In situ markers of *Crassostrea gigas* oyster sensibility to pathogen infection : toward the selection of early indicators of summer mortality

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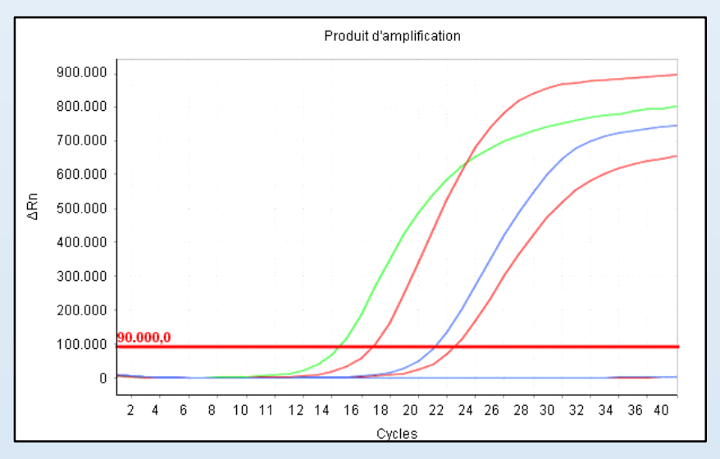
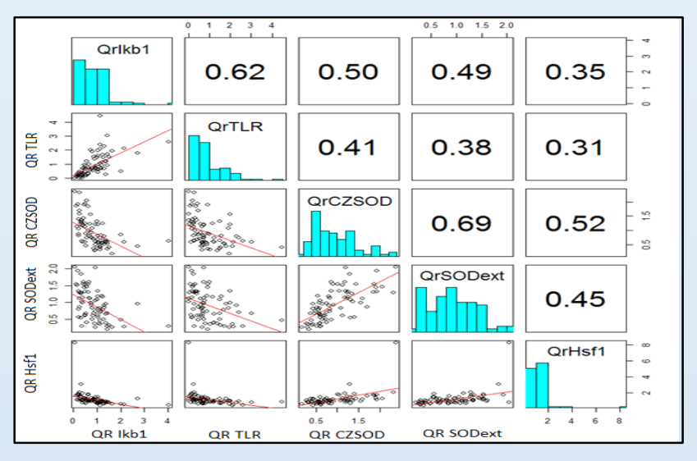
Massive mortalities of *Crassostrea gigas* spat associated with OsHV-1 virus occurred every year since 2008, all along the French coasts. Previous experimental studies lead to identify physiological functions of the oyster metabolism that responds to infection, denoting infection routes and symptoms or indicating immune response. Among these functions, studies highlighted **several genes** whose expression was significantly **down or up-regulated before the mortality event**. The expression of these genes should be then used as **predictive indicator of oyster response to pathogen infection**, including marker-associated selection.

