Electron transport system activity in the presence or absence of its substrates, in the mysid, *Leptomysis lingvura*

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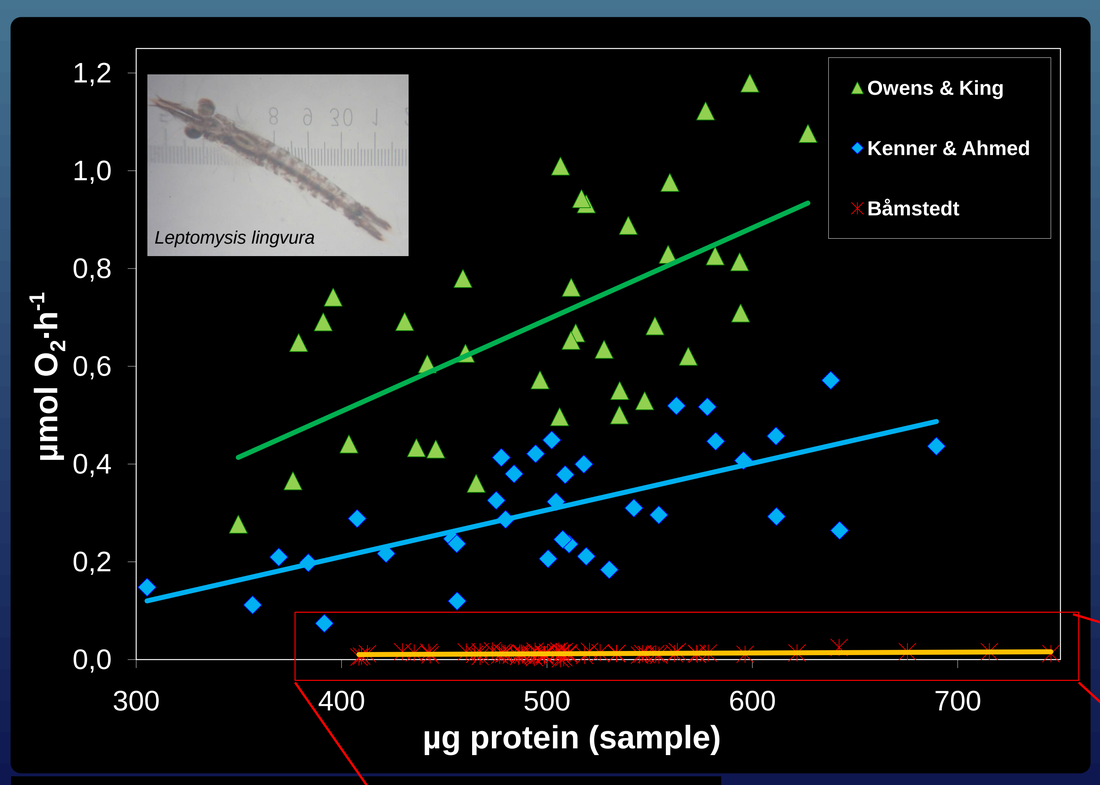
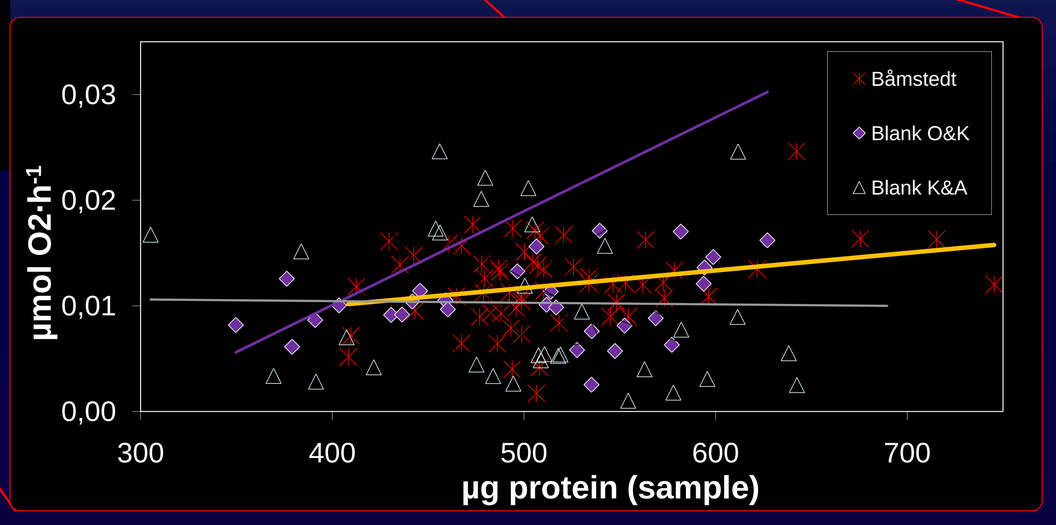
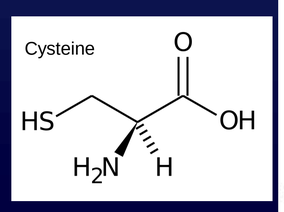
Introduction

