From the concept of health to the determination of reference intervals in *Crassostrea gigas*

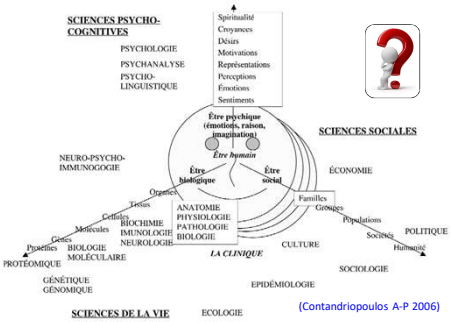
Cyrille François, Joël Haure
Ifremer, SG2M-LGPMM, Station de La Tremblade, Avenue de Mus de Loup, F-17390 La Tremblade, France
Corresponding authors : cyrille.francois@ifremer.fr ; joel.haure@ifremer.fr

Health(s)?

❖ "(Human) Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity. " (OMS 1946)

… **Lack of an official definition of animal health**. (Nicks et Vandenheede 2014)

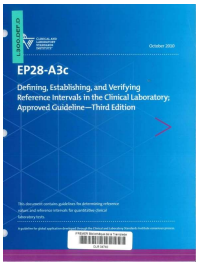
❖ Which scientific discipline(s) for the study of health in marine molluscs?
Choice of **pathophysiology** for the study of the **biological (physical) dimension of health.**

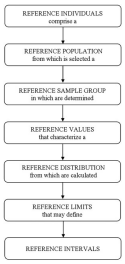


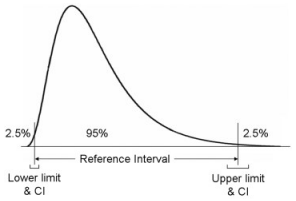
(Contandriopoulos A-P 2006)

Theory of reference values

❖ Starting with the **description of a "healthy" population.**







(CLSI 2008, Geffré *et al.* 2009)

① Biomarkers

❖ Evaluating biological health through **biomarker** monitoring: "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic process, or pharmacologic responses to a therapeutic intervention."
(Atkins *et al.* 2001)

Biomarkers of food intake and respiration in *C. gigas*

Clearance rate (CR)
Volume of 100% filtered water, free of all effectively retained particles per unit of time

CR ($l.h^{-1}$) = F x [(I-O)/I]

Oxygen consumption rate (OCR)

OCR ($mgO_2.h^{-1}$) = F x (I-O)

where F = flow rate ($l.h^{-1}$), I = fluorescence or concentration of oxygen ($mgO2.l^{-1}$) measured at the outlet of the control unit; O= fluorescence or concentration of oxygen ($mgO2.l^{-1}$) measured at the outlet of the experimental unit (Haure *et al.* 2003)

② Reference population & sample group

Reference population

❖ Taking into account the availability of "healthy" animals and the biological, pre-analytical and analytical variations factors of biomarkers: **2000 diploid F1 oysters, produced and maintained in safe conditions in an Ifremer hatchery, by crossing broodstock of wild origin.**

Reference sample group

❖ Choice within the reference population based on **inclusion/exclusion criteria** (biological, zootechnical, medical), with a **sample size greater than the 120 individuals** recommended to estimate the 90% confidence intervals (CI) for reference limits using non-parametric statistical analysis. (CLSI 2008)

③ Acquisition of reference values (RV)

Acclimatization of the reference sample group

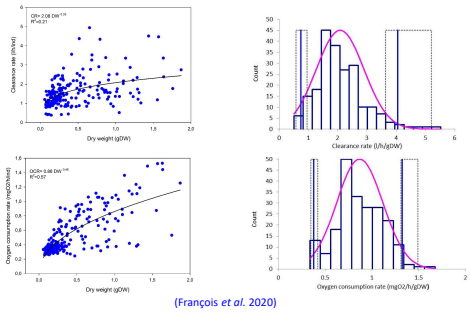
❖ Gradual adjustments during **8 days**.