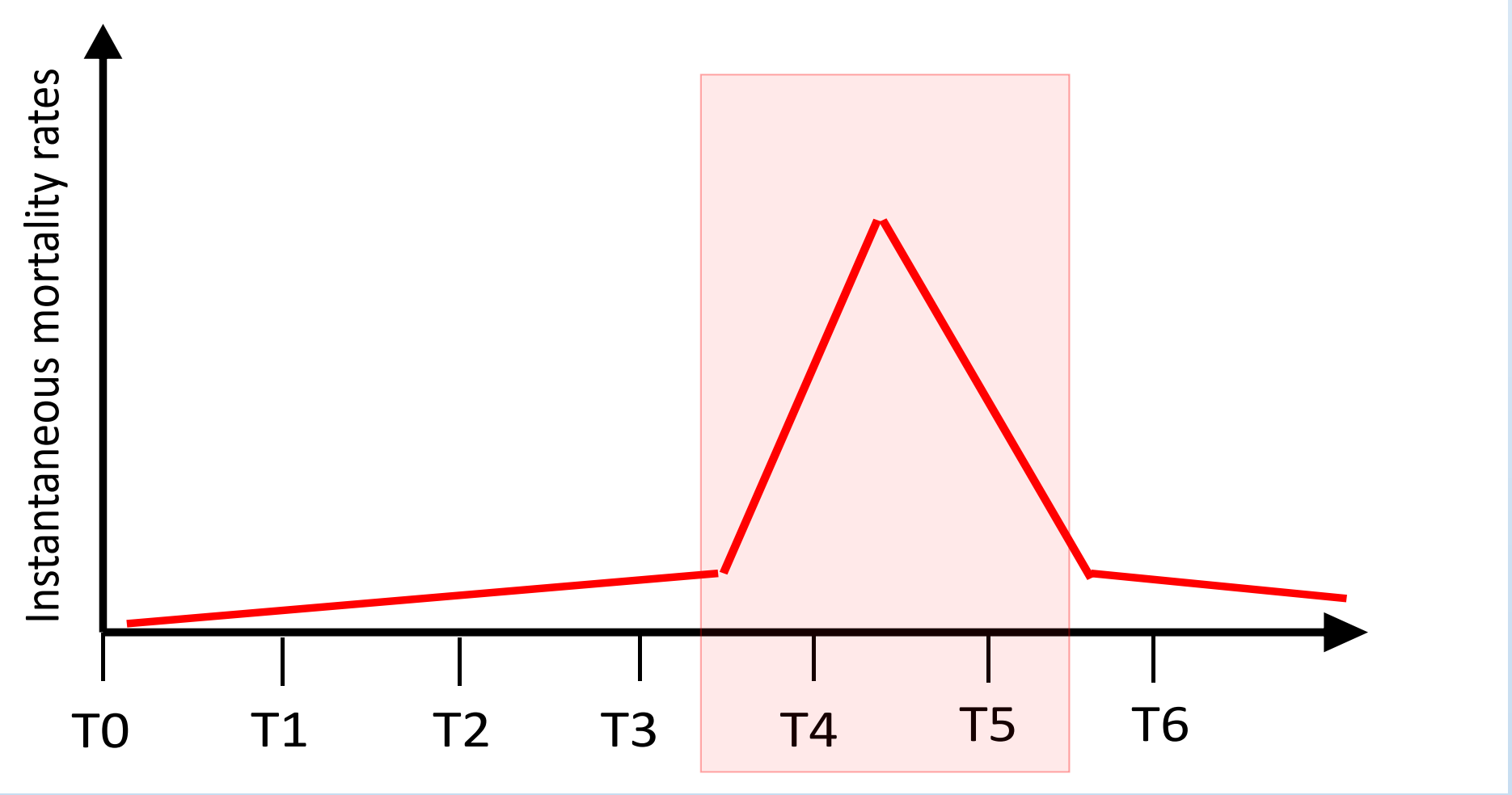
In this study, two experiments were designed to **identify genes linked to early infection response of Pacific oyster spat** during *in situ* mortality. For several oyster batches, we looked for **statistical associations** between, on one hand, ***in situ* variation of candidate gene expression**, and, on the other hand, **variation in kinetic** (exp. 1) or **intensity** (exp. 2) **of the mortality outbreak**.

First experiment : relation between gene expression variation and kinetics of mortality outbreak

