

# The secret suffering underwater

## Does Human-Made Noise Trigger Oxidative Stress in *Ciona intestinalis*?

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### Background

Noise pollution is an emerging threat to marine life, but its full impact remains unclear (1). Preliminary data show that *Ciona intestinalis* (figure 1) exhibits behaviour responds to human-made underwater noise indicating elevated stress. Oxidative stress triggers the production of antioxidant enzymes superoxide dismutase (SOD). By analyzing changes in SOD gene expression after noise exposure, we can uncover how noise induced stress affects marine invertebrates at the molecular level.

**Aim: Analysing changes in SOD gene expression in different tissue after noise exposure.**



Figure 1: *Ciona Intestinalis*, Photo credit Hans Leijnse for MindenPictures

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### The SOD gene

When an organism is stressed, it produces excessive amounts of Reactive Oxygen Species (ROS), which can damage lipids, proteins, and DNA(3). The SOD gene encodes an antioxidant enzyme that helps neutralize ROS. By measuring mRNA expression, we can assess the organism's stress level.

### *Ciona intestinalis*

1. Small compact genome
2. locally and easily accessible
3. small and easy to handle
4. abundant in oceans worldwide
5. Molecular and behavioural tools available (2)

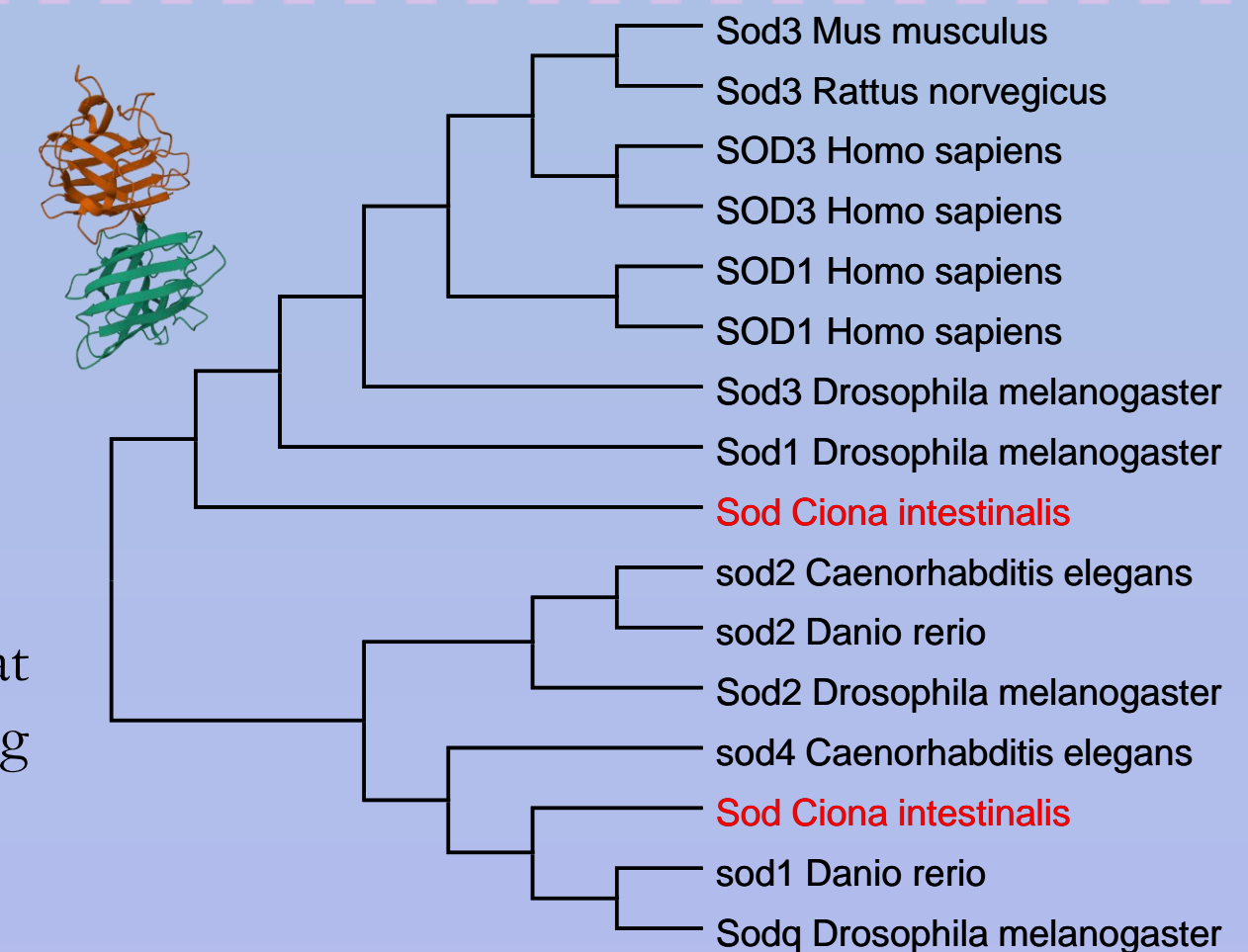


Figure 2: Phylogenetic tree of model organisms based on the SOD gene.

