., 1996; Nell, 2002) and survival (Degrémont, 2003)
- limited propagation in the wild

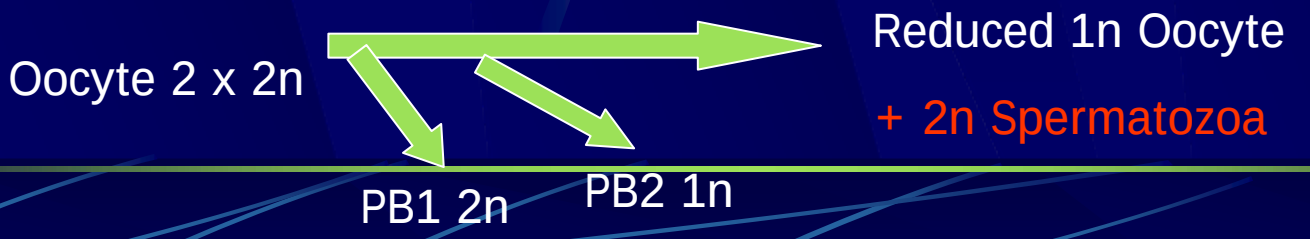
Triploidy induction in *C. gigas*

« Normal » diploid fecondation



« Chemical » induction of triploidy (inhibition of the 2nd PB release) (Beaumont and Fairbrother, 1978)**:**

« Natural » induction of triploidy (2n x 4n cross) (Guo and Allen, 1994):



Part one: Comparative histological study of reproduction in 2n, 3n c & 3n n spat

Material and Methods

BREEDERS

M 4n F 2n M 2n

Chemical induction

STUDIED OYSTERS

3n n 2n 3n c

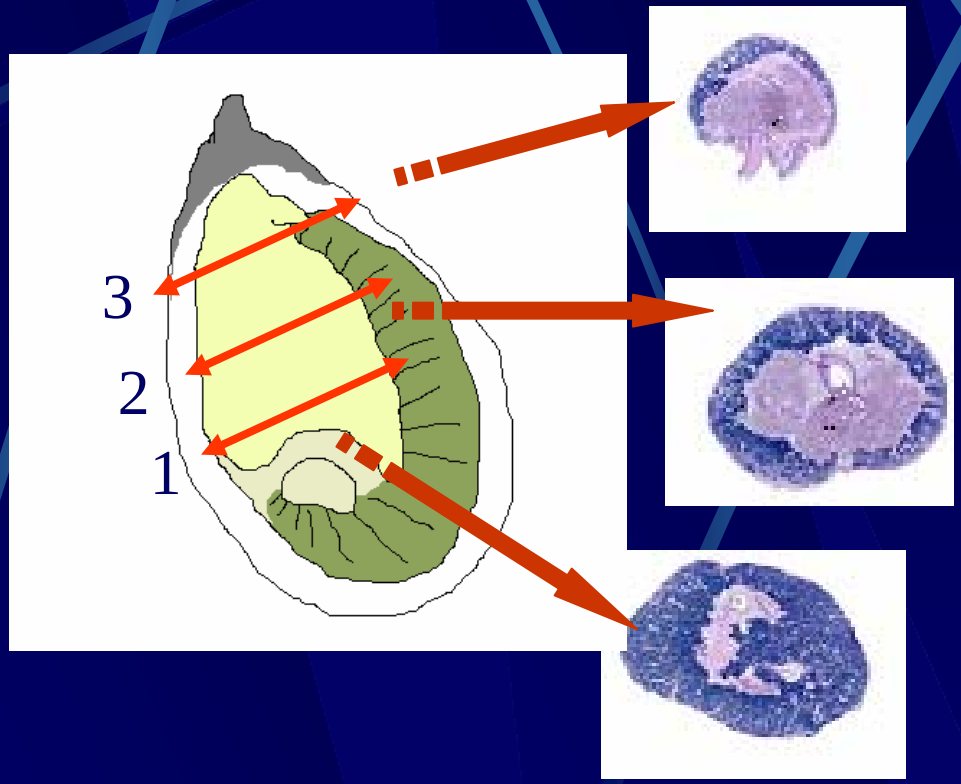
5 months in nursery

Ploidy (flow cytometry)