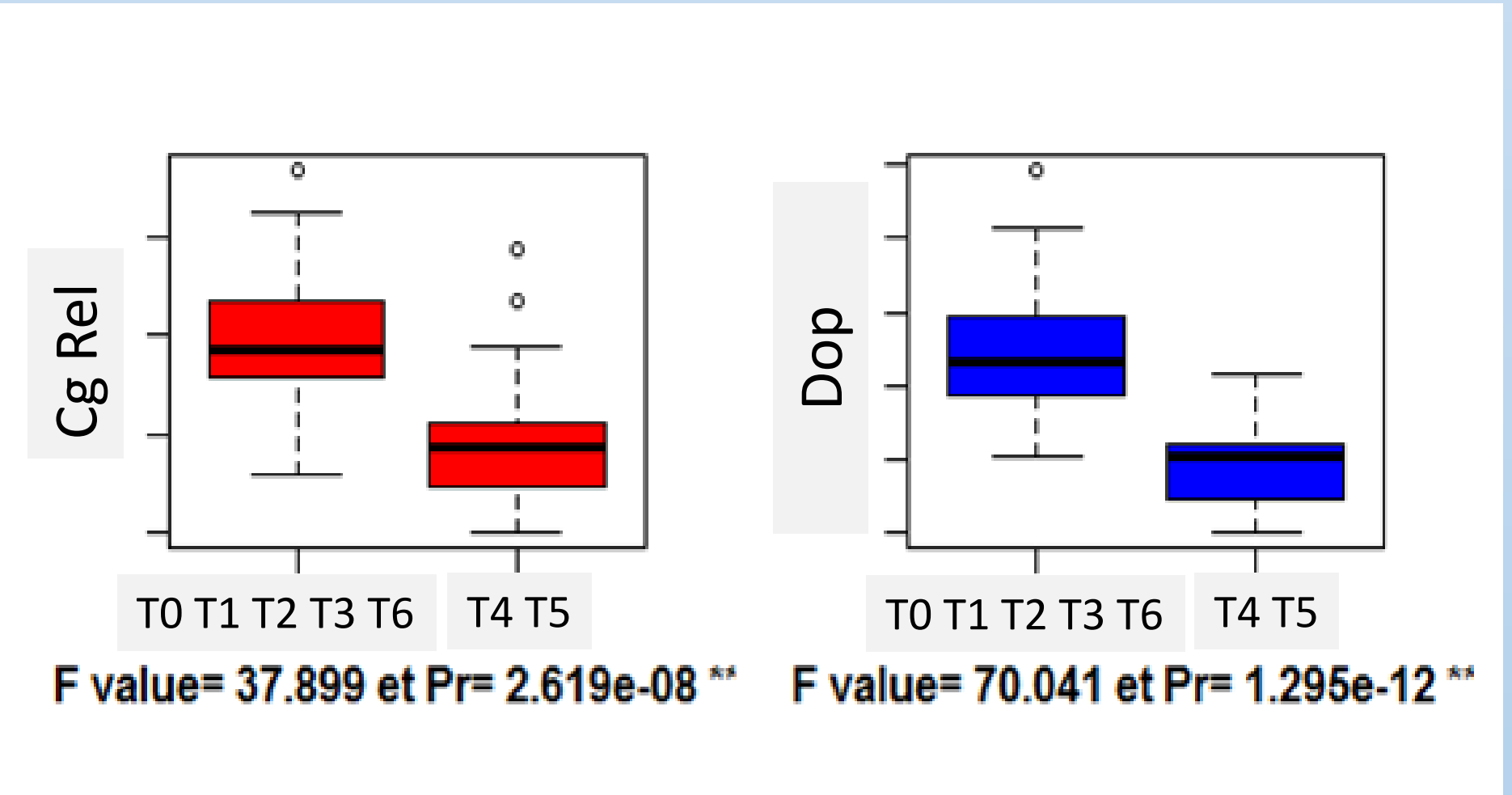
Two batches of spat oyster have been **sampled during an *in situ* mortality event**. For seven dates (from May to July), **mortality rates** were estimated and **mRNA expression levels of 14 genes** were measured on sampled spat oysters of each batches.



