

# STUDIES ON THE CHARACTERISATION OF BIOMARKERS OF NUTRITIONALLY-DERIVED STRESS IN PARALARVAL CULTURES OF THE COMMON OCTOPUS

## (*Octopus vulgaris*)

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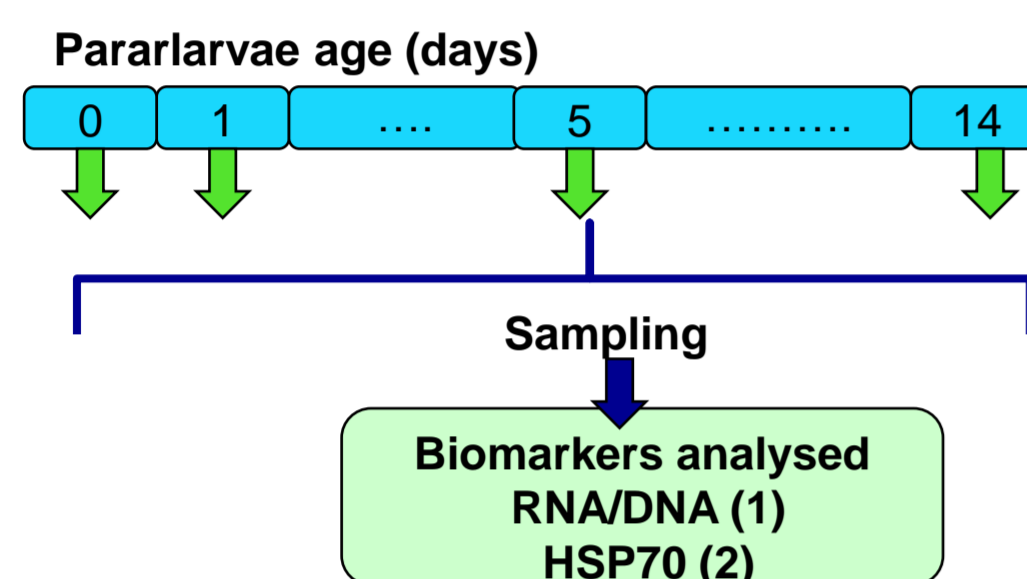
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## INTRODUCTION

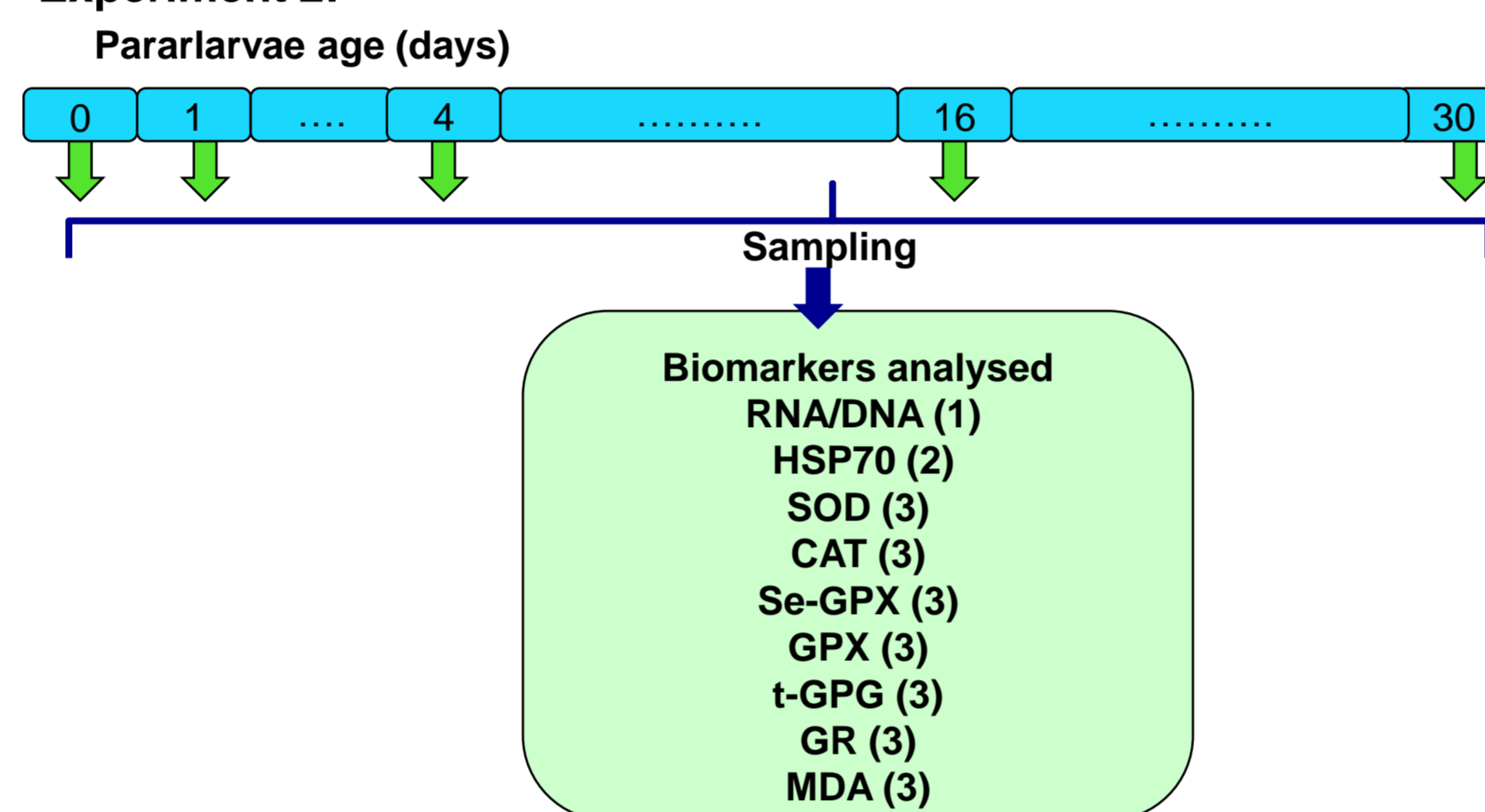
Nowadays, due to the high mortality within the first 30 days of life, octopus paralarvae culture represents the main obstacle for commercial production of this species. The causes of such mortality are not yet well defined and understood. **As a part of a broader project aimed at characterising the causes of such massive mortality, we envisaged the study of nutritionally-derived stress, through the selection of biomarkers capable of its detection and quantification.**

