UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA

Ciclo de Ciencia Compartida

# PYRIDINE NUCLEOTIDE LEVELS IN ZOOPLANKTON: LABORATORY AND FIELD SAMPLES

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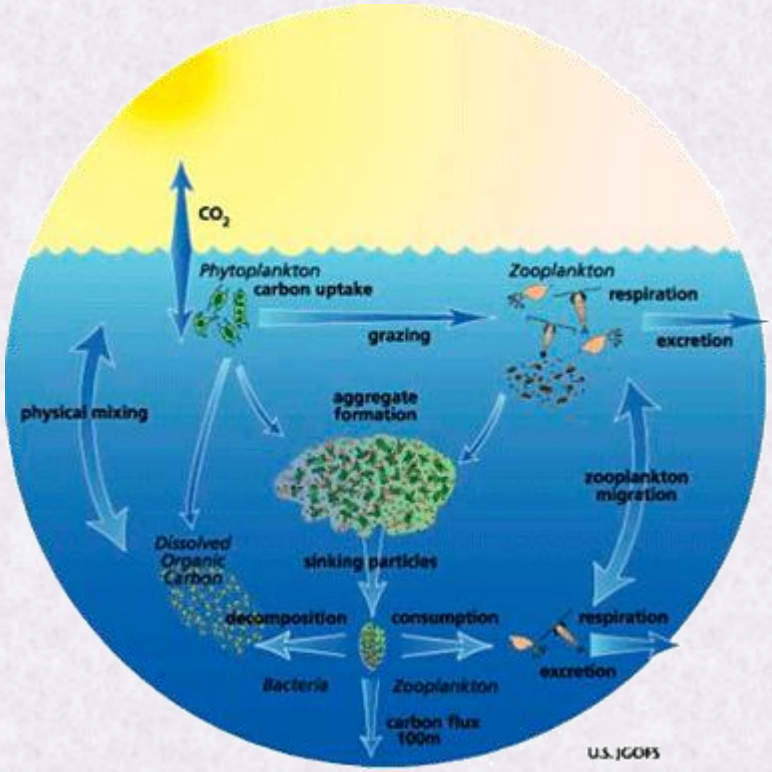
# Respiration

- Ubiquitous process
- Key component in the estimation of the carbon flux.

**Nevertheless…**

¿¿OCEANIC RESPIRATION ??




**Due to**

- Difficult to quantify *in situ* respiration rates.
- Direct measurements → Incubations

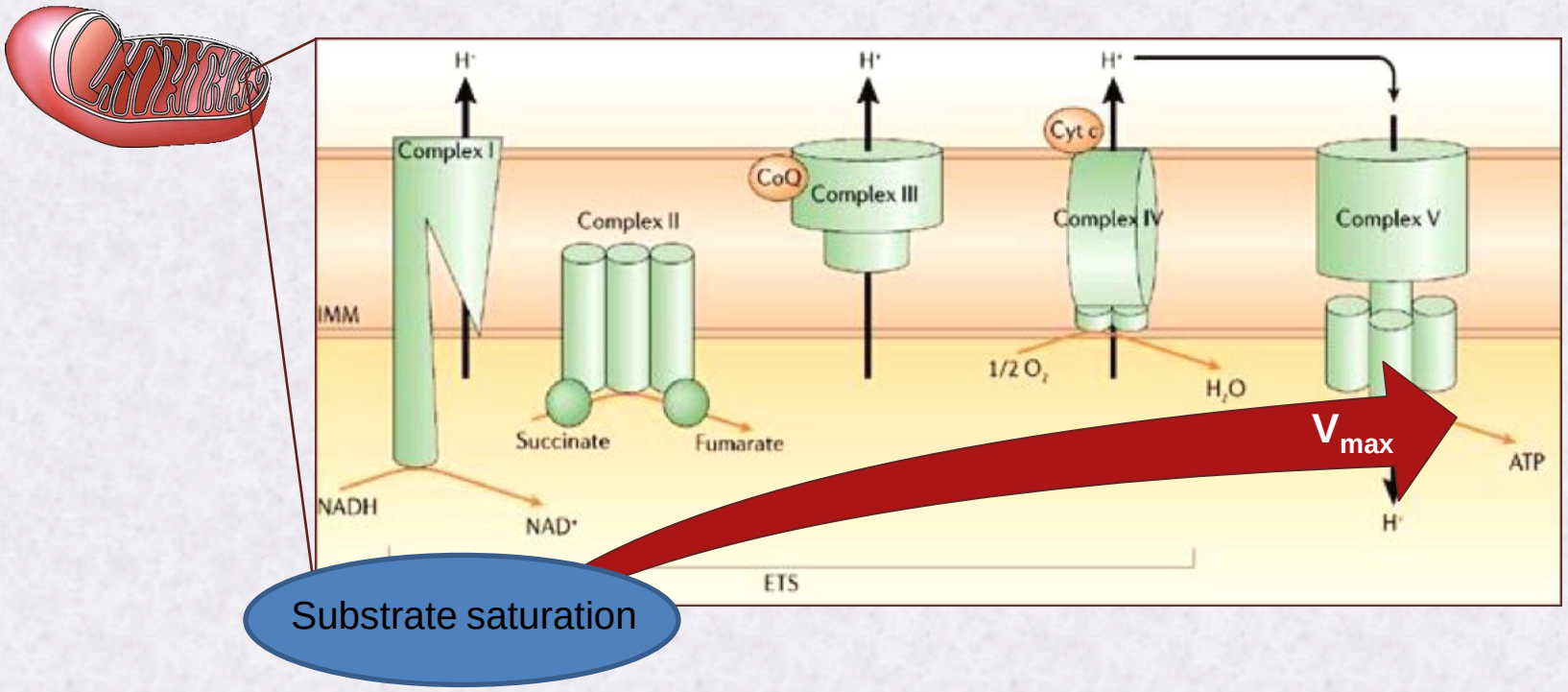
**SOLUTION!!**

**Biochemical assays**

Del Giorgio and Duarte, 2002

# Potential Respiration (Φ)

Electron transport system (**ETS**) activity to estimate respiratory oxygen consumption ($\mathbf{R_{O2}}$)