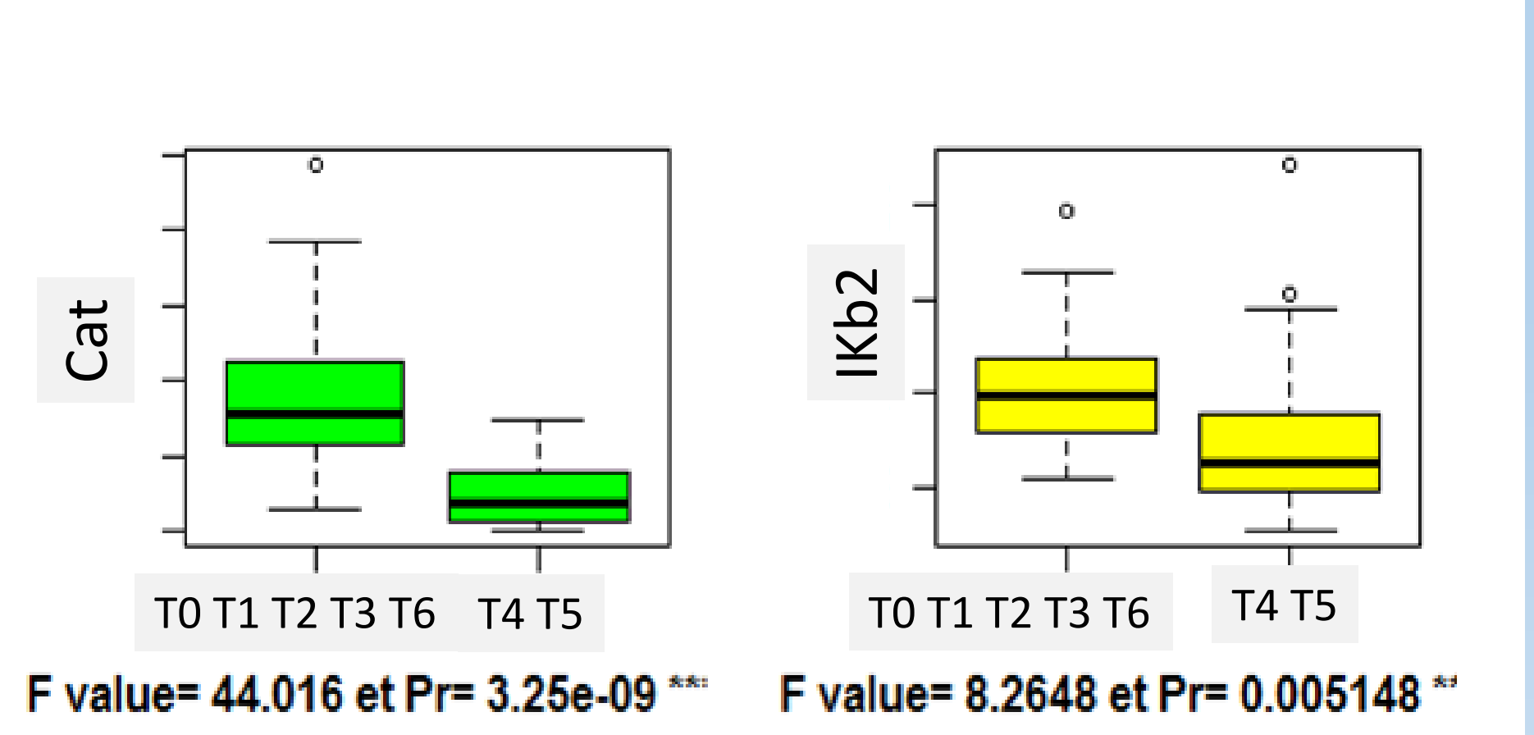




Surveys of mortality were the same for both batches. Kinetics showed **high mortality rates** during **T4 and T5**. Otherwise, no mortality was registered (T0, T1, T2, T3 and T6).



F value= 37.899 et Pr= 2.619e-08 ** F value= 70.041 et Pr= 1.295e-12 **



F value= 44.016 et Pr= 3.25e-09 *** F value= 8.2648 et Pr= 0.005148 **

Statistical analyses between the kinetics of mortality and candidates gene mRNA expression levels measured by qPCR showed that 4 genes :

- **Transcription factor Rel (Cg-Rel),**
- **Dopamine receptor 1 (Dop),**
- **Catalase (Cat)**
- **NF-kappa-b inhibitor 2 (IKb2)**