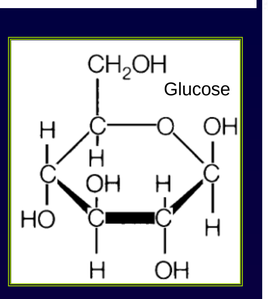
Respiratory electron transport system (ETS) activities have been used to study oxygen consumption in a myriad of marine organisms and communities. Normally, the methodology follows standard practices of enzymology, by measuring the maximum ETS velocity (Packard, 1971). However, in the last decade an ETS assay was developed that deviated from these practices by omitting the biochemical reactants (substrates) that makes the ETS assay specific for respiratory O2 consumption. The rationale was to measure *invivo* ETS activity and hence in-situ respiration (Båmstedt, 2000). This assay used the tetrazolium salt, INT, to detect electron flux, but excluded the substrates (NADH, NADPH and succinate) required for the ETS to function. Instead it relied on the cytoplasm in the cell-free extract of the sample to supply these substrates. Unfortunately dilution during the extract preparation reduces the concentrations of these substrates far below their *invivo* level and below the level required by the ETS enzymes. Here we examine this zero-substrate method by comparing it to ETS activity measured by standard methods.

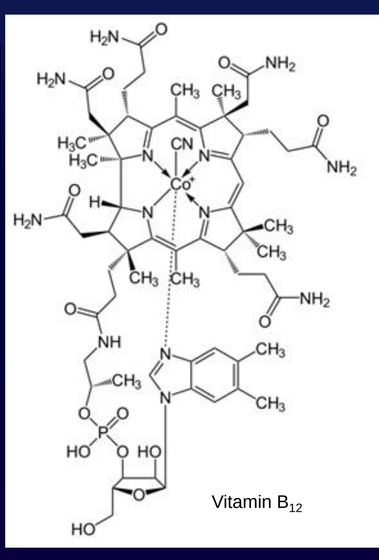


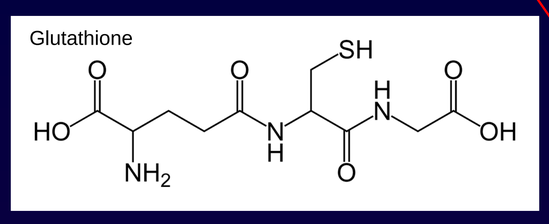
METHOD	SLOPE
OWENS & KING	0,001
KENNER AHMED	0,001
BåMASTEDT	0,00005





