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Carla Hernández-Cabanyero, Leïla Parizadeh, Elyna Bouchereau, Agathe Marot, Gaëlle Courtay, Juliette Pouzadoux, Sébastien De La Forest Divonne, Cristina Peris Perales, Guillaume Charrière, Emmanuel Vignal, et al.

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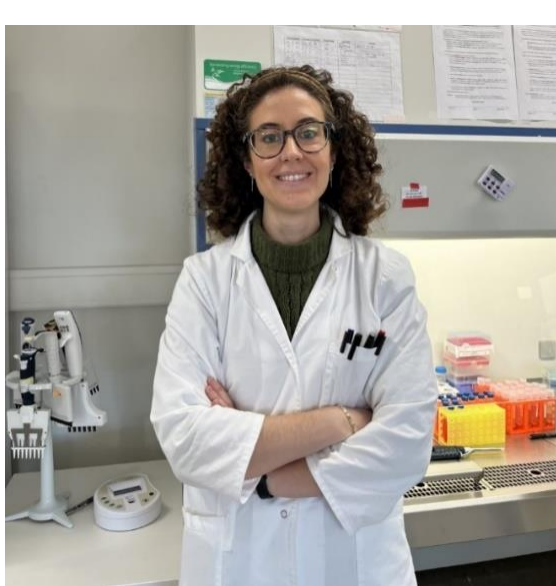
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IMPACT OF COPPER ON THE INTERACTION BETWEEN THE OYSTER *Magallana gigas* AND THE BACTERIAL PATHOGEN *Vibrio aestuarianus*

Hernández-Cabanero, C.¹, L. Parizadeh², E. Bouchereau¹, A. Marot¹, G. Courtay¹, J. Pouzadoux¹, S. de la Forest Divone¹, C. Peris-Perales¹, G. Charrière¹, E. Vignal¹, D. Destoumieux-Garçon¹, M.A. Travers¹

¹Interactions Hôtes-Pathogènes-Environnements (IHPE), UMR 5244, CNRS, Université de Montpellier, Université de Perpignan, IFREMER, Montpellier, France. ²Université de La Rochelle, UMR CNRS 7266, LIENSs, F-17031 La Rochelle, France.

BACKGROUND

Human activities, copper pollution and pathogen emergence

- Widespread use of copper as a biocide in French vineyards has led to the accumulation of heavy metals in coastal environments.
- Oysters (*Magallana gigas*), marine bioaccumulators of copper, can accumulate 719 - 14,000 µg/g of dry weight.
- While copper is an essential micronutrient required for enzymatic functions, elevated levels can exert strong selective pressure on microbial populations, promoting the evolution of metal-resistance mechanisms.

Vibrio aestuarianus subsp. *francensis*

- Emergent marine pathogen described in France in 2001, with a re-emergence in 2011–2012. Now widely distributed in European coasts.
- Responsible for mass mortality events in market-size oysters, causing real and growing economic losses in aquaculture.
- V. aestuarianus* subsp. *francensis* strains are clonal and characterized by oyster-specific pathogenicity and a notable copper-resistant phenotype compared to the environmental non-virulent strains of the species.

Oyster immune response and copper

- In oysters, copper homeostasis is closely tied to immune function and contribute to resistance against other vibrio species infections.
- The immune response of adult oysters during *V. aestuarianus* pathogenesis and the role of environmental factors such as copper levels remains poorly understood.

OBJECTIVE: To investigate whether accumulated copper influences the outcome of the host–pathogen interaction, by deciphering copper-induced immune modulations in oysters and their impact on disease progression.