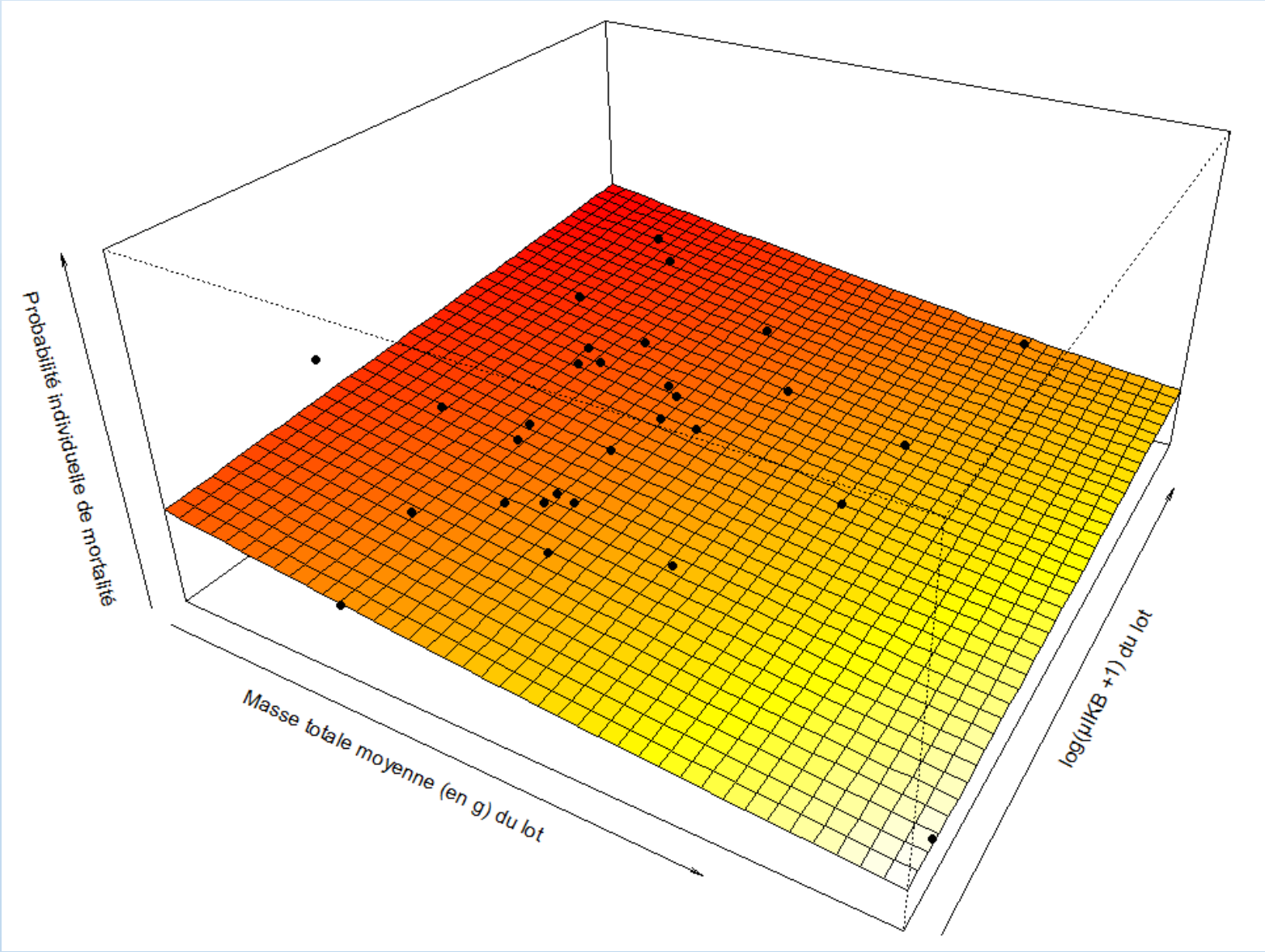
were significantly **down-regulated during the mortality peak** (T4 and T5) compared to time without mortality (T0, T1, T2, T3 and T6).