Reproductive Effort (histology)

Ifremer

Quantitative analysis of reproductive effort



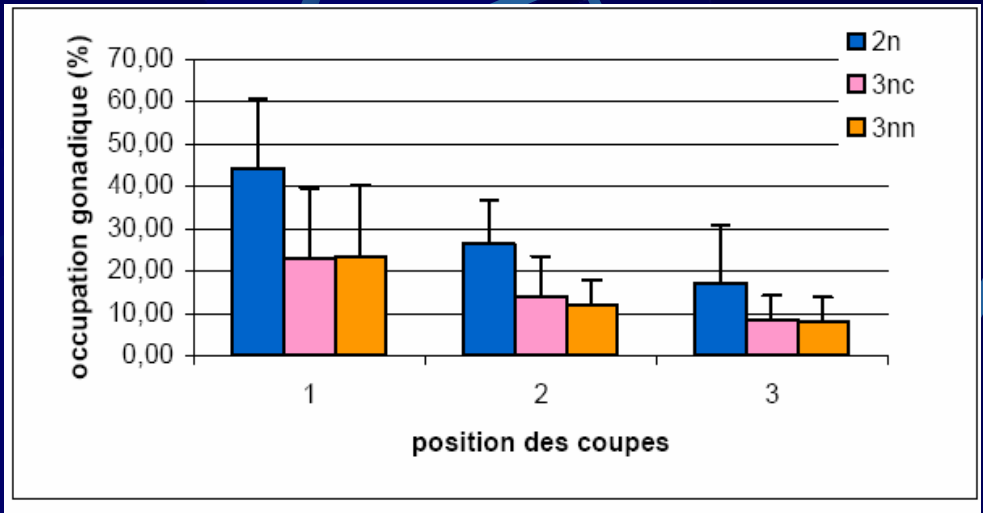
Histological slides:

- Davidson Fixator
- Hematoxilin-eosin staining
- 3 cross-sections of the visceral mass /ind.

30 ind. /group

Gonad occupation = gonad surface / total surface

Results



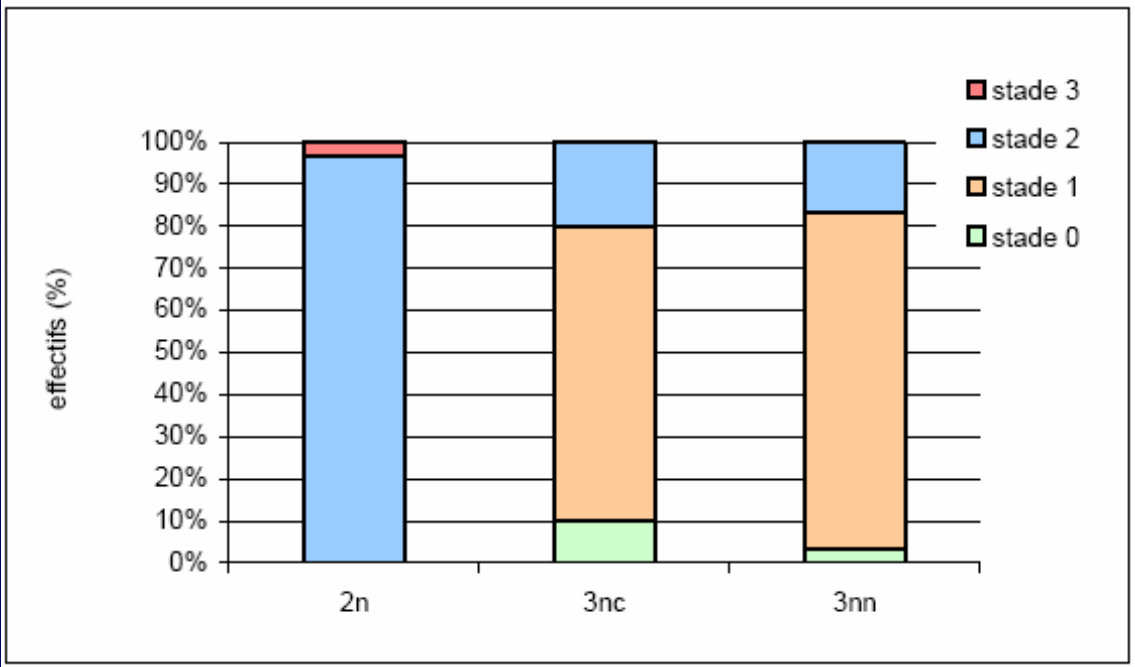
Group	Gonad occupation
2n	29.18% ± 10.61
3n n	14.57% ± 7.27
3n c	15.24% ± 8.11