METHODOLOGY

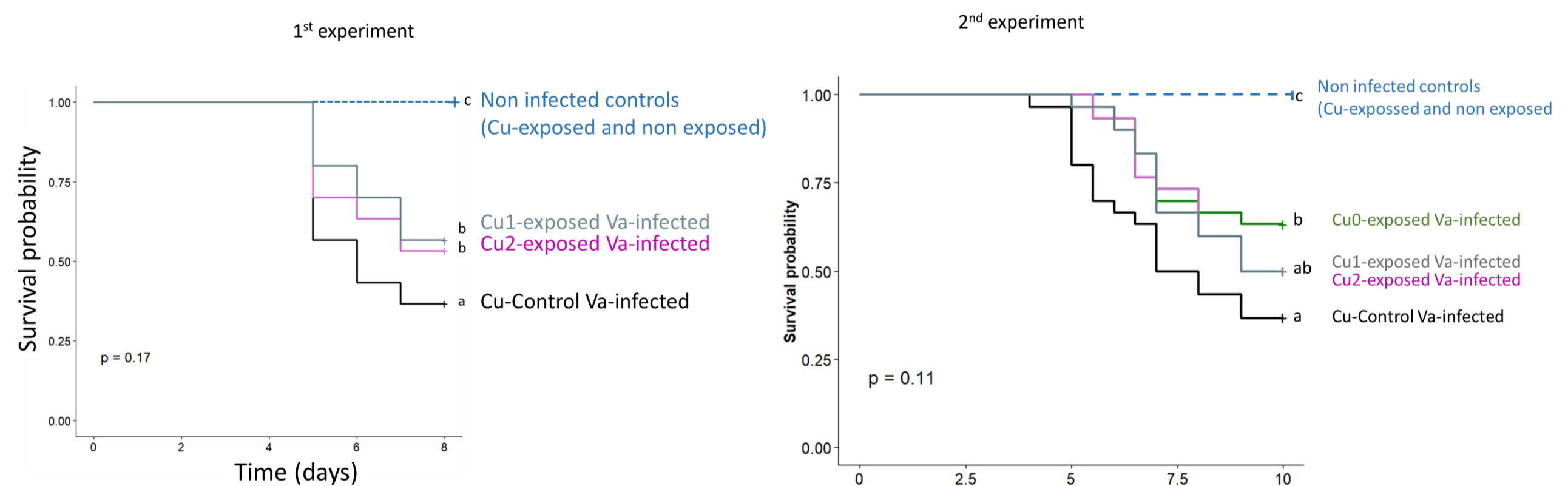
- Adult *M. gigas* oysters were exposed to copper (CuSO₄ 0 [CTL], 30 [Cu1], 60 [Cu2] and 120 [Cu3] µg/L; 3 groups of 40 animals per condition) via immersion for 1 month.
- 15 oyster per copper condition were collected at 0, 14 and 28 days to quantify copper content and determine microbiota composition.
- Oysters were bath-challenged with *V. aestuarianus* strain 12/016 or in the absence of bacteria (control group) (3 replicates of 10 animals per condition).
- Mortality was monitored daily during 14 d.
- Hemolymph was collected before and after infection for downstream analyses (hemocyte composition and bacterial multiplication).
- Gills and hemocytes were collected for transcriptomic analysis by RNAseq.

CONCLUSION

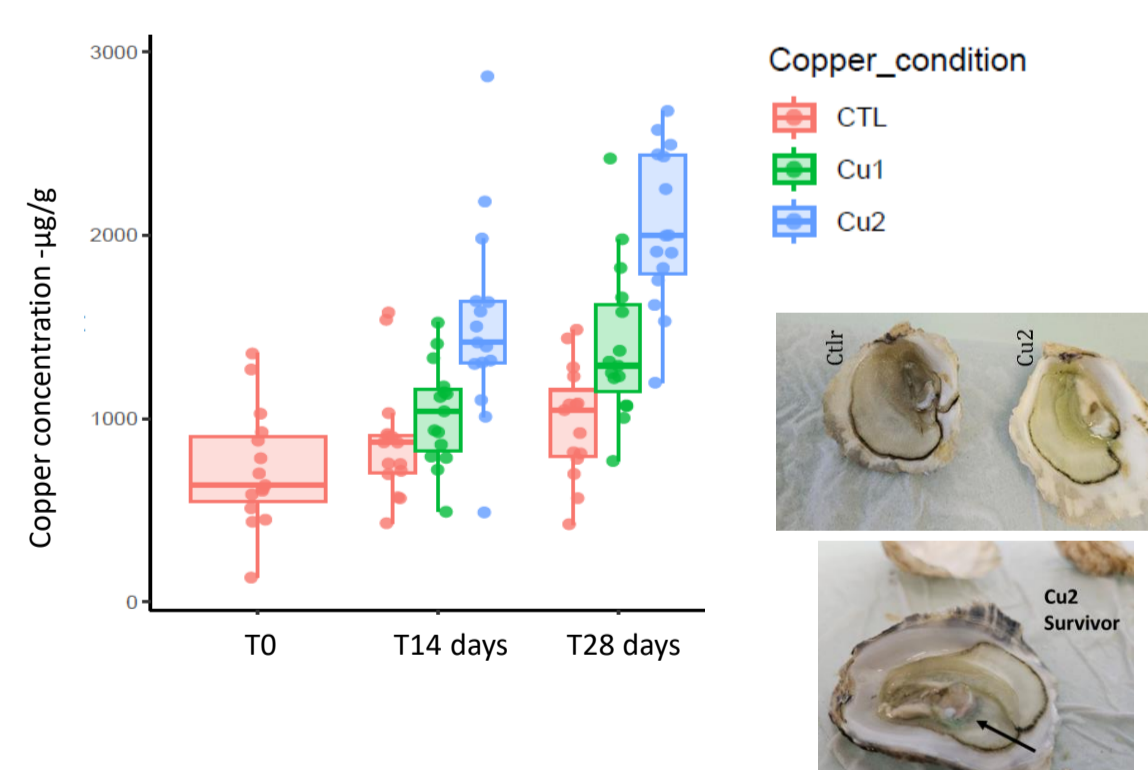
- Copper accumulation in oysters contributes to the host–pathogen dynamic during *V. aestuarianus* infection.
- Reduced intracellular bacterial survival in hemocytes of copper-exposed oysters' points toward copper-mediated enhanced cellular immune defenses.
- Ongoing microbiota analyses and localization studies will help clarify whether copper's impact is mediated by host immunity alone or also involves shifts in microbial communities.
- This work highlights the complex interplay between environmental metal exposure, immune modulation, and pathogen success, emphasizing the need to consider abiotic stressors in the management of oyster health in aquaculture settings.