## MATERIAL and METHODS

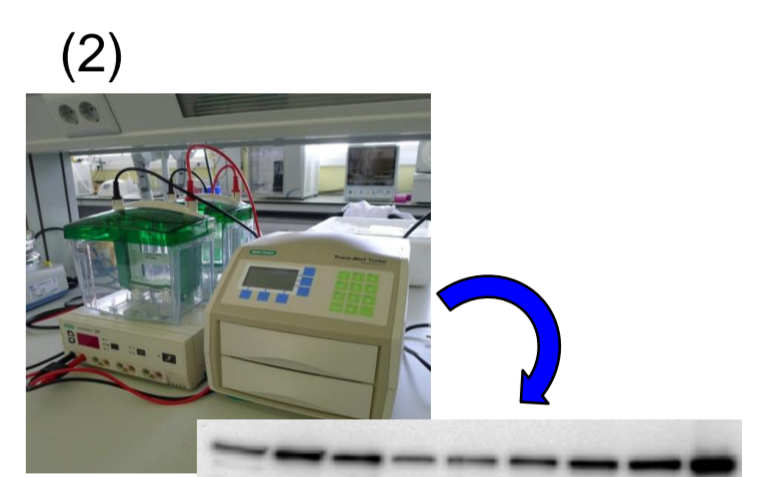
### Experiment 1:



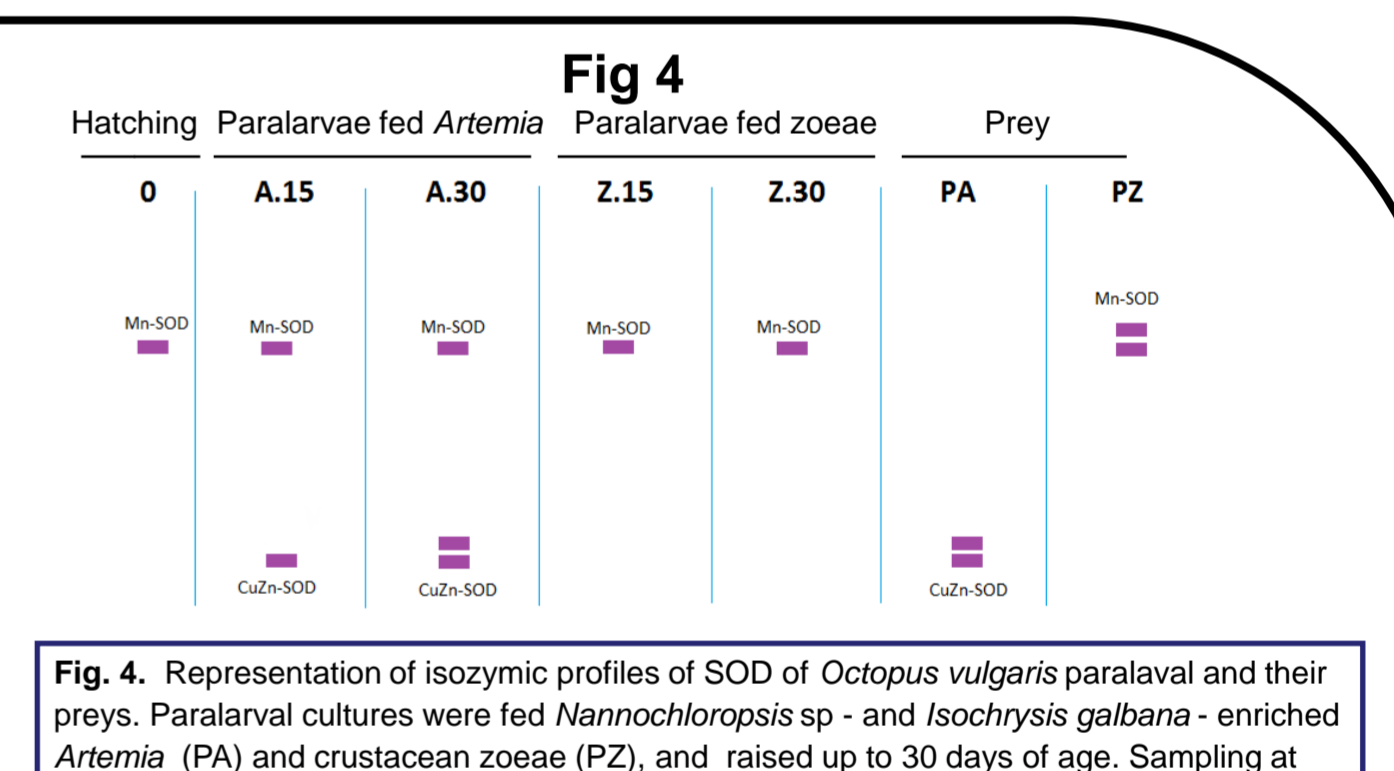
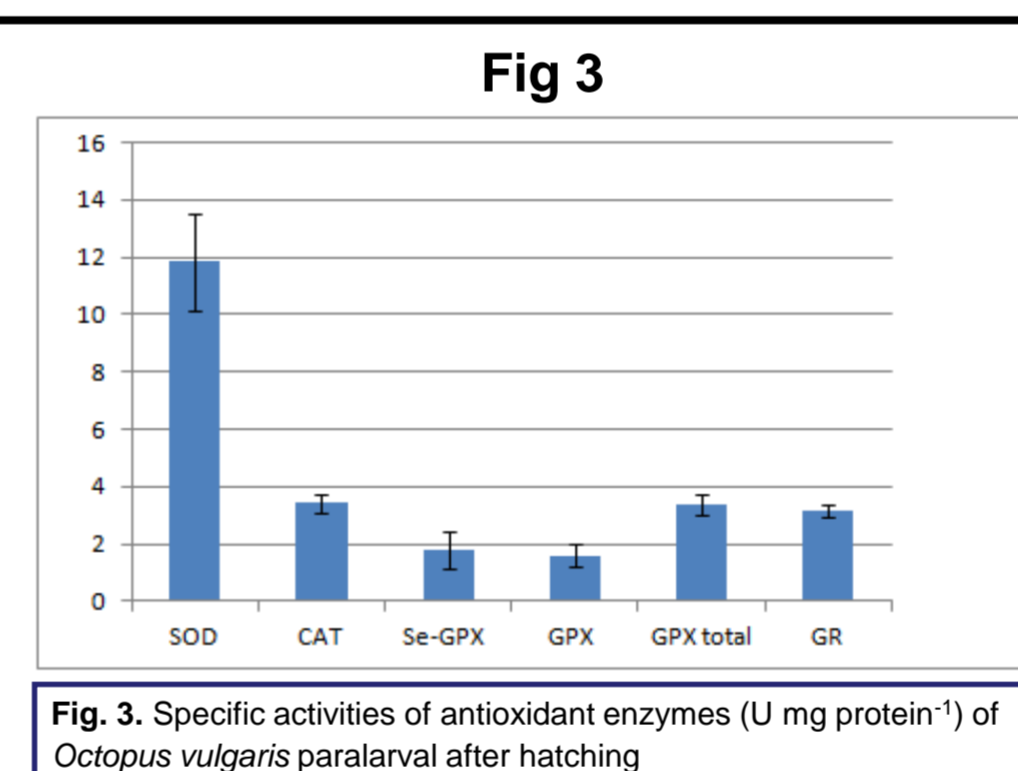