High individual variation

Reduced gonad development in 3n oysters (≈ -50%)

No difference between 3n n and 3n c

Results



Stage 0 : proliferation of germ cells

Stage 1 : proliferation of gonias

Stage 2 : maturation of germ cells

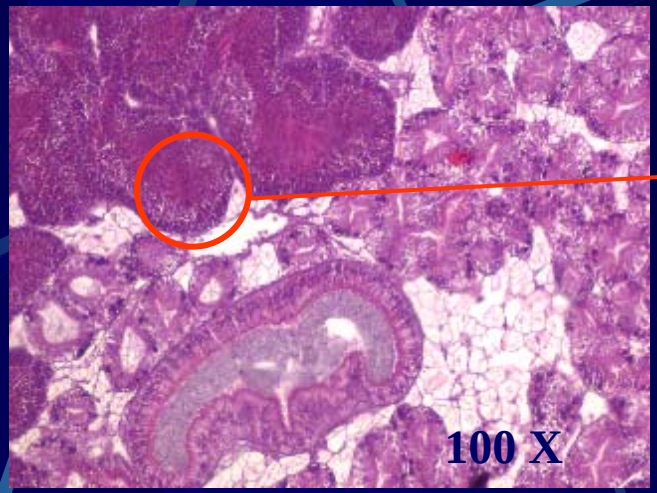
Stage 3 : maturity

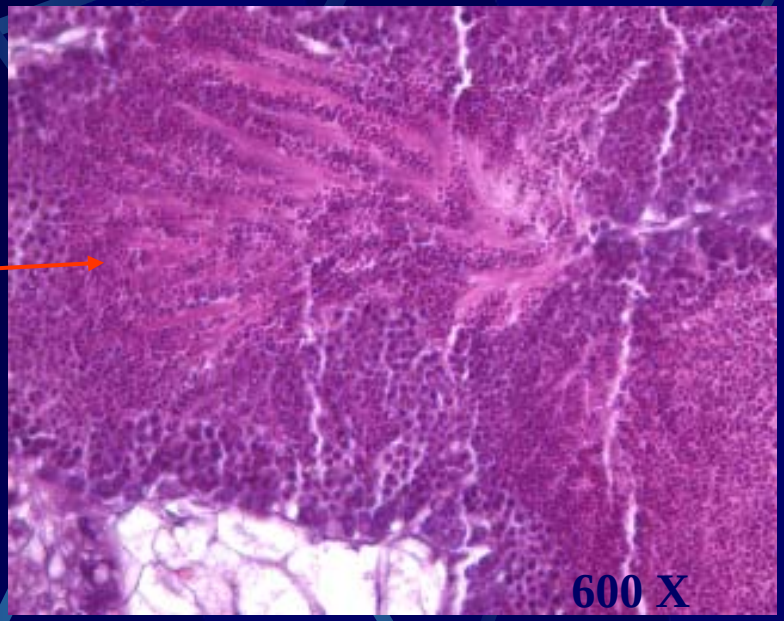
Stage 4 : spawning and resorption

Retardation in gonad development of triploids

- Some 3n individuals showed active gametogenesis (i.e. stage 2)