RESULTS

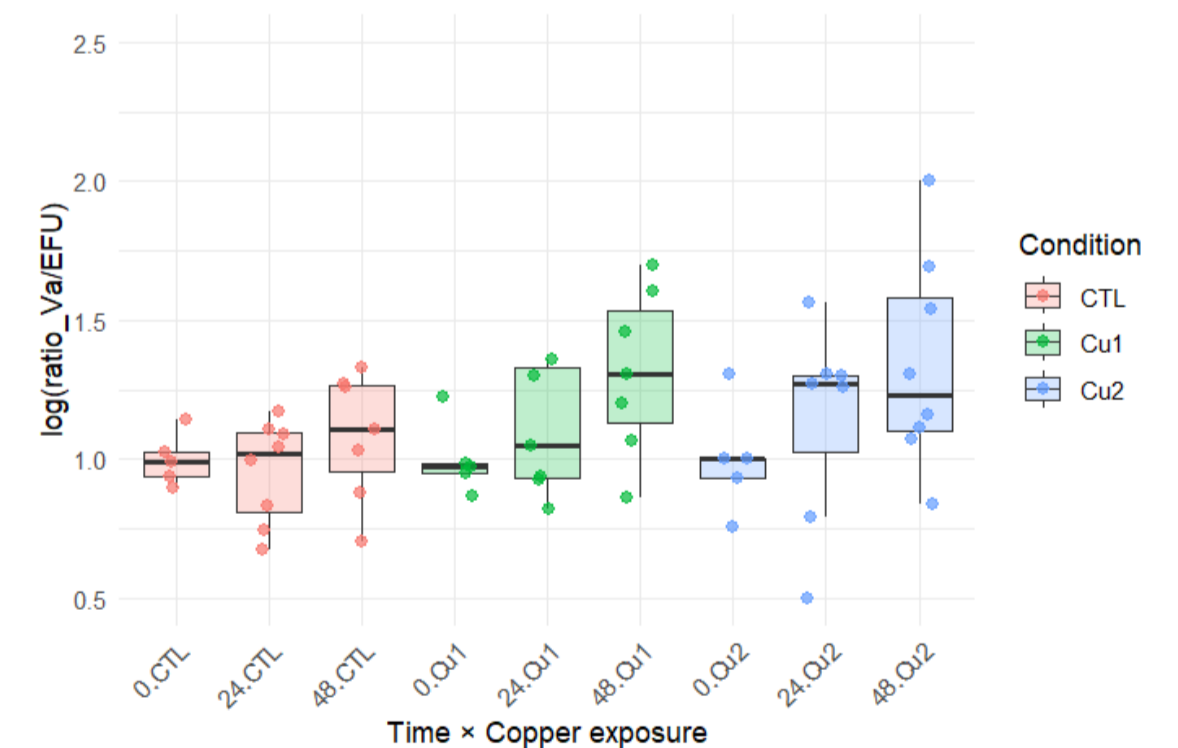
1. Copper pre-exposure confers moderate protection to *V. aestuarianus* in vivo