Packard et al., 1971

## What mechanism controls the $R_{O_2}$?

Physiological $R_{O2}$ ⟺ ETS activity

**$H_o$**: *In vivo* potential respiration determined by the $V_{max}$ and the substrates availability .

**RESPIRATION MODEL**

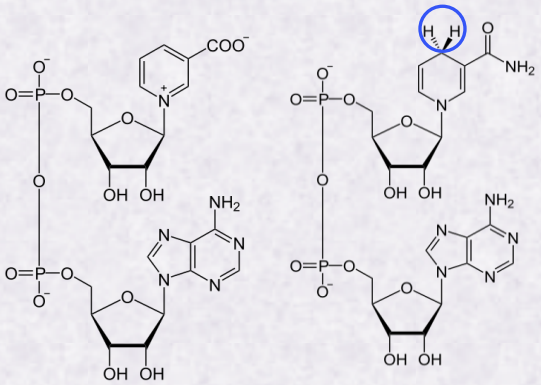
**BASED ON SUBSTRATE LIMITATION**

**Pyridine nucleotides** and succinate

Packard et al., 1996

# Pyridine Nucleotides (PNs)

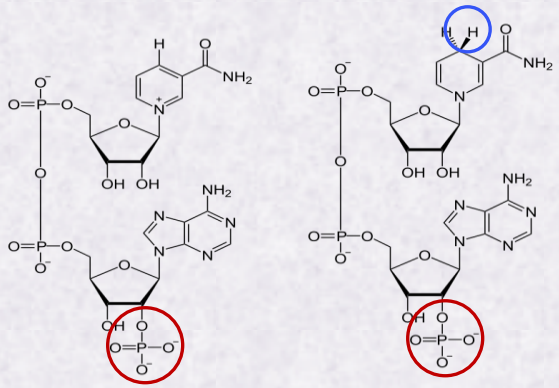
NADt



**$NAD^+$** **NADH**

Catabolism

NADPt



**$NADP^+$** **NADPH**

Anabolism

✓ Reducing equivalents for hundred of cellular reactions.

✓ **Functions**:

- Modulating energy metabolism, reductive biosynthesis and antioxidation processes.
- Recent studies, importance in cell signaling in animals, plants and fungi.

(Berger et al., 2004; Ziegler, 2005; Pollack et al., 2007; Ying, 2008)

# Previous Work

**Animal physiology**

- During 50's and 60's, development of different techniques to measure PNs (Chance et al.,1955,1962; Lowry et al. 1961).
- Recent clinical applications (Mayevsky and Chance, 2007).

**Plant physiology**

- Knowledge increasing steadily → Levels and roles under different metabolic conditions.

(Moller and Rasmusson, 1994; Hagerdon, 2004; Noctor et al., 2006)

**Bacterial activity**

- Wos and Pollard (2009): NADH as an index of bacterial metabolic activity in activated sludge.

➢ **Few studies in marine organism**

- NADP+ in relation to isocitrate dehydrogenase activity (Roy and Packard, 1998).
- Attempted to estimate marine primary production with the NADPH (Steigenberger et al., 2004), .

# Previous Work

**Animal physiology**

- During 50's and 60's, development of different techniques to measure PNs (Chance et al.,1955,1962; Lowry et al. 1961)
- Recent clini

**Plant physio**

- Knowledge

ctor et al., 2006)

**Up to now, NO work in marine organisms**

Describing intracellular levels of PNs

Comparing PNs levels with respiration rates

**Bacterial activity**

- Wos and Pollard (2009): NADH as an index of bacterial metabolic activity in activated sludge.

➢ **Few studies in marine organism**

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# Laboratory Work

# *Oxyrrhis marina*

- Heterotrophic dinoflagellate.
- High tolerance to change in temperature, salinity and nutrients → Good for culturing!

**Why??**

✓ Easy to culture

✓ Model organism → respiratory metabolism??

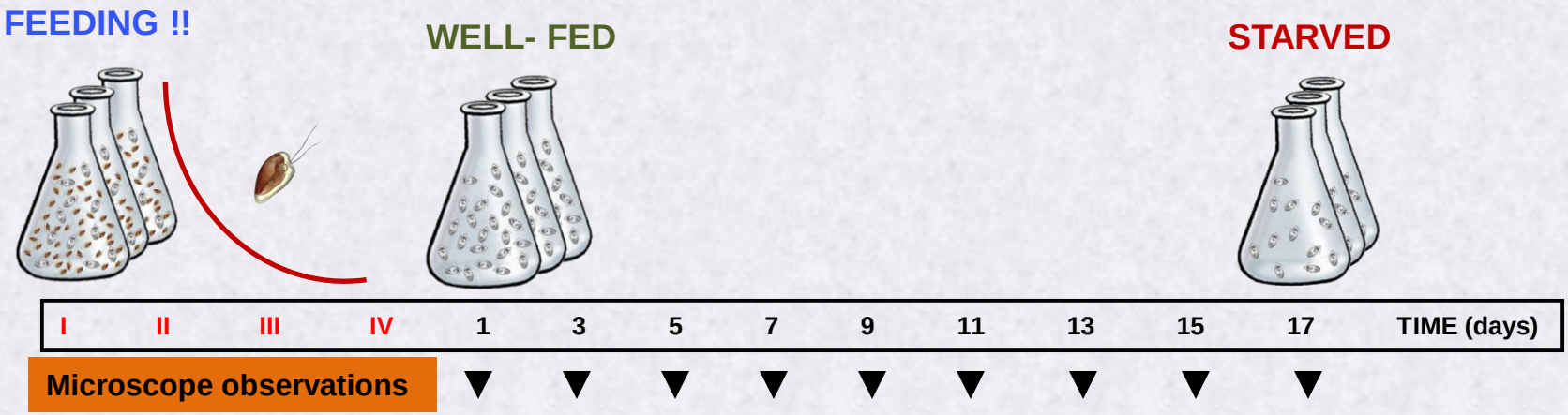

*Oxyrrhis marina,*
Aristizabal (2009)