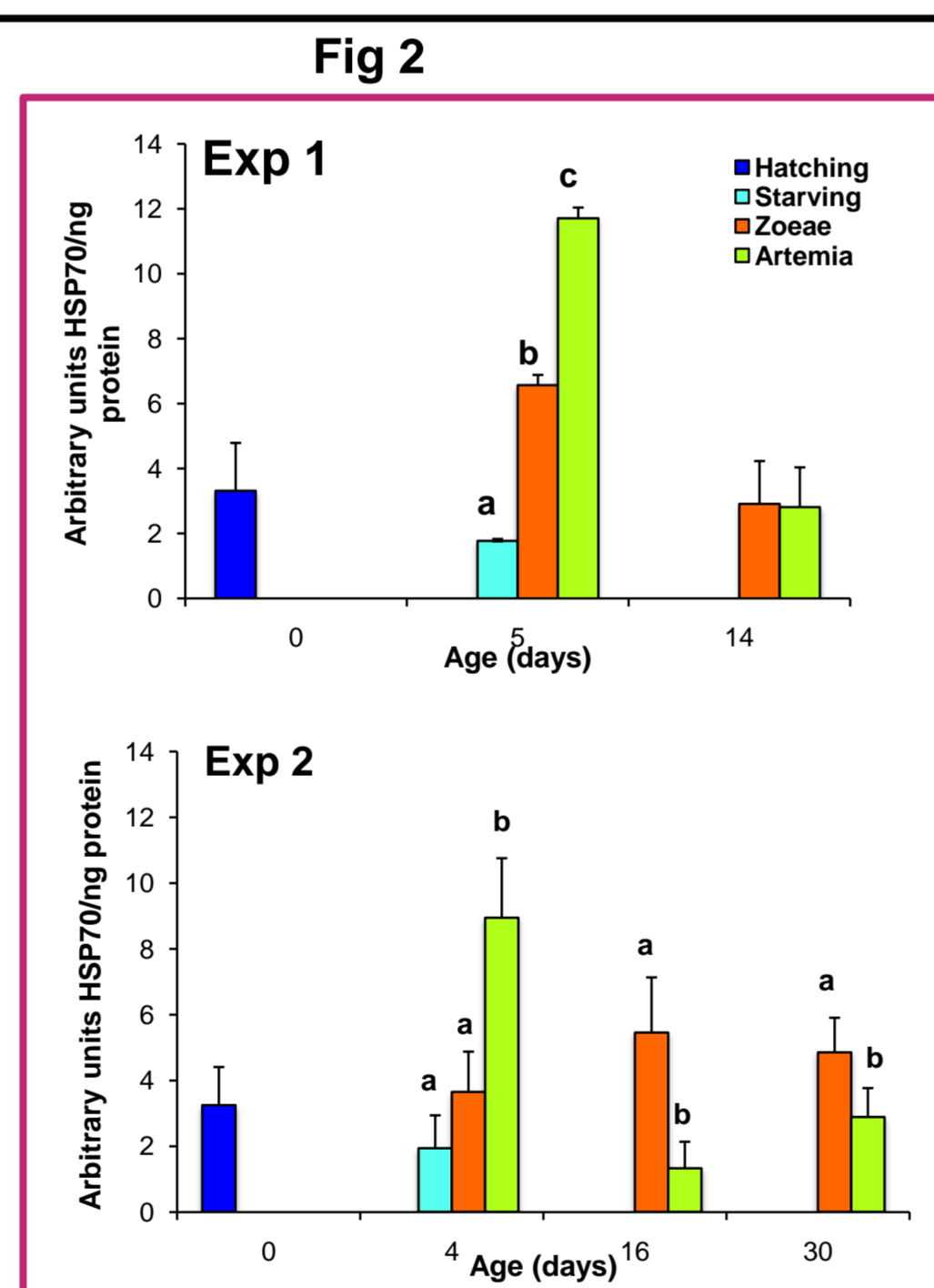