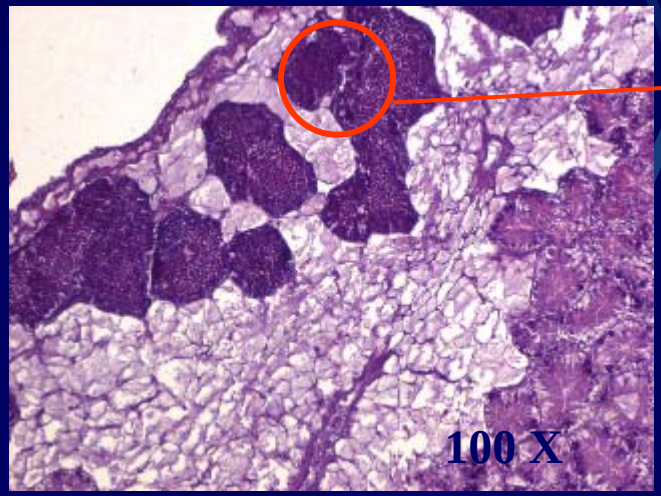
Stage 2

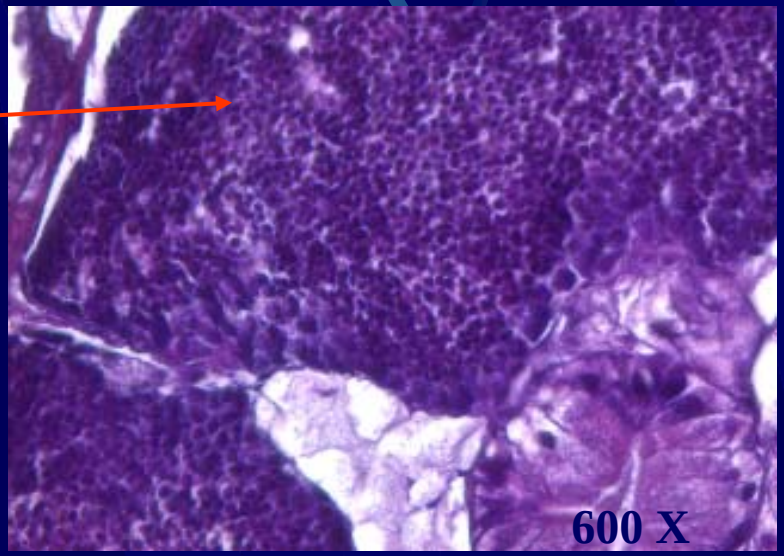




2n

3n





Stage 1

Conclusions (part one)

- Triploid oysters are not fully sterile, even at the spat stage (study on 1 year-old oysters currently in progress)
- No difference between 3n n and 3n c oysters

Altered gonadogenesis in 3n compared to 2n oysters:

- reduction of gonad development
- retardation of gonad development

} *correlated ?*

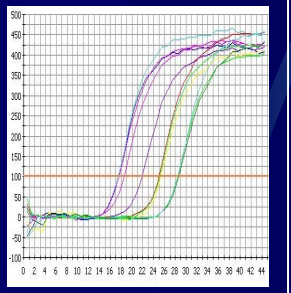
-… but normal pre-meiotic proliferation of the germ cells

Part two:
Study of the expression of the gene Oyster Vasa-like (Oyvlg) in 2n & 3n spat

Oyvlg: specific marker of the germline (Fabioux et al, 2004)

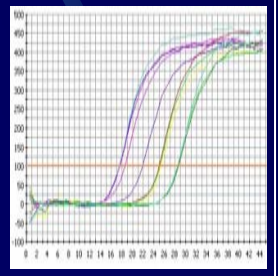
- ATP-dependent RNA helicase DEAD-Box protein
- Vasa-expression restricted to Germline lineage:
  - in the embryo (ontogenesis)
  - in the adult gonad (proliferation and differentiation of germ cells)