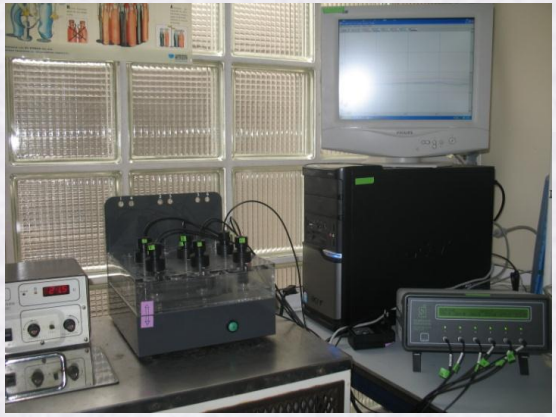
*Rhodomonas salina*

- Fed with *Rhodomonas salina*
- Same culturing conditions, except for light

Droop,1959; Kimmance et al., 2006; Jeong et al., 2003

# Experimental Design



**Unfiltered samples**

Respiration

Cell number

**Filtered samples**

ETS activity

PN levels

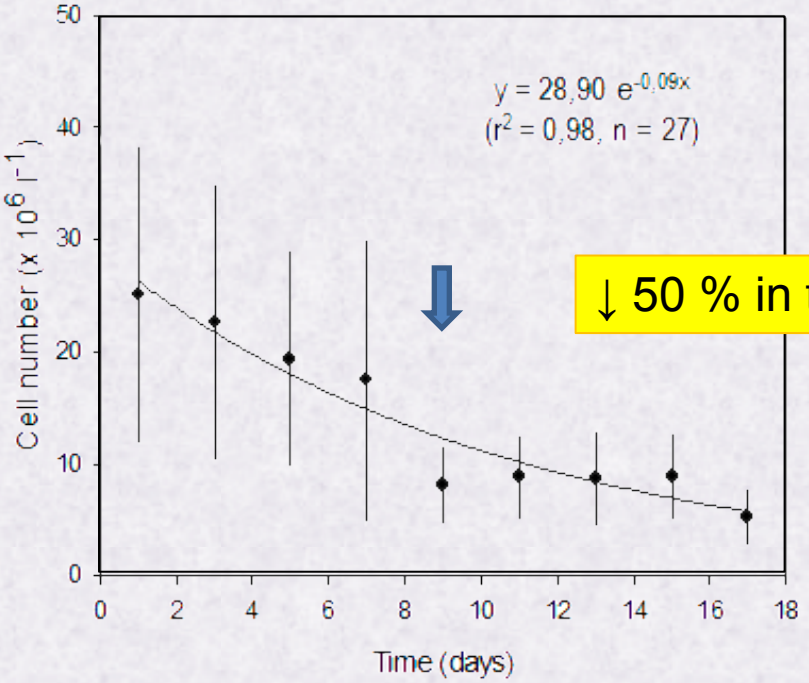
Protein

Chlorophyll

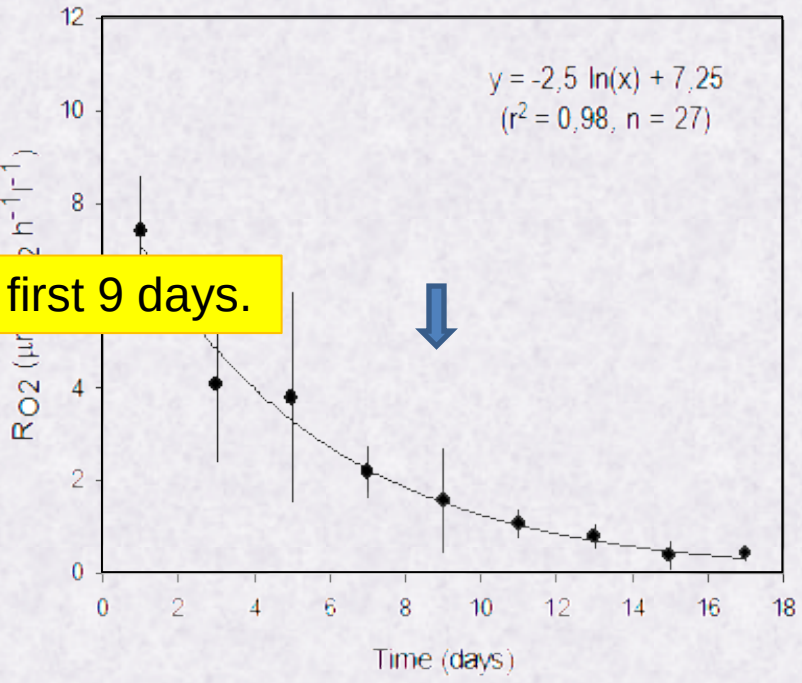
# Results & Discussion

*Time profiles (I)*

Cell number




Respiration




↓ 50 % in the first 9 days.

