

## Development of a fluorescent method for the detection in marine organisms of the respiratory electron transport system

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### Abstract

Respiration consumes oxygen, produces CO<sub>2</sub>, and utilizes organic carbon[1]. It is fundamental to marine life and key to assessing metabolism in the ocean[2]. However, because ocean respiration is nearly undetectable by current technology, an enzymatic method based on the electron transport system (ETS) technique was developed[3, 4]. The problem in using this approach stems from the diffuse nature of ocean plankton and the difficulty and cost in concentrating it. Methodology to minimize these two factors is necessary and explored here.

To improve ETS detection, we test a sensitive fluorometric method using diaphorase as proxy for plankton potential respiration. The detection uses the dye, resazurin that is reduced by the ETS, to form resorufin a fluorescent compound. We optimized the reactant concentrations involved in this reduction.

The reactants and the range of concentration for the reduced pyridine nucleotides were: nicotinamide adenine dinucleotide (NADH), 0.1 to 3 mM; nicotinamide adenine dinucleotide phosphate (NADPH), 0.05-0.5 mM; and the salt of the tricarboxylic acid, sodium succinate, 0.5 to 0.5 M. Diaphorase from *Clostridium kluyvery* was used as reference standard (0.02U).

A series of preliminary tests with zooplankton comparing the classic spectrophotometric method with the one we want to introduce shows that both methods are equivalent. The spectrofluorometric assay shows promise in the detection of the respiratory electron transport of the marine plankton, reducing biomasses, minimizing the time, the effort and the costs of the sampling.

### References

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