Reproducible laboratory conditions

❖ Measuring bench equipped with a Hach Lange Orbisphere 410 oximeter / M1100 LDO probe and a Seapoint SCF fluorometer, in a closed circuit filled with seawater from natural source previously filtered (sand, bag filters), treated by ultraviolet sterilizer, at a temperature of 22°C, at the salinity of 32 ‰, supplemented with 30-40 cells/µl of *Isochrysis affinis galbana.*

④ Proposal for reference intervals (RI)

❖ **Reference Value Advisor**, a freeware set of macroinstructions was used to generate **distribution histograms** and propose the **RI limits with a corresponding 90% confidence interval (CI)**. The 90% CIs were obtained using a non-parametric bootstrap method after verifying the symmetric distribution of transformed data with the Box-Cox technique. (Geffré *et al.* 2011)



(François *et al.* 2020)

Variables	Units	N	Median	Mean	SD	Min	Max	Centile 2.5 (90% CI)	Centile 97.5 (90%CI)
CR	$l.h^{-1}.gDW^{-1}$	214	1,9	2,1	0,8	0,5	5,5	**0,7** (0,6-1,0)	**4,1** (3,6-5,2)*
OCR	$mgO2.h^{-1}.gDW^{-1}$	214	0,8	0,9	0,2	0,3	1,7	**0,4** (0,3-0,4)	**1,3** (1,3-1,5)*

Abbreviations: * the 90% CI of one (or more) limit is wider than recommended (CLSI 2008); CI confidence interval; Max maximum; Min minimum; N number of reference individuals in the sample group; SD standard deviation.

Applications

❖ **Methodological harmonization of physiology studies and inter-laboratory validation.**

❖ Comparison and understanding of **observed values** of biomarkers under non-aggressive / aggressive conditions.

❖ **Assessment of animal health and welfare.**

Références :

Atkins AJ, Magnuson WG, Colburn WA, Degruttola VG, Demets DL, Downing GJ, Hoth DF, Oates JA, Peck CC, Schooley RT, Spilker BA, Woodcock J, Zeger SL (2001). Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. *Clinical Pharmacology & Therapeutics,* 69, 89-95 p.
Clinical and Laboratory Standards Institute (CLSI) (2008). Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, document C28-A3. Third edition, Clinical and Laboratory Standards Institute (CLSI), Wayne, PA, USA.
Contandriopoulos A-P (2006). Eléments pour une « topographie » du concept de santé. *Ruptures, revue transdisciplinaire en santé*, 11(1), 86-99 p.
François C, Haure J, Billy J-C, Renault T (2020). Toward reference intervals for shellfish: An illustrative case of feeding and respiratory activities in the Pacific cupped oyster, *Crassostrea gigas*. *Veterinary Clinical Pathology*, 49(2), 345-353 p.
Geffré A, Friedrichs K, Harr K, Condorcet D, Trumel C, Braun J-P (2009). Reference values: a review. *Veterinary Clinical Pathology*, 38(3), 288-298 p.
Geffré A, Concordet D, Braun J-P, Trumel C (2011). Reference Value Advisor: a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. *Veterinary Clinical Pathology*, 40(1), 107-112 p.
Haure J, Huvet A, Palvadeau H, Nourry M, Penisson C, Martin JLY, Boudry P (2003). Feeding and respiratory time activities in the cupped oysters *Crassostrea gigas*, *Crassostrea angulata* and their hybrids. *Aquaculture*, 218(1), 539-551 p.
Nicks B et Vandenheede M (2014). Santé et bien-être des animaux : équivalence ou complémentarité ? *Revue Scientifique et Technique de l'Office International des Epizooties*, 33(1), 97-101 p.
Organisation mondiale de la santé (OMS) (1946). Constitution de l'Organisation Mondiale de la Santé. Open Access version : http://www.who.int/governance/eb/who_constitution_fr.pdf