Embryos



in toto hybridization *qPCR*

Adults



in situ hybridization *qPCR*

Material and Methods

BREEDERS



M 4n F 2n M 2n

STUDIED OYSTERS

3n n 2n

2-6 months in the field

Ploidy

Expression of Oyvlg

3 sampling dates:

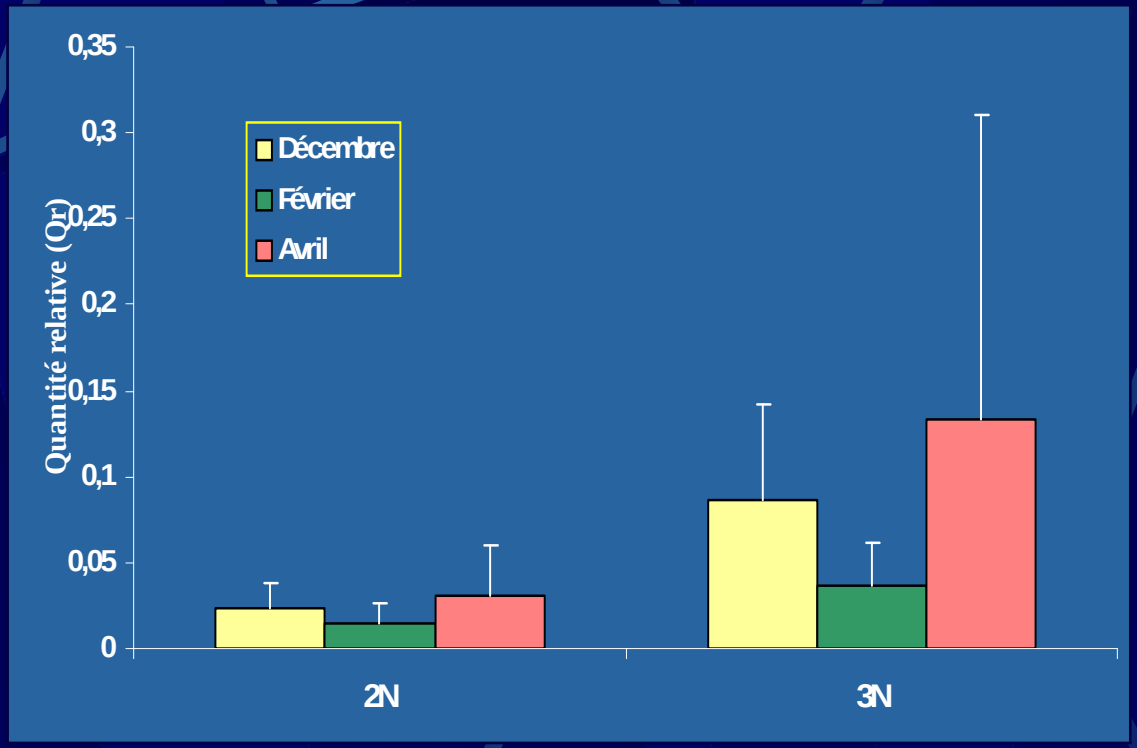
(December / February / April 2006)