Constructed using Maximum Likelihood in MEGA12, with *Ciona intestinalis* highlighted. Upper right: 3D structure of superoxide dismutase (SOD), retrieved from the Protein Data Bank (PDB ID:1CB4)

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### Material and Method

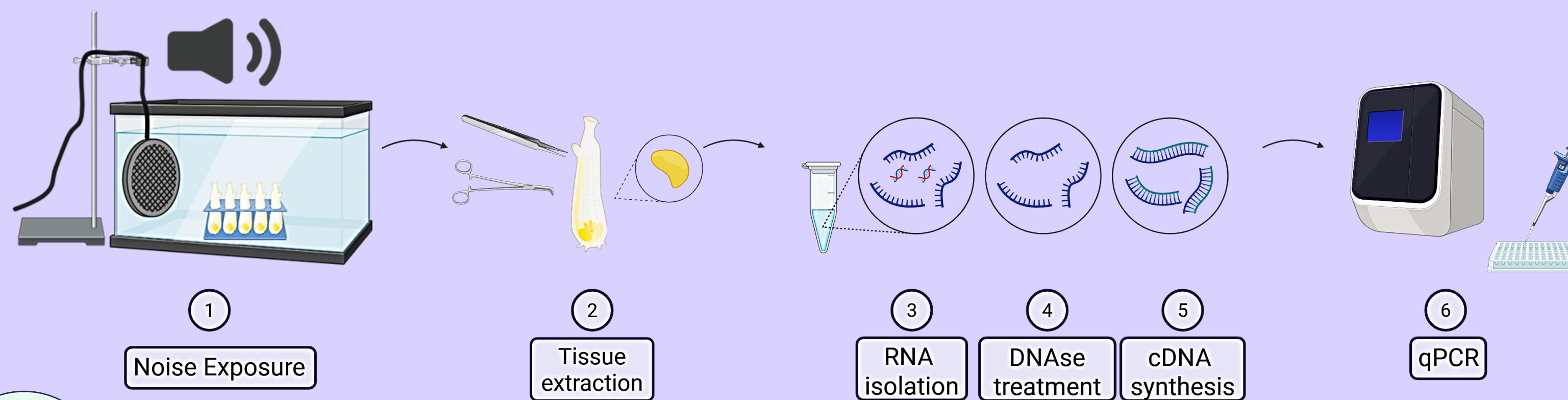


Figure 3: *Ciona intestinalis* were exposed to 149 decibel for 2 hours, tissue from one *Ciona intestinalis* were dissected 1 hour after noise exposure. RNA-isolation, DNase treatment and cDNA synthesis were done. qPCR were run with gene-specific primes triplicates of all different tissue, testing the two different SOD genes.

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### SOD gene 1

### SOD gene 2



Figure 4:  $C_q$  values for SOD gene 1 and gene 2, across different tissues. One animal in Control group (green) and one animal in two hours noise exposed group (red)

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### Result

*In silico* analysis identified two SOD genes in *Ciona intestinalis*, *Sod1* and *Sod2*, revealing their evolutionary relationship to SOD homologs from commonly used model organisms. Phylogenetic analysis showed that *Ciona's* SOD genes cluster with those of both invertebrates and vertebrates, supporting their conserved function across species. (figure 2)

The mRNA expression of *Sod1* and *Sod2* was measured by qPCR across different tissues from one *Ciona intestinalis* (figure 3 and 4). *Sod1* showed consistent increase across all tissues after noise exposure, while the response of *Sod2* expression varied. Heart tissue exhibited the most marked increase of *Sod1* expression, indicating elevated stress to human made noise.

### Conclusion:

SOD expression in heart tissue indicates elevated stress, shown in more expression of SOD genes, after 1 hour noise exposure. Future research should include tissue from multiple *Ciona intestinalis* to be tested using qPCR. Additionally, testing SOD expression from wild animals to exclude potential false heightened SOD expression from animals in captivity should also be considered.