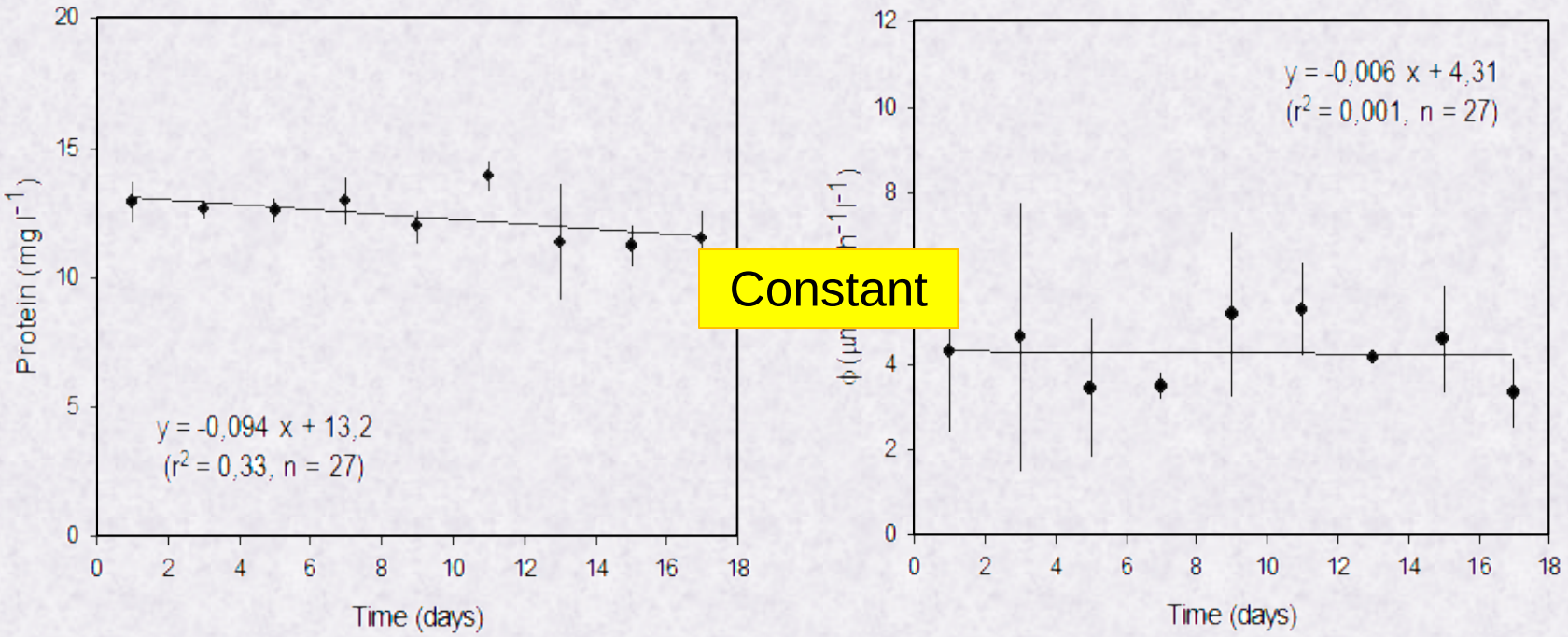
- Exponential decrease of cell number and respiration rates.

## Results & Discussion

*Time profiles (II)*

Protein

Potential respiration



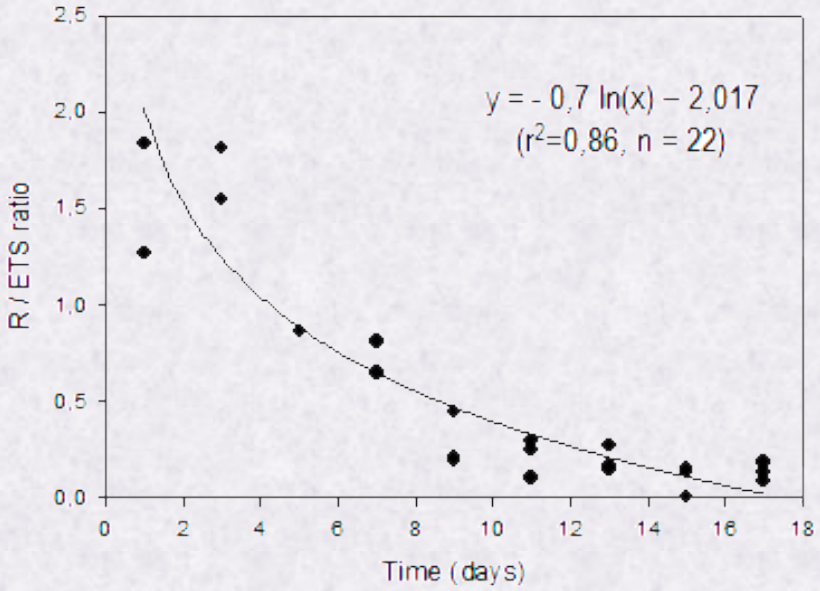

Constant

- Nearly constant during food deprivation period
- Parallel behavior of protein and ϕ → ETS activity good index of living biomass

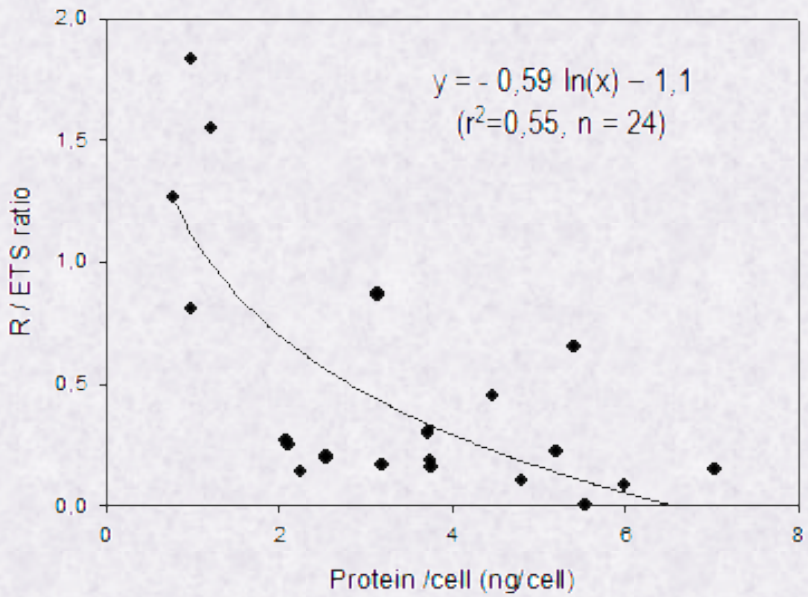
# Results & Discussion

*Variability of R/ϕ*

**During starvation …**




**With cell size …**




R / ϕ ratios

- Index of the physiological state
- Well- described variability in laboratory cultured organism and marine samples