Control by flow-cytometry

q-PCR

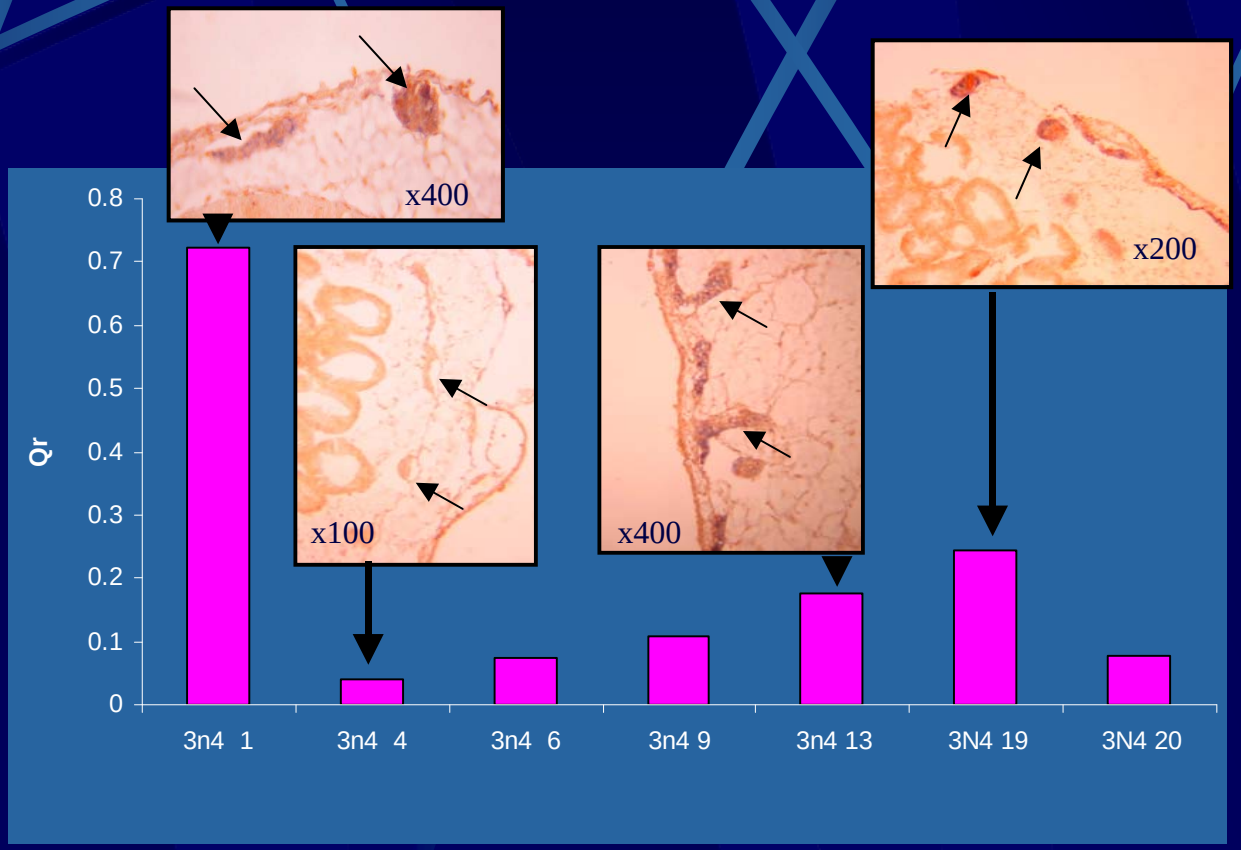
ISH (localisation)

Oyvgl expression in 2n & 3n



- Mean level of expression is higher in 3n than in 2n oysters (x 3.6) : the expression of Oyvlg is additive in triploids but dosage effect varies over time
- Variation is higher between triploids than between diploids

Variation of Oyvlg expression between 3n



Results / Perspectives of part two:

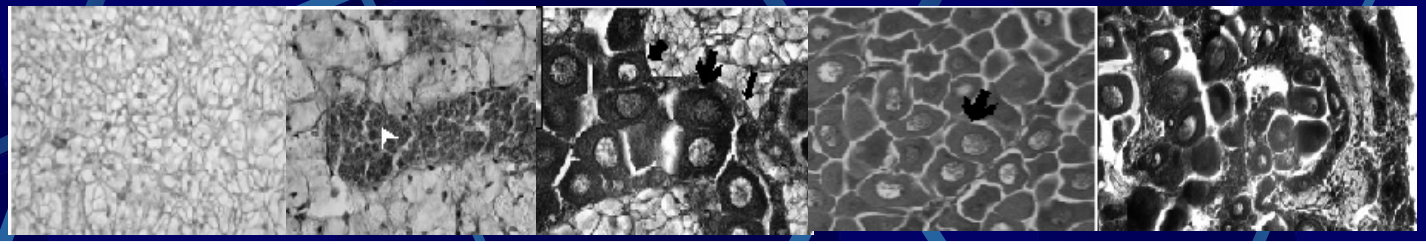
- Paradoxal « dosage effect »: the mean reproductive effort is lower in 3n vs. 2n, but the expression of Oyvlg in additive

