Robsonella fontaniana LARVICULTURE: ONTOGENIC CHANGES OF THE MORPHOLOGY AND DIGESTIVE ENZYMES

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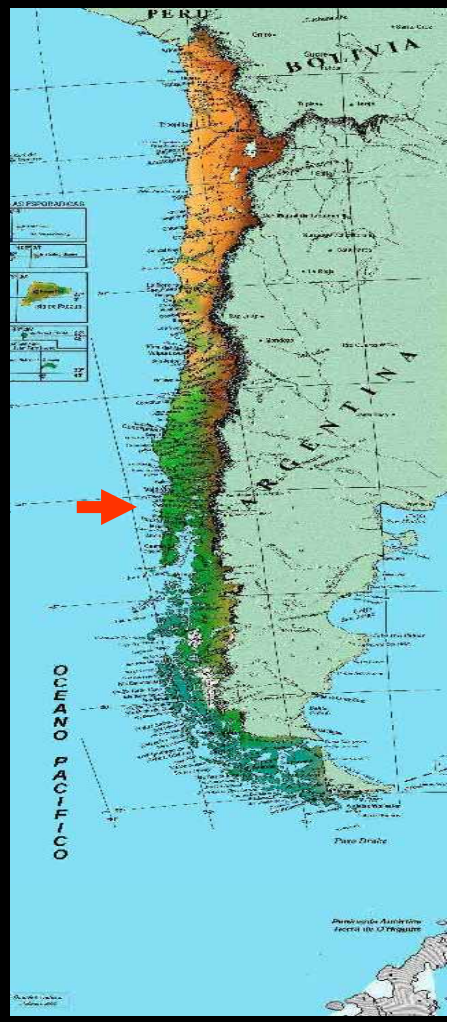
Objectives

The main objective was to study the development of embryos, paralarvae (planktonic stage/phase) and newly-settled juveniles (benthonic stage/phase) of *Robsonella fontaniana* by analyzing both rearing/farming conditions and morphometric and biochemical changes occurring during the different phases.

Material and Methods

Robsonella fontaniana (Orbigny, 1834) is a small-sized octopus found off the Chilean coast and the south of Argentina.



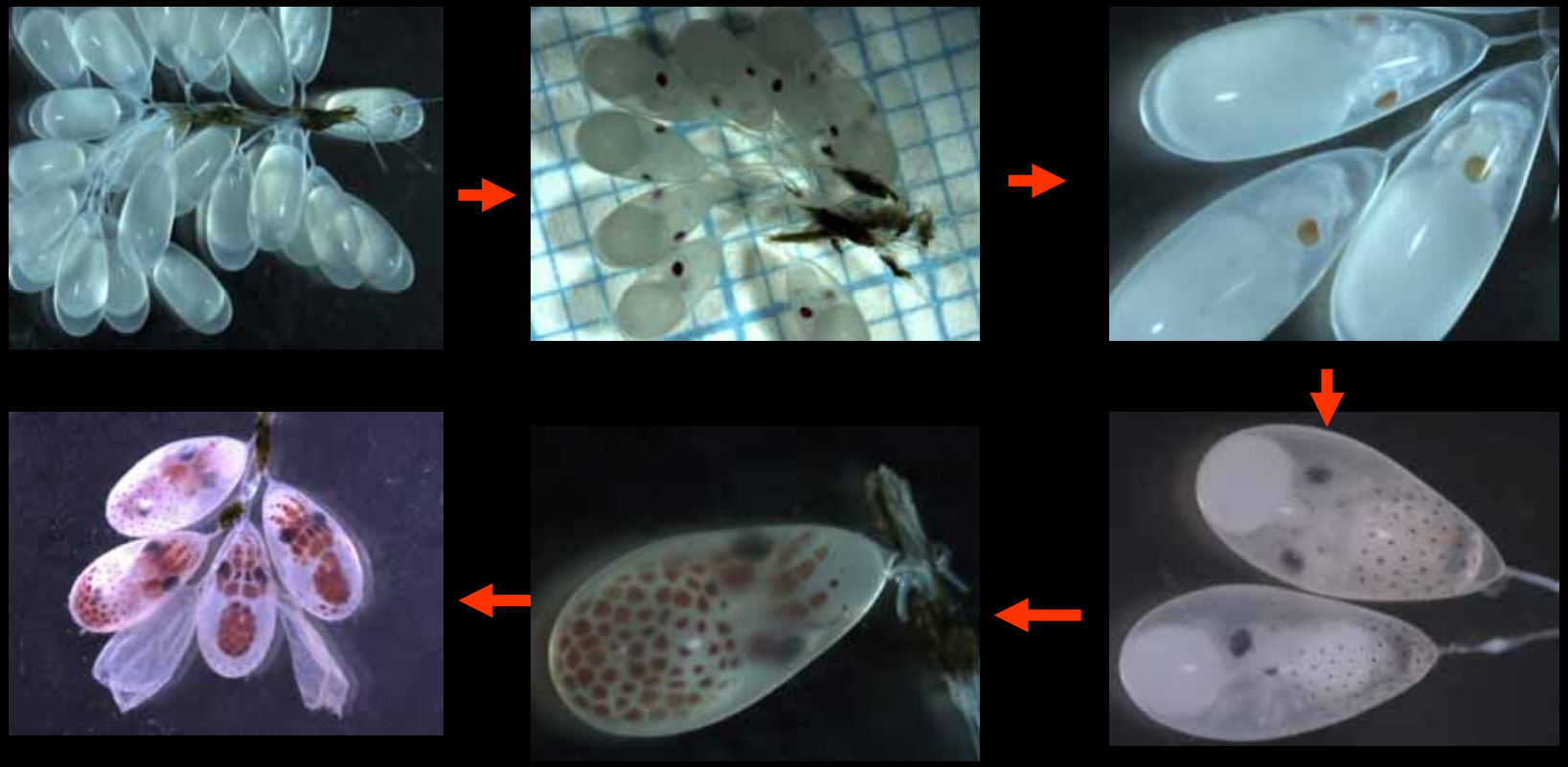
R. fontaniana females with eggs were collected at Hueihe, Chilean Patagonia, (41°52’ S; 73°51’ W), X Region, Chile. Octopuses and their spawn (attached to stones) were transported in spring to the Marine Invertebrate Hatchery Laboratory of the Universidad Austral de Chile (HIM-UACH) in 70-L tanks of aerated sea water.