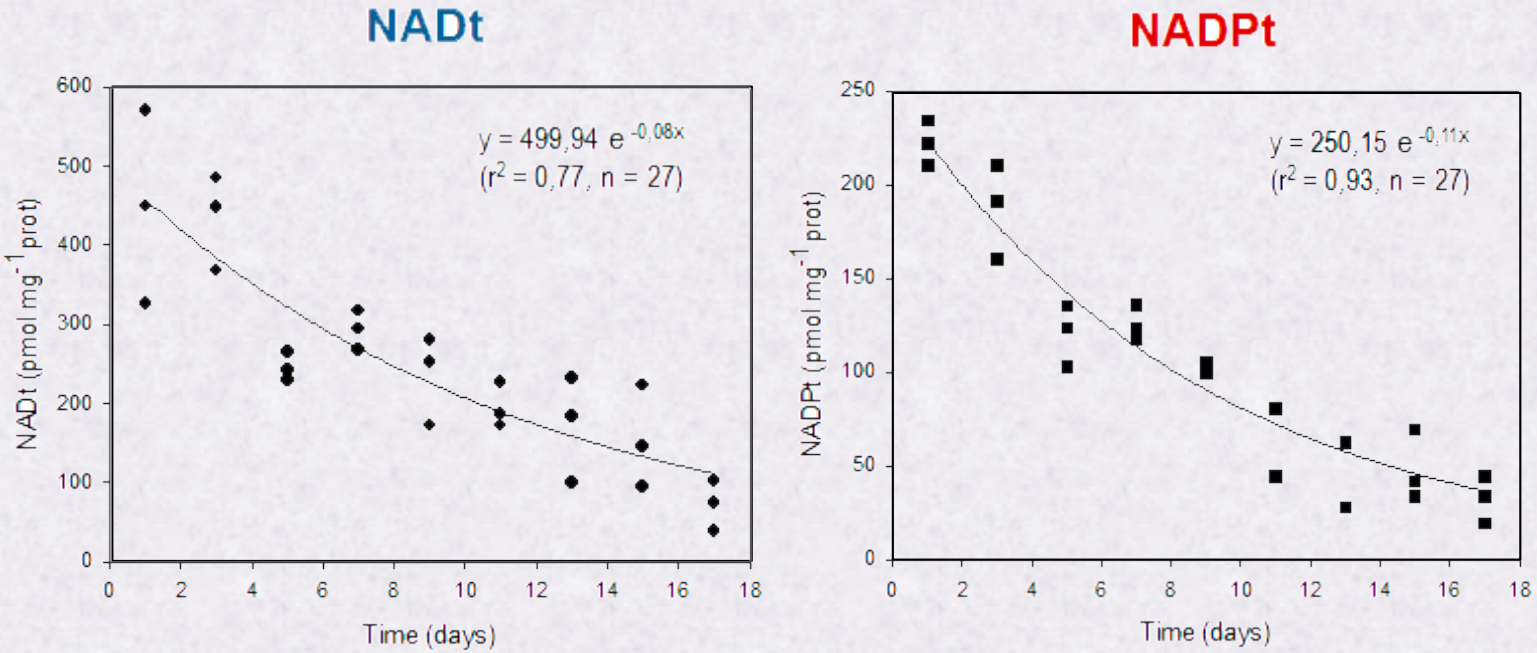
Higher R/ϕ in well-fed and smaller organism

↑ **R/ϕ** ↑ Primary production and ↓ size fractions

Christensen et al., 1980; Packard, 1985; del Giorgio,1992; Arístegui and Montero, 1995; Hernández-León and Gómez, 1996

# Results & Discussion

*PNs levels*




**First determination of intracellular PN levels in marine organism !!**

In the same range that animal and plant cells

**First demonstration in marine organism !!**

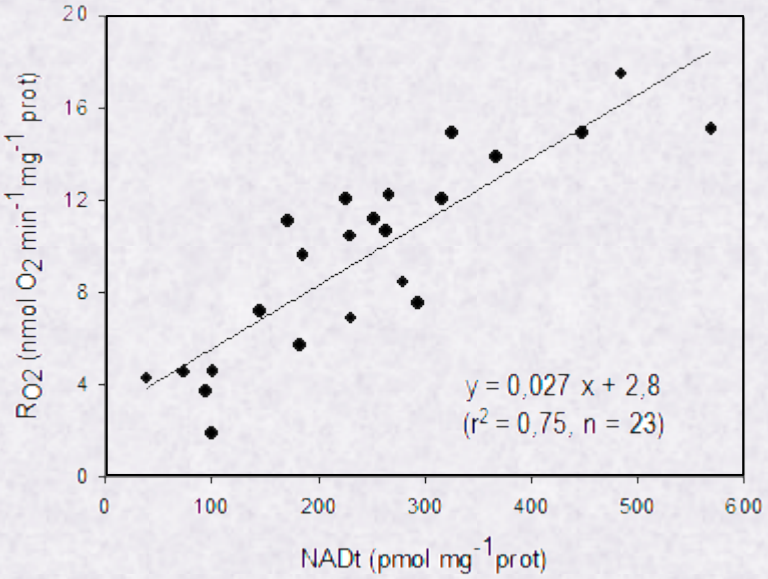
↑ Food starvation, ↓ NADt and NADPt concentrations.