- Can Oyvlg be used as a predictor of R.E. within triploids ? (temporal study in progress)

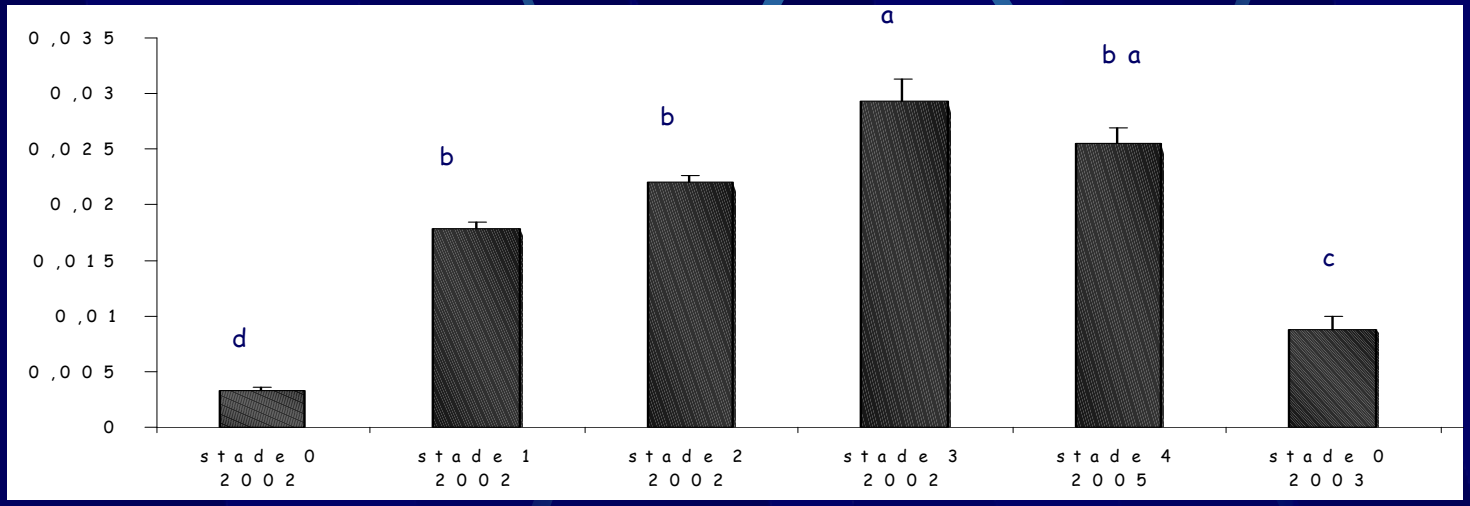
- At what stage is gonadogenesis affected by triploidy ? (after stage 1)

- Which other gene might better follow and predict gonadogenesis in triploids ? (TGFβ , vitellogenine…)

TGFβ expression in diploid oysters



stage 0 stage 1 stage 2 stage 3 stage 4



Expression of TGFβ increases with maturity stage, it is maximal for ripe oysters (E. Fleury, unpublished datas)

Conclusion

Triploid oysters are not fully sterile :

- Mitotic proliferation of germ stem cells (and gonias?) seems to occur normally

- Their reproduction process is however altered at a later stage but some individuals showed active gametogenesis

- Environmental factors (food, temperature..) are likely to strongly influence reproductive effort in triploids (Shpigel et al., 1998)

- Previously published results about reproductive allocation in diploid oysters (Ernande et al., 2004; Samain et al., in press) supports the possible existence of a genetic basis of the variation of gonadogenesis between triploids (currently under study)