Development of a clinical prognostic scale for the health state of the Pacific cupped oyster *Crassostrea gigas*

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Clinical prognostic scale ?

Among the clinical prediction rules (CPR) used in epidemiology and human/veterinary medicine (Grobman and Stamilio 2006;) scores and scales are simplified tools for predicting the risk of an event (diagnostic tool) or the risk of progression (prognostic tool). They qualify the intensity of a clinical phenomenon such as a functional gene, the intensity of a symptom, the extension of a disease (Laporte 2014).
Although the clinical approach is commonly applied in terrestrial domestic animals, physical examination is insufficiently considered in marine molluscs. This study proposes an original approach by highlighting the interest of a **clinical scale** based on **physical examinations** of ***Crassostrea gigas*** to **predict the outcomes of a disease** involving the pathogen **OsHV-1** (Oyster Herpesvirus type 1).

① Experimental design

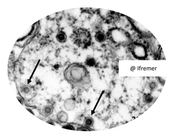
❖ *Crassostrea gigas:*

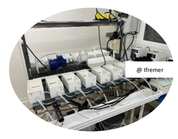
Broodstocks came from wild deposit of Marennes Oléron. Conditioning, fecondation, breeding were conducted at the Marine Molluscs Experimental Platform of La Tremblade (Ifremer PMMLT), protected from pathogens, with filtered and UVC treated seawater.

Acclimatization of progeniture during 8 days at 19 ± 1.0 °C, fed with *Isochrysis affinis galbana* supplied continuously at 30-40 cells μl^{-1}. One day before experiments on 1-year-old diploid oysters, temperature progressively increased to reach the target temperature of 22 °C, without phytoplankton.

❖ OsHV-1:

Isolates of OsHV-1 provided by pathology staff of Marine Invertebrate Health Adaptation Unit (Ifremer ASIM) and production of seawater contaminated by OsHV-1 according to Cordier et al 2020.

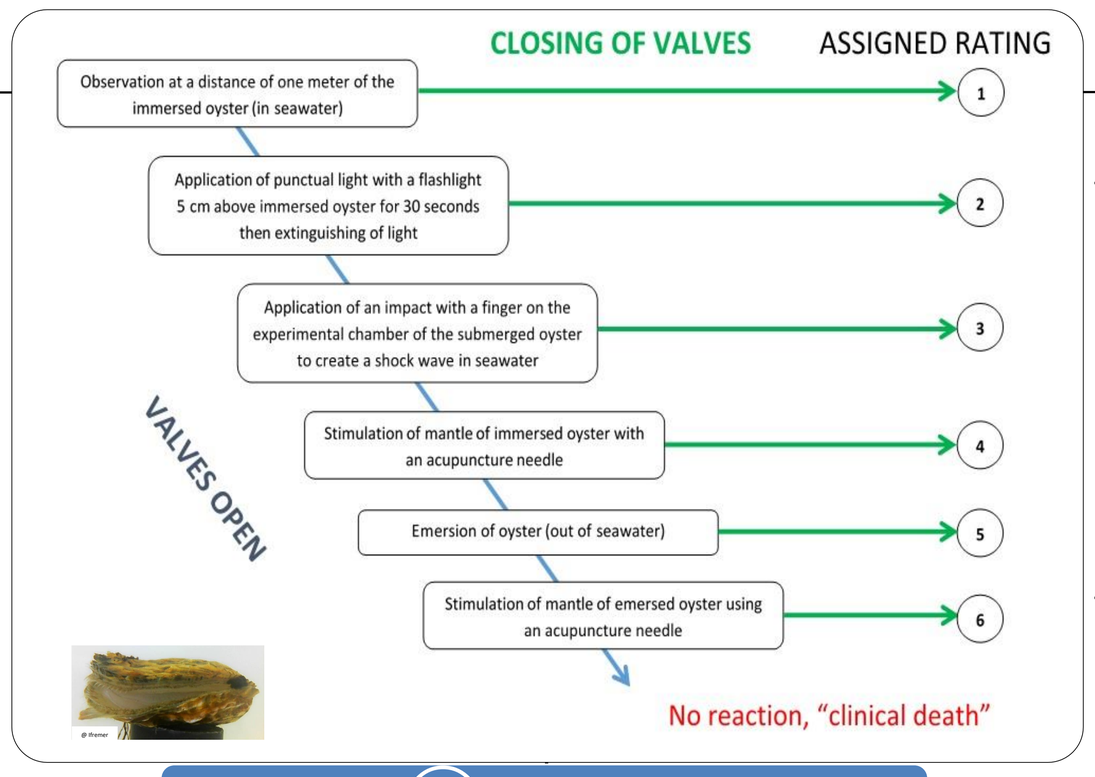




❖ Experimental device:

Oysters placed in individual chambers described in François et al. (2020) to facilitate the physical examination of each animal over 7 days using a clinical scale, (Food: 30-40 cells.μl^{-1} *Isochrysis affinis galbana;* T: 22°C; S: 35 ‰).