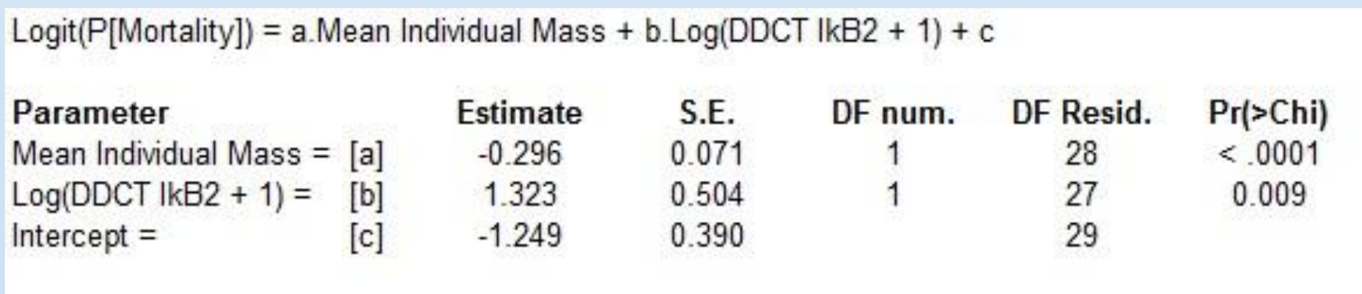
Second experiment : relation between gene expression variation and intensity of mortality outbreak

Thirty batches of 500 spat oysters have been identified by labels glued on the shells, and pooled in a **common garden**. **Individual sampling** of 10 oysters per batch have been realized **just before the *in situ* mortality** event for biometry, candidate gene and Herpes virus OsHV-1 quantification. Remaining oysters have been conserved in common garden until the end of the outbreak for **cumulative mortality rates estimation**.

➢ Results at group level

Statistical analyses at group level highlighted that both "**Mean individual mass**" and "**IKb2 expression levels**" are two significant **predictive variables** of the **mortality probability**.





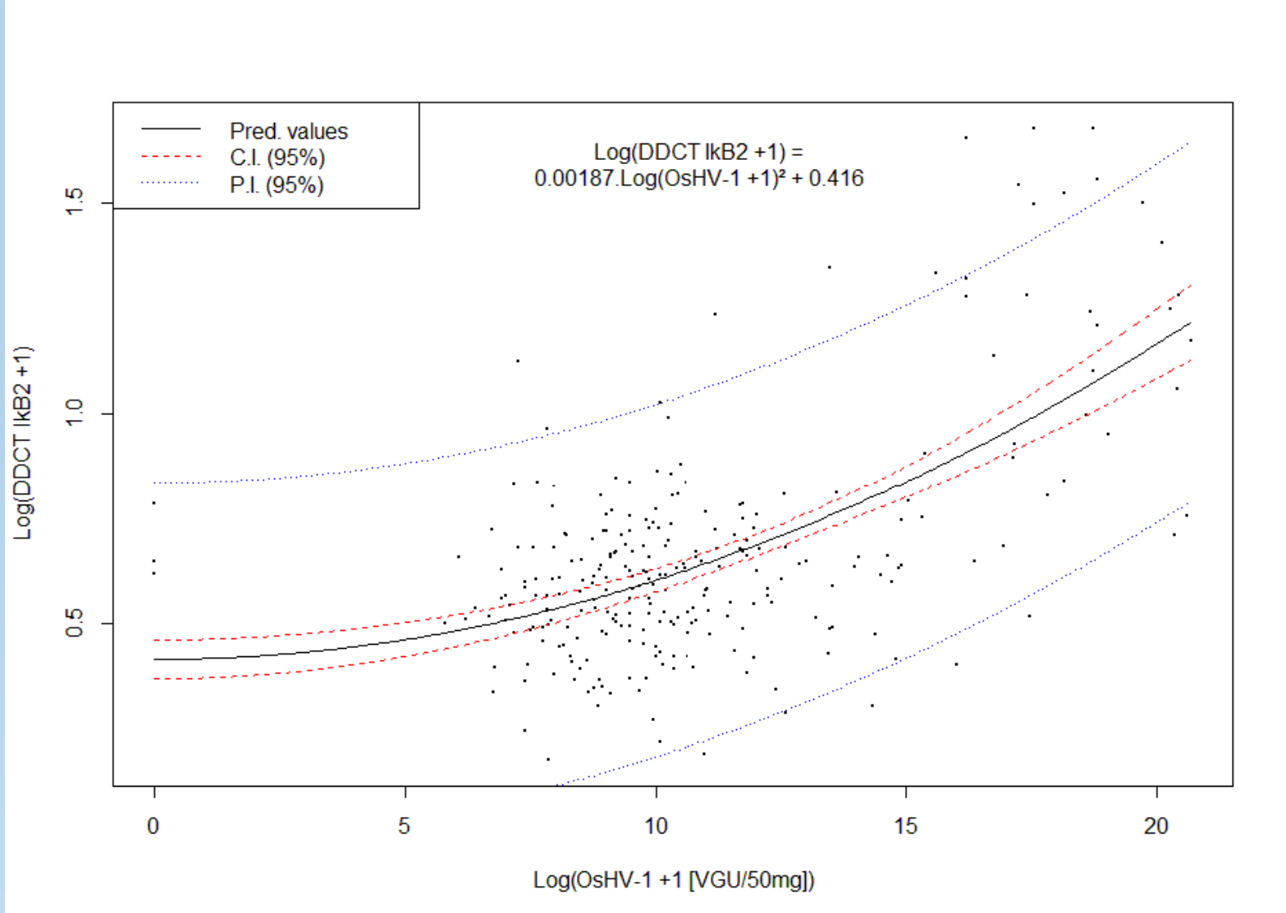
Logit(P[Mortality]) = a.Mean Individual Mass + b.Log(DDCT IkB2 + 1) + c