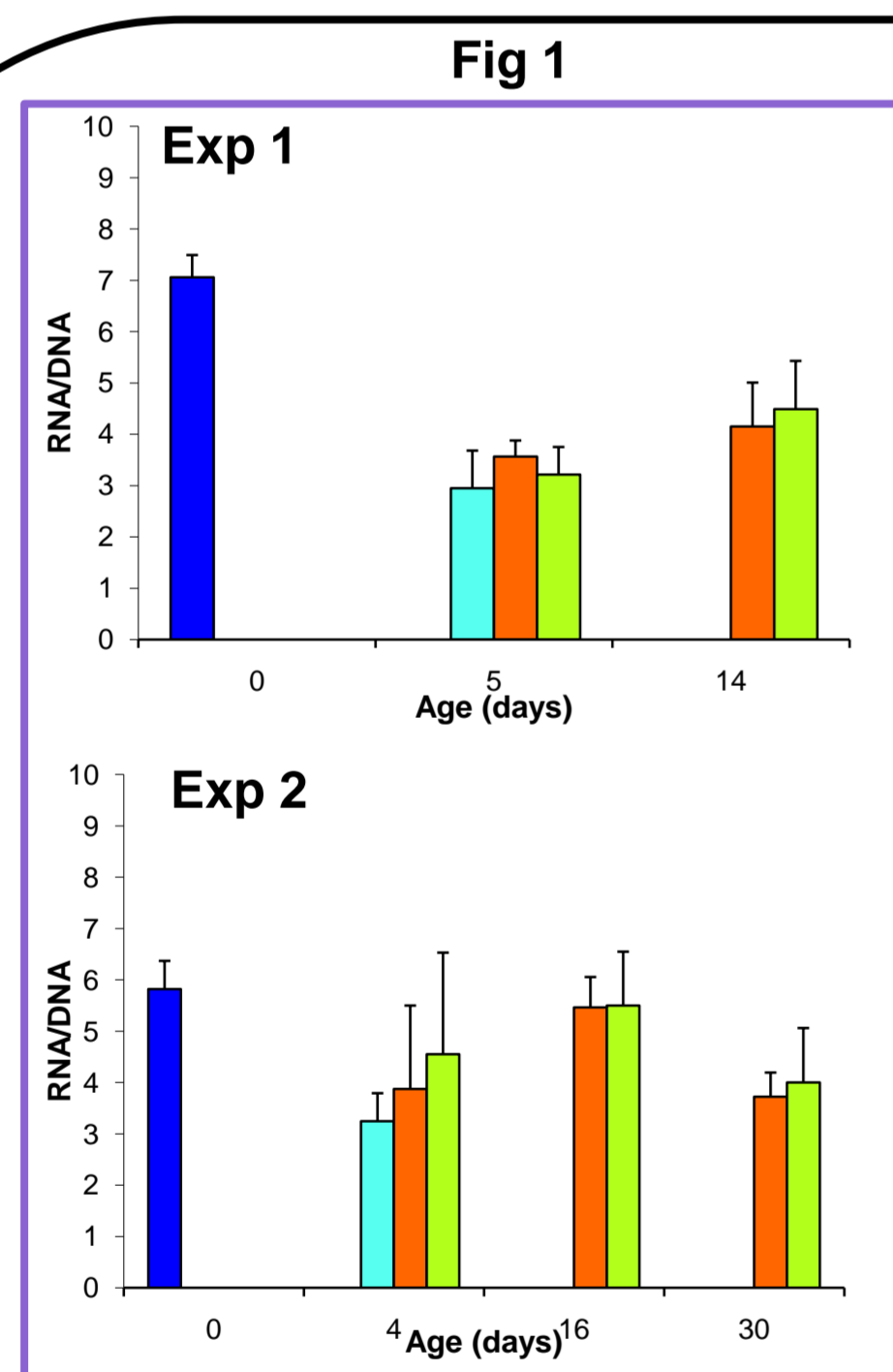
### Experiment 2:



- (1) RNA/DNA ratio was quantified following the procedure described in Varó et al. (2007).
- (2) HSP70 was determined by western blot using a Mini-Protean Tetra cell system and a Trans-Blot<sup>®</sup> Turbo Blotting system (Bio-Rad).
- (3) Antioxidant enzymes and levels of MDA were determined as described in Pérez-Jiménez et al. (2009).



## RESULTS and DISCUSSION



**Fig. 1 & 2.** RNA/DNA ratio and levels of HSP70 of *Octopus vulgaris* paralarval cultures, either starved, or fed *Nannochloropsis* sp - and *Isochrysis galbana* - enriched *Artemia*, or crustacean zoeae, raised up to 30 days of age. Different letters denote significant differences between diets for each age. Values are means  $\pm$  std

- ✓ After hatching, 4 to 5 days of starvation show up in a decrease in the values of RNA/DNA ratio and HSP70 as a consequence of nutritional stress.
- ✓ From days 4 to 30 the RNA/DNA remained unchanged irrespective of diet.
- ✓ The levels of HSP70 were higher in the dietary groups, and especially higher in the paralarvae fed *Artemia* up to 5 days.
- ✓ At the end of experiment 1 (14 days) lower values of HSP70 were found, probably as a consequence of mortality.
- ✓ Paralarvae fed zoeae in the second experiment, showed higher HSP70 values, possibly reflecting a better nutritional status that correlated with higher growth (data not shown), and pointing at this biomarker as a sensitive indicator.

**Fig. 3.** Specific activities of antioxidant enzymes (U mg protein<sup>-1</sup>) of *Octopus vulgaris* paralarval after hatching

	Day 16		Day 30	
	Zoeae	Artemia	Zoeae	Artemia
SOD	8.35 $\pm$ 3.22	7.50 $\pm$ 0.76	7.54 $\pm$ 0.49	11.41 $\pm$ 0.79
CAT	4.35 $\pm$ 0.21	5.74 $\pm$ 0.26*	6.73 $\pm$ 0.45*	6.49 $\pm$ 0.20*
Se-GPX	3.59 $\pm$ 0.35	n.d.	5.26 $\pm$ 1.19 <sup>ab</sup>	3.41 $\pm$ 0.23 <sup>a</sup>
GPX	1.27 $\pm$ 0.17 <sup>a</sup>	2.97 $\pm$ 0.30 <sup>b</sup>	1.55 $\pm$ 1.36	1.06 $\pm$ 0.27
t-GPX	4.86 $\pm$ 0.18 <sup>b</sup>	2.97 $\pm$ 0.30 <sup>b</sup>	6.80 $\pm$ 0.17 <sup>b</sup>	4.04 $\pm$ 0.43 <sup>a</sup>
GR	2.08 $\pm$ 0.07	2.61 $\pm$ 0.02	2.30 $\pm$ 0.19	1.92 $\pm$ 0.05*
MDA	4.09 $\pm$ 0.45	4.48 $\pm$ 0.23	3.82 $\pm$ 0.71	4.67 $\pm$ 0.45

**Table I.** Specific activities of antioxidant enzymes (U mg protein<sup>-1</sup>) and levels of MDA (nmol MDA g tissue<sup>-1</sup>) of *Octopus vulgaris* paralarval cultures fed *Nannochloropsis* sp - and *Isochrysis galbana* - enriched *Artemia* and crustacean zoeae raised up to 30 days of age. Different letters denote significant differences between diets for each age. Asterisk indicates differences with hatching. Values are means  $\pm$  std

- ✓ After hatching paralarvae possess a complete enzymatic pool of enzymes studied
- ✓ The enzymatic activities are high (especially Mn-SOD) when are compared with other cephalopods
- ✓ There is an increase in CAT and Se-GPX activity during development irrespective of diet
- ✓ There are not clear changes in enzymatic activities associated to diet, except Se-GPX activities that were higher in the paralarvae fed zoeae, in agreement with a better growth and survival (data not shown).
- ✓ The SOD isozymic profiles seem to reflect those of live preys, as well as Se-GPX activities.

## CONCLUDING REMARKS

The results point at the RNA/DNA ratio as an indicator of starvation, and at the levels of HSP70 and Se-GPX activity as more sensitive biomarkers of the nutritional status of paralarvae. There is not evidence of a prooxidative status promoted by diets, but a detailed analysis of oxidative damage to proteins could help to associate oxidative damage to mortalities and growth.

## REFERENCES

Pérez-Jiménez, A., Hidalgo, C., Morales, A.E., Arizcun, M., Abellán, E. and G. Cardenete. 2009. Comparative Biochemistry and Physiology, Part C 150: 537–545.  
Varó, I., J.C. Navarro, B. Nunes, and L. Guilhermino. 2007. Aquaculture 266: 87–96.

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