Agius et al., 2001; Umemura and Kimura, 2005

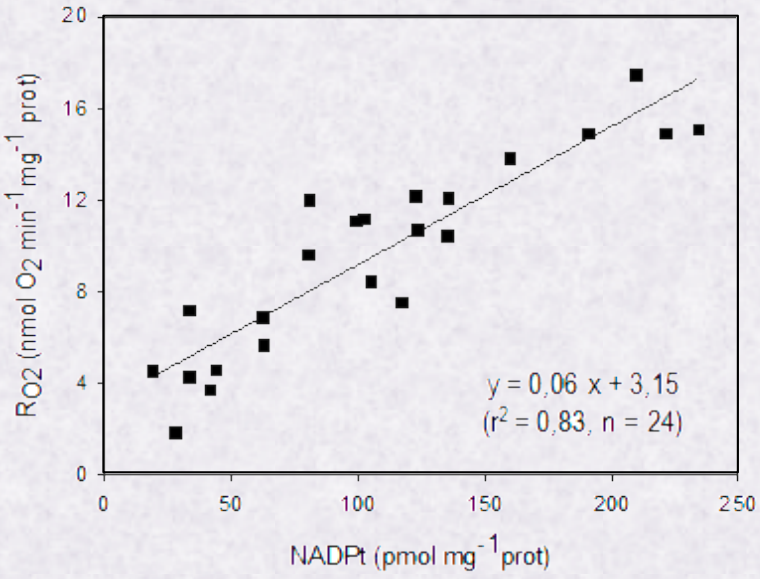
# Results & Discussion

*PNs levels*

**NADt**




**NADPt**

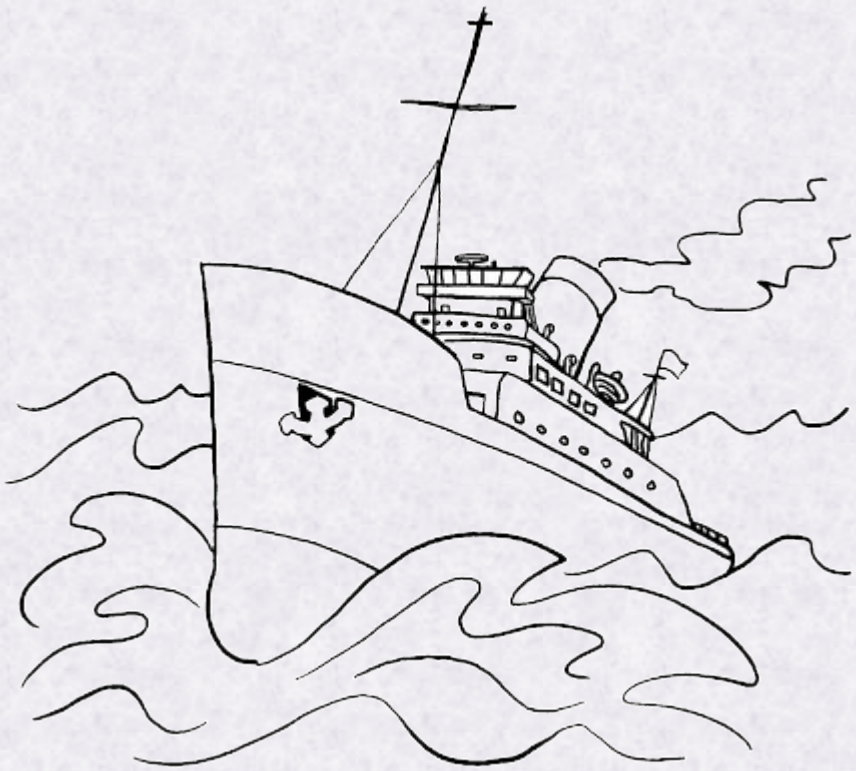



High correlation between Respiration and PN levels

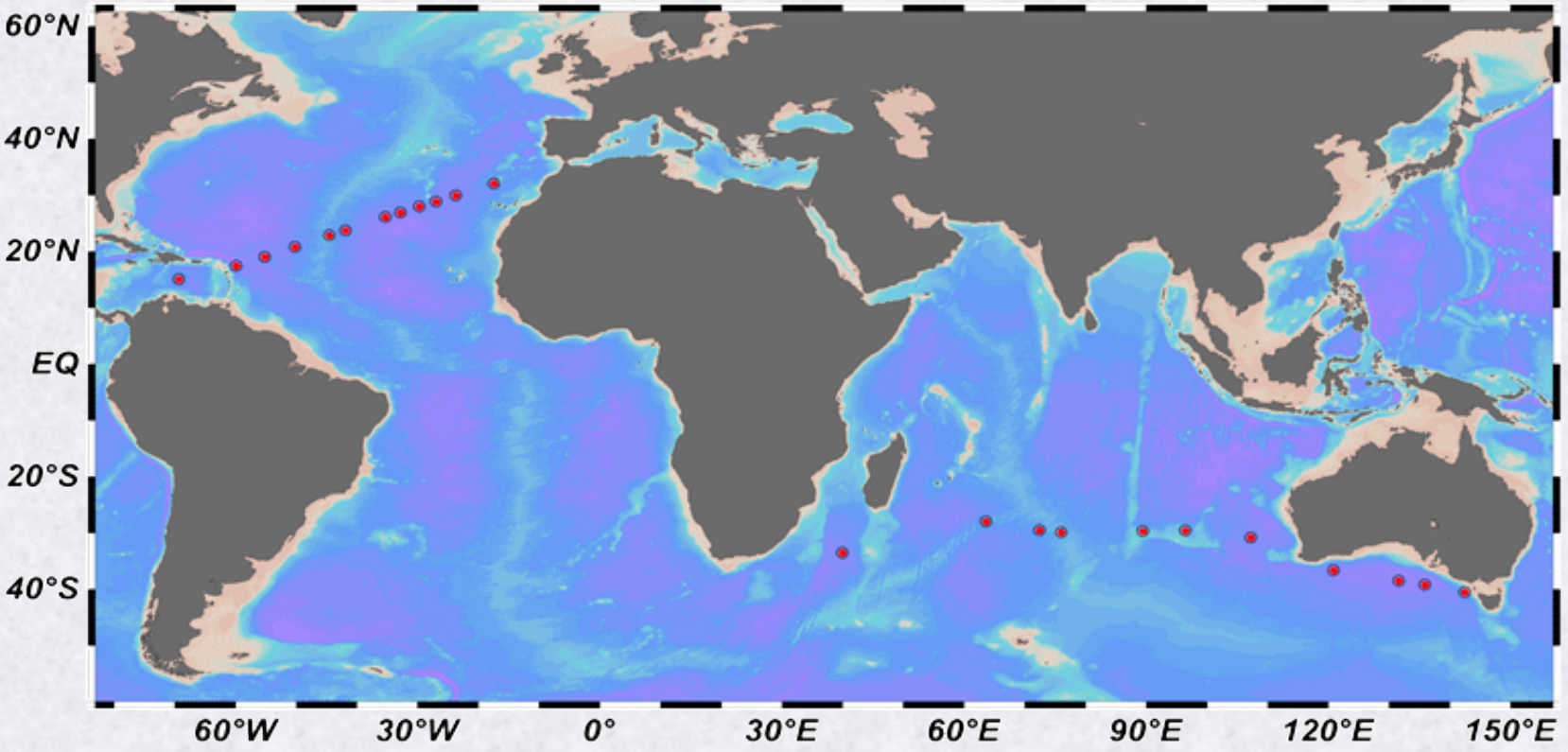
**Supports the respiration model based on substrate limitation**