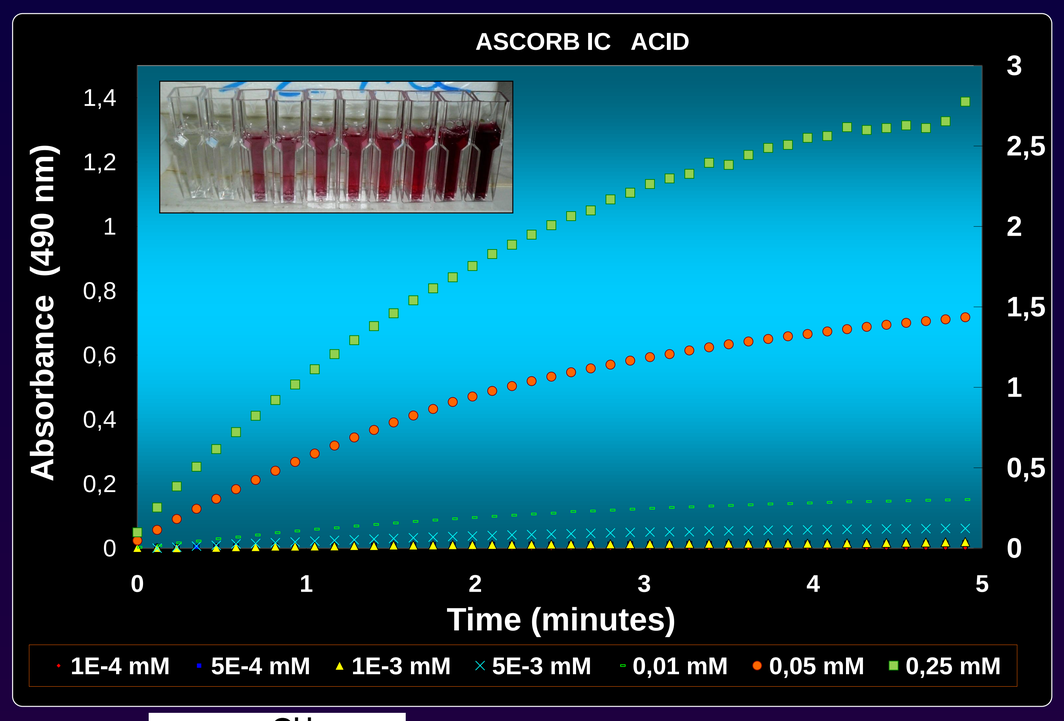


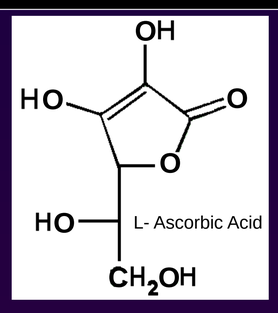


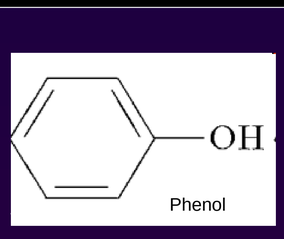


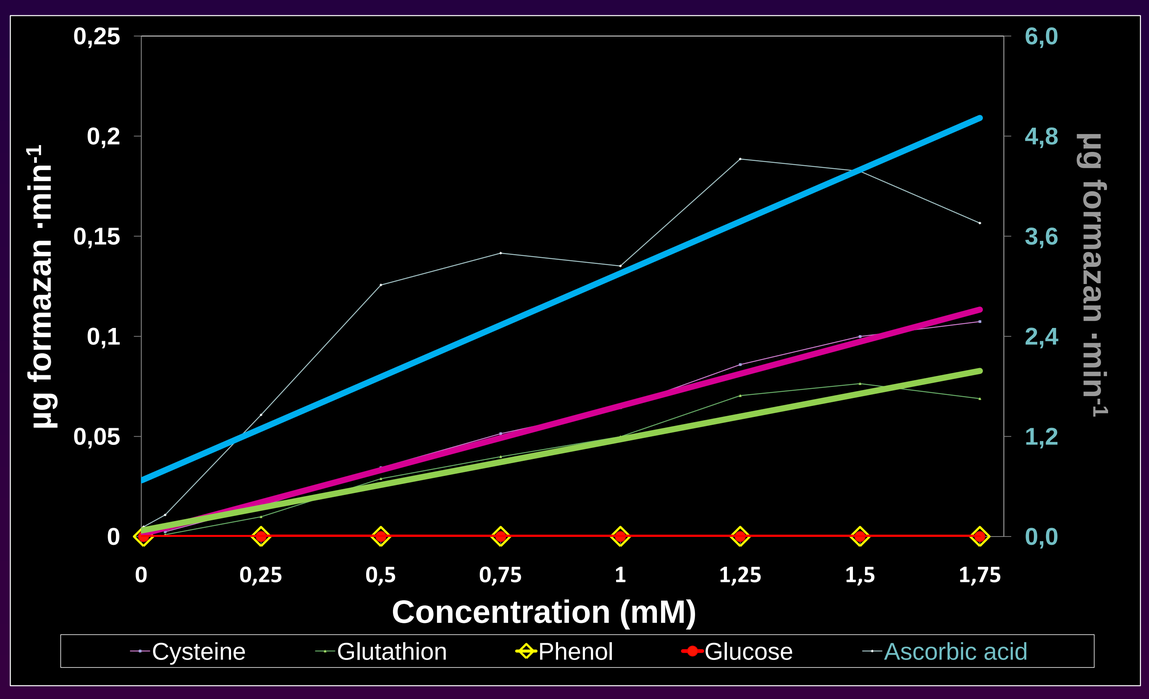
Results

Tests were performed with wild adult males of *L. lingvura*. ETS activity was measured by two methods that use substrates and the one method that does not (Fig 1). The zero substrate ETS method displayed weak activity (Fig 1B), comparable to the blanks of the standard methods. As a result this assay is much less sensitive that the standard assay. Furthermore, it was not clear whether this activity was caused by the ETS or by an non-enzymatic redox reaction between INT and reducing agents in the mysid cells. To test the possibility that INT was reduced by some natural biochemical reducing agents (i.e., ascorbic acid, vitamin-B12, glutathione, cysteine, glucose and phenol), these substances were tested for their natural ability to reduce INT to its formazan (Fig 3). Ascorbic acid, cysteine, glutathione, and vitamin-B12 all reduced INT non enzymatically. (Fig 2) Thus the presence of these substances in sample extracts will confuse the interpretation of an ETS activity measurement made without substrate addition. These substances are ubiquitous in marine organisms practically guaranteeing this confusion.









Conclusions

From our results we conclude that:

1.There is a difference of two orders of magnitude between the ETS rates of the methods using substrates [Owens & King (1975) and Kenner & Ahmed (1975)] and of the method that omits them (Båmstedt, 2000). The latter was equal to the blanks of the first two methods.

2.The method suggested by Båmstedt (2000) is not specific for the ETS, because INT reacts with many substances present in the cell that may or may not be related to the ETS. It measures the background reducing capacity of the extracted cytoplasm.

3.Three of the six substances (glutathione, cysteine and ascorbic acid), reacted strongly and non-enzymatically with INT. INT reduction was directly related to the concentrations. Vitamin B_{12} likely has the same potential to reduce INT as ascorbic acid (vitamin C).

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