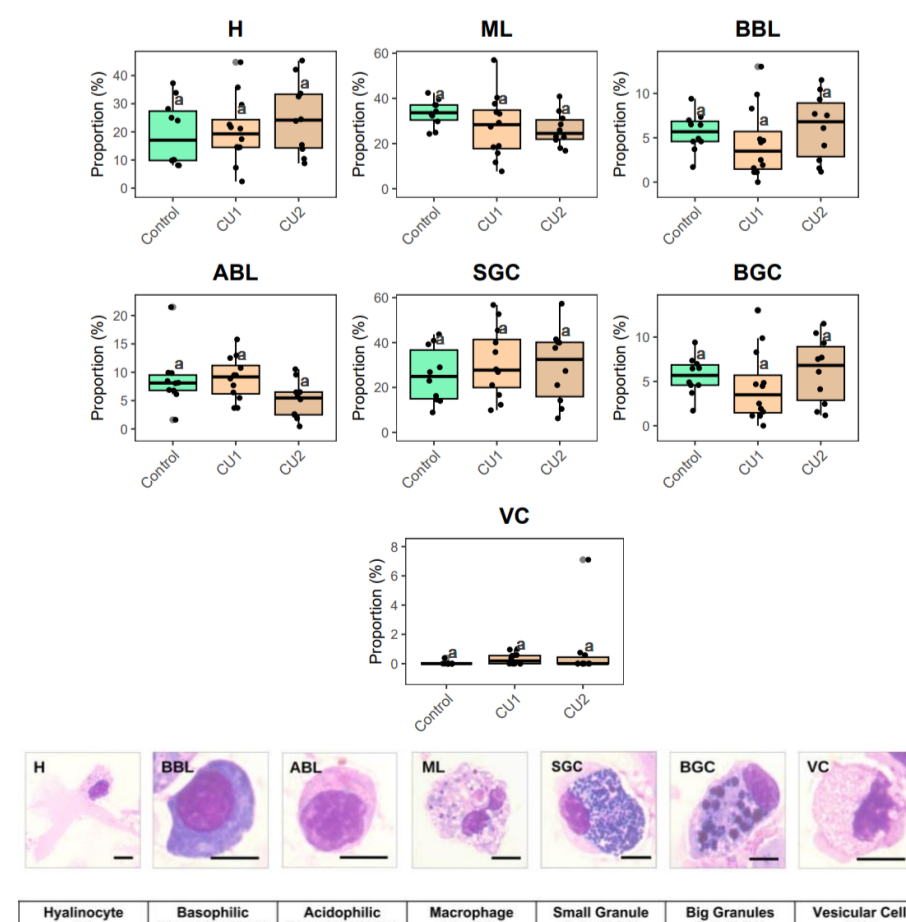
2. Copper pre-exposure leads to copper accumulation in whole oysters



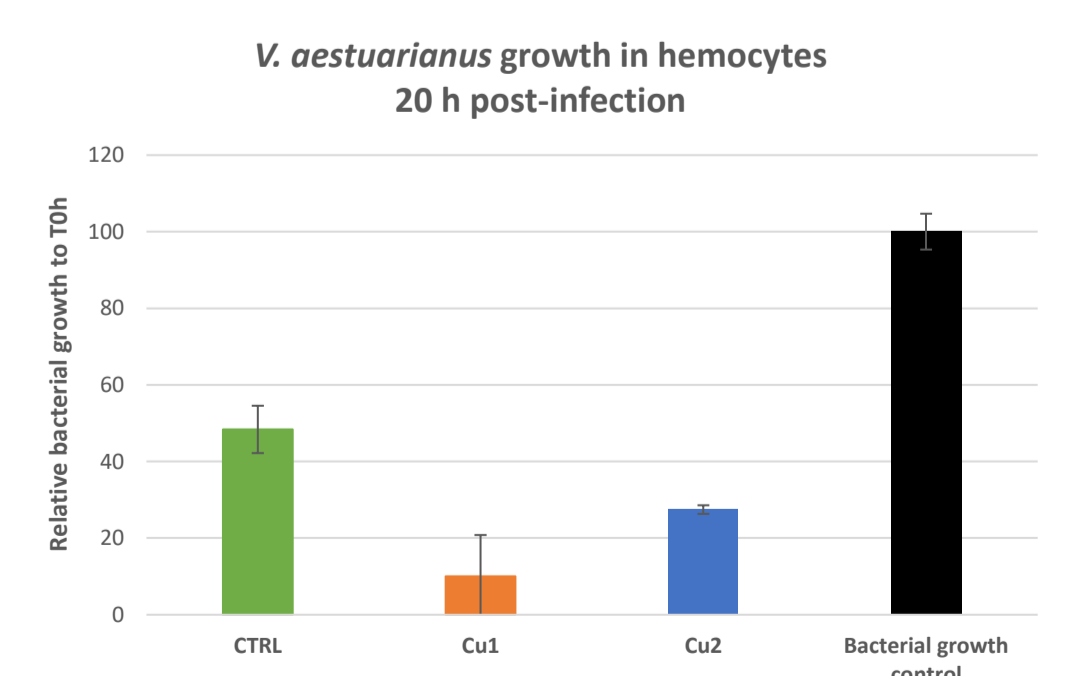
3. Copper pre-exposure does not impact *V. aestuarianus* growth in plasma in vivo



4. Copper pre-exposure does not affect hemocyte population composition



5. Copper pre-exposure enhance hemocytes' intracellular killing of *V. aestuarianus*



Transcriptomic analyses are ongoing to evaluate if copper modulates the oysters' immune-related genes

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