Packard et al. 1996

# Field Samples



# Sampling




Cruise track of the R/V Hesperides during the LEG 3, 4 and 7.

- LEG 3,4: February - April 2011.
- LEG 7: June - July 2011.

# Methods

100 µm mesh size WP-2 net

150 - 200 m sampling

SIZE FRACTIONATION

> 1000 µm

500 – 1000 µm

100 – 500 µm

**0 h**: Well fed

**24 h**: Starved

**RESPIRATION MEASUREMENTS ON BOARD**

**Storage at - 80°C**

**LABORATORY WORK AT INSTITUTE**

ETS ACTIVITY

INTRACELLULAR SUBSTRATES

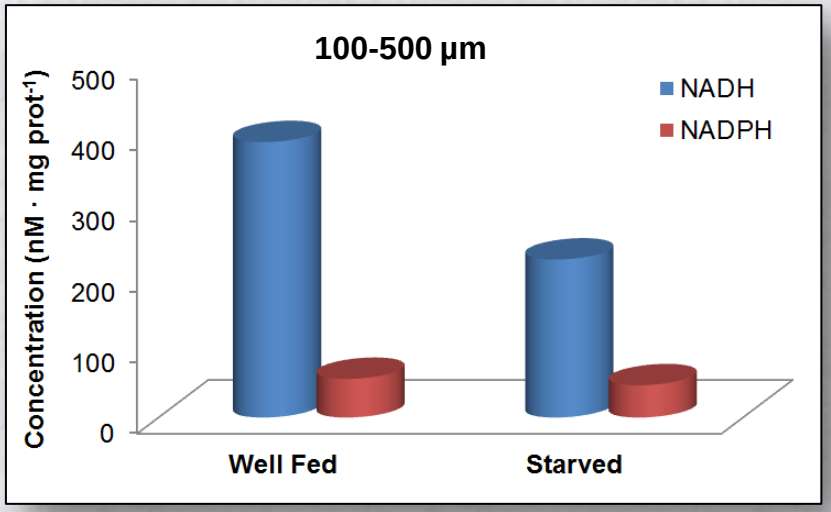
PROTEIN MASS

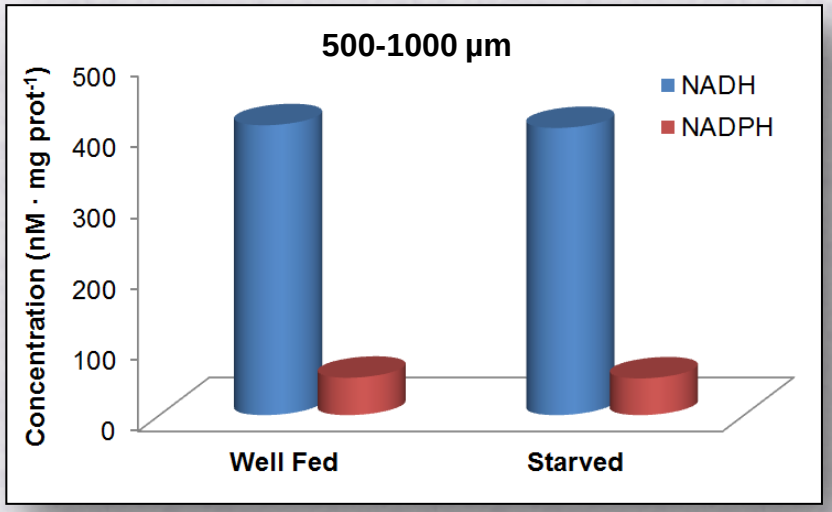
# Results & Discussion

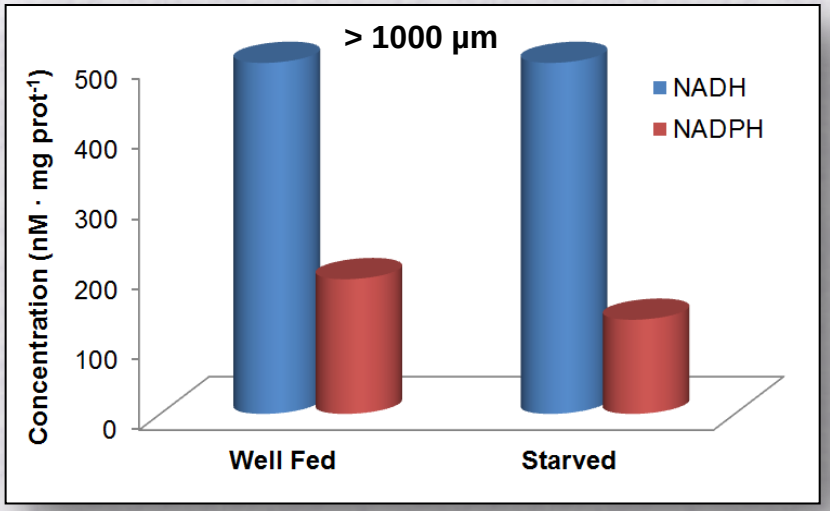
| | Size | n | R (µmol $O_2$/ h · mg prot) | Φ (µmol $O_2$/ h · mg prot) | R / Φ |
|---|---|---|---|---|---|
| ***Well Fed*** | | | | | |
| | **100- 500 µm** | 38 | 2.584 ± 2.426 | 2.190 ± 1.317 | 0.888 ± 0.621 |
| | **500- 1000 µm** | 29 | 2.303 ± 1.637 | 2.494 ± 1.743 | 0.923 ± 0.939 |
| | **>1000 µm** | 15 | 1.351 ± 1.077 | 1.239 ± 0.542 | 1.09 ± 1.987 |
| ***Starved*** | | | | | |
| | **100- 500 µm** | 9 | 3.053 ± 2.173 | 3.323 ± 2.021 | 0.919 ± 1.075 |
| | **500- 1000 µm** | 6 | 1.452 ± 1.189 | 2.073 ± 0.759 | 0.700 ± 1.566 |
| | **>1000 µm** | --- | --- | --- | --- |

- Specific respiratory oxygen consumption decreases with size fraction, both in well-fed and starvation conditions.