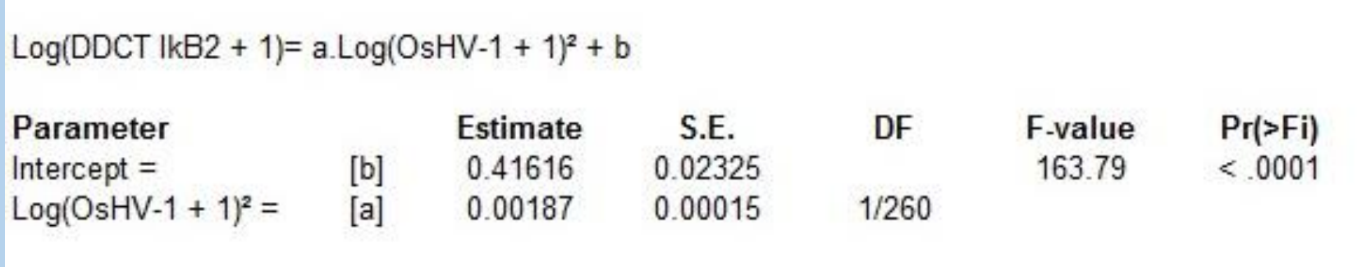
Parameter		Estimate	S.E.	DF num.	DF Resid.	Pr(>Chi)
Mean Individual Mass =	[a]	-0.296	0.071	1	28	< .0001
Log(DDCT IkB2 + 1) =	[b]	1.323	0.504	1	27	0.009
Intercept =	[c]	-1.249	0.390		29	

The probability of mortality is higher when the oyster mass is elevated, and when the expression of IKb2 gene is down-regulated.

➢ Results at individual level

Statistical analyses at individual level highlighted that "**Ikb2 expression levels**" and "**DNA OsHV-1 Herpes virus**" quantification are **significantly correlated**.





Log(DDCT IkB2 + 1)= a.Log(OsHV-1 + 1)² + b

Parameter		Estimate	S.E.	DF	F-value	Pr(>Fi)
Intercept =	[b]	0.41616	0.02325		163.79	< .0001
Log(OsHV-1 + 1)² =	[a]	0.00187	0.00015	1/260		

The IKb2 expression level is positively correlated to the OsHV-1 viral load during an *in situ* mortality outbreak. These results confirmed previously experimentally obtained outcomes *(Segarra et al., 2014).*

These studies allowed to identified **one predictive gene, Ikb2, whose expression correlated to *in situ* final mortality rates** (at the group scale), and **OsHV-1 viral load** (at the individual scale). These results are consistent with the Ikb2 function in oyster (*Montagnani et al., 2008*) and will be useful for **alert mortality threshold**.

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