To predict the outcome of an infection with OsHV-1 and to verify the hypothesis of the loss of motor function and sentivity of organs during OsHV-1 disease , a **clinical scale** has been proposed with **6 stage ranked according an ordinal nested method**.



❖ Mortality recording and OsHV-1 exposure:

	1rst Experiment Control	2nd Experiment Development	2nd Experiment Internal validation
OsHV-1 in seawater copies of DNA per µL at D0 (day of infection)	No exposure	$3.47E^{+03}$ [$3.07E^{+00}$; $2.56E^{+04}$]	
Mortality	0/32	12/26	14/26
OsHV-1 in oysters copies of DNA per mg of tissue	0	$4.26E^{+06}$ [$0.00E^{+00}$; $3.98E^{+07}$] in 24/26	$1.09E^{+07}$ [$0.00E^{+00}$; $1.02E^{+08}$] in 24/26

❖ Development of the clinical scale:

a Cox univariate regression was applied to assess the pertinence of the model and estimate the **Hazard Ratio (HR)** aimed at predicting the **duration of survival of the Pacific oysters exposed to OsHV-1** as a function of the variable "rating assigned to the clinical scale".

	Estimation of values	95% conf-L	95% conf-U	p value
Cox's coefficient	0,369	0,171	0,567	< 0.001
Hazard Ratio	1,446	1,186	1,762	

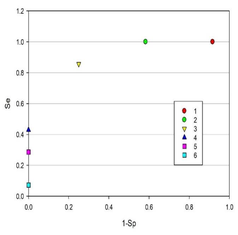
Cox regression and proportional hazards model.

→ Based on the daily examination of the development sample :
Duration of survival =10.122-(1.446*Rating of clinical scale)
[p-value < 0.001]

❖ Internal validation:

- the **discrimination** (capacity of separating subjects at risk of an event) was studied by expressing the c-statistic (Tripepi et al. 2010) and drawing a Receiver Operating Characteristic curve) each day of the experiment (Tripeppi et al. 2009; Grunkemeier and Jin 2001).
- the **calibration** (quality of the regression model to predict events) by using a Hosmer - Lemeshow test (Hosmer and Lemeshow 2013) and graphically by representing predicted / observed data.

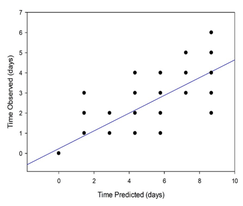
② Results



Discrimination: ROC curve at D3 post exposure to OsHV-1 (Se : sensitivity, Sp : specificity, 1-6 : rating).

Area	Accuracy
0.9-1.0	Excellent
0.8-.09	Very good
0.7-0.8	Good
0.6-0.7	Sufficient
0.5-0.6	Bad
< 0.5	Test not useful

Relation between the area under the ROC curve and discrimination (according to Šimundić, 2009).



Graphic representation of the relation between the observed and the predicted survival durations. Y = 0.441 X + 0.234 with Y= observed; X= calculated survival duration (n=77; R2=0.721; p<0.001).

Scale rating	Mean observed	Mean predicted	H-L test (chi2)
1	4.00	8.68	2.52
2	3.83	7.23	1.60
3	2.88	5.78	1.46
4	1.88	4.34	1.40
5	1.33	2.89	0.84
6	1.56	1.45	0.01
			Sum = 7.83

Hosmer-Lemeshow test confirmed that with this model, the observed values were not significantly different from the predicted values at a threshold of 5% (ddl=5; chi2<11.7).

→ Based on the daily physical examination of the validation sample, the clinical scale exhibits **very good discrimination** on the 3rd day (D2 post exposure) **with c-statistics** of **0.86.** Regarding **calibration** and according Huang et al. (2020), our model presents, on one hand an underestimation (regression slope b<1) of low risk with an overestimation of high risk, and on the other hand an average underestimation of risk with an intercept >0 (a=0.234).

Thanks to the scale, prediction of the outcome of the experiment on the 7th day based on physical examination of *C. gigas* from the 3rd day

③ Optimization

To improve the clinical prognostic scale:
i) reducing the number of stages, in particular for values ≤ 3 which indicate a healthy animal,
ii) adding to the model other non-destructive parameters (clearance rate, oxygen consumption, etc.) for a multivariate risk analysis.

Références :

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