- No significantly differences of the R/Φ ratio between well-fed and starved organisms.

# Results & Discussion



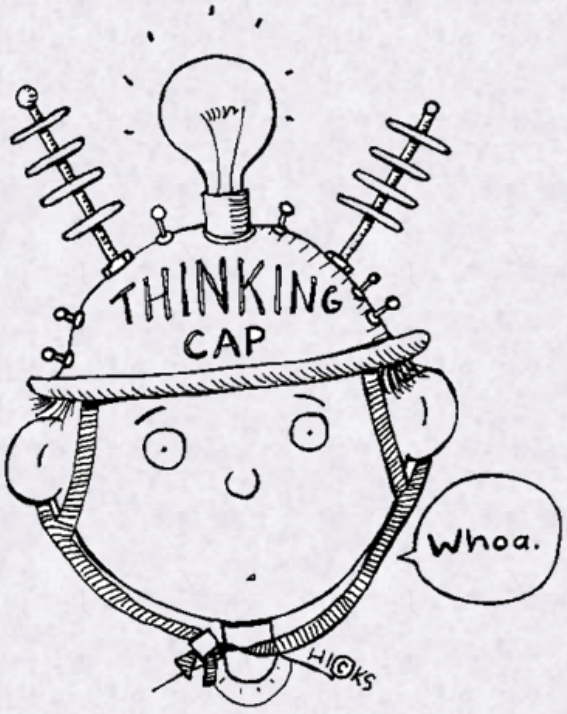




- Specific NADH and NADPH concentration not significantly different in the three size classes.

- No significantly diferences between well-fed and starved organisms,

  - *Exception*: NADH levels in 100-500 m size fraction

# Conclussions



# Conclusions

*Laboratory work …*

- The fall in the respiration during the onset of starvation suggests that respiration is substrate limited during this period.
- Total PNs and respiration are well correlated during starvation in a marine dinoflagellate. This observations supports the use of a respiration model based on substrate limitation.

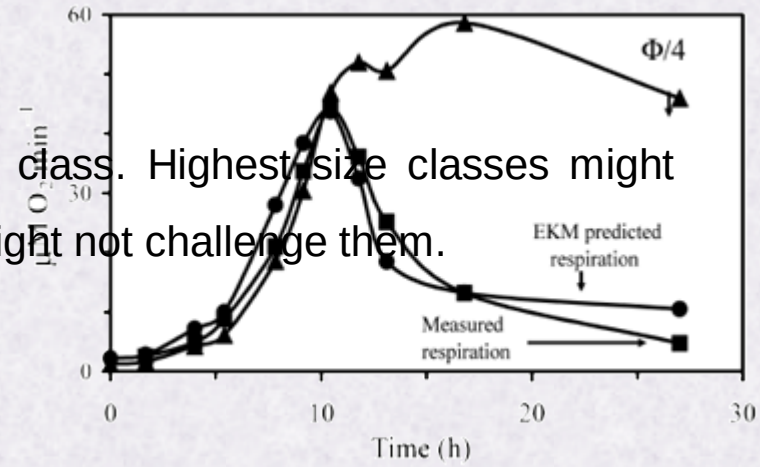
*Field data …*

**ENZYME KINETIC MODEL**

$$R = \frac{Vmax \cdot [S1 \cdot S2]}{Kia \cdot K1 \cdot K2 + K2 \cdot [S1] + K1 \cdot [S2] + [S1 \cdot S2]}$$

***Vmax****:* Maximum velocity of the reaction
***S1, S2****:* Substrate concentration of NADH and NADPH
***K1, K2****:* Michaelis constant of NADH and NADPH
***Kia****:* Apparent dissociation constant

- Effect of starvation seen in 100-500 µm size class. Highest size classes might have a larger reserve and 24 h of starvation might not challenge them.




Packard and Gomez, 2008

# Acknowledgements

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