Female ***R, fontaniana***

Eggs attached on stones *R. fontaniana*

Once in the laboratory, the animals and stones with eggs were placed in aerated sea water tanks kept at 11° C and 30 ppm salinity and connected to a sea water re-circulation system. Females were put in a semi-dark environment with 12:12 h light:dark photoperiod and were fed daily with white fish (*Odontestes sp*.) to keep them from eating their spawn.

Uriarte et al., 2009 Vie Milieu. *in revision*

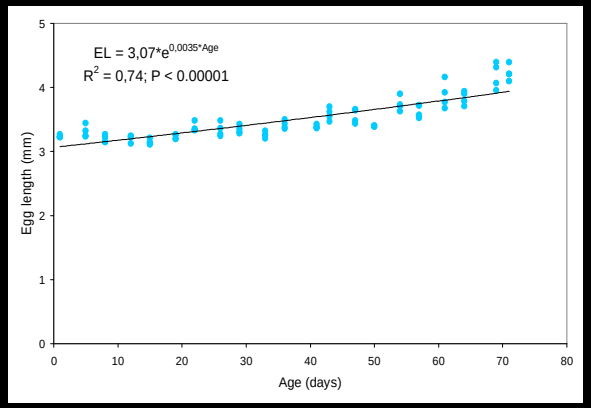


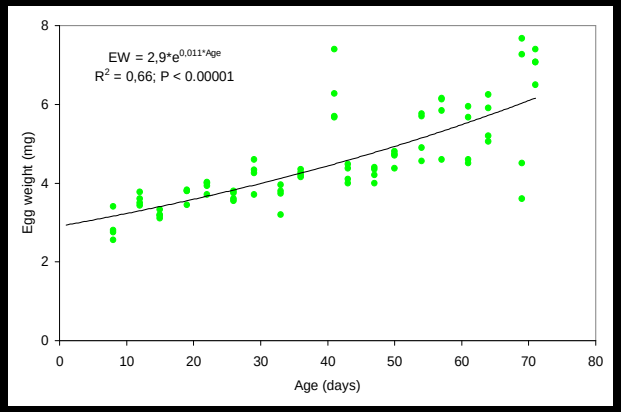
Samples of 30 eggs were taken from each of the seven clutches collected through the year to evaluate the growth rates of the eggs between the 30-day periods and related the change in perivitelline fluid protein concentrations to the egg size and age. The embryonic development took 70 days. Egg size was 3.45 mm.

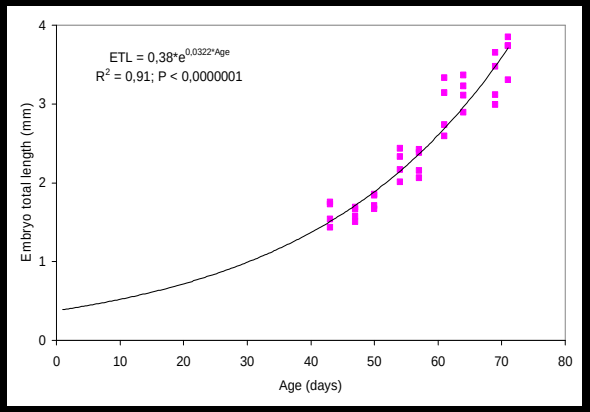
In this study, the *R. fontaniana* eggs fluctuated between 3.13 and 3.77 mm in length in the clutch used for the descriptions and between 2.4 and 4.7 mm (3.5 and 6.8% of adult ML, respectively) in the seven clutches used for gravimetric characterization. The entire embryonic development took 71 to 76 days, until the paralarvae hatched, the hatching period lasted an average of 4 to 5 days at 12 ºC.

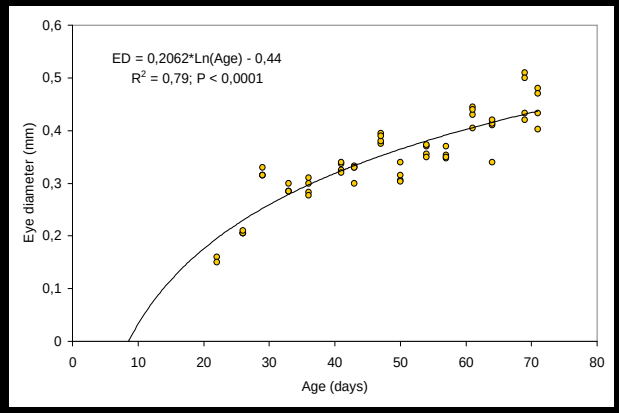
During development, the embryo rotated twice into the eggs. The cephalic organs were visible after day 22 and the eyes were well defined after day 26. The mantle was nearly 95% of the total embryonic length on day 54, whereas the arms varied between 15 and 24% of the total length, peaking just before hatching.

The length of the eggs and its weight increase exponencially throughout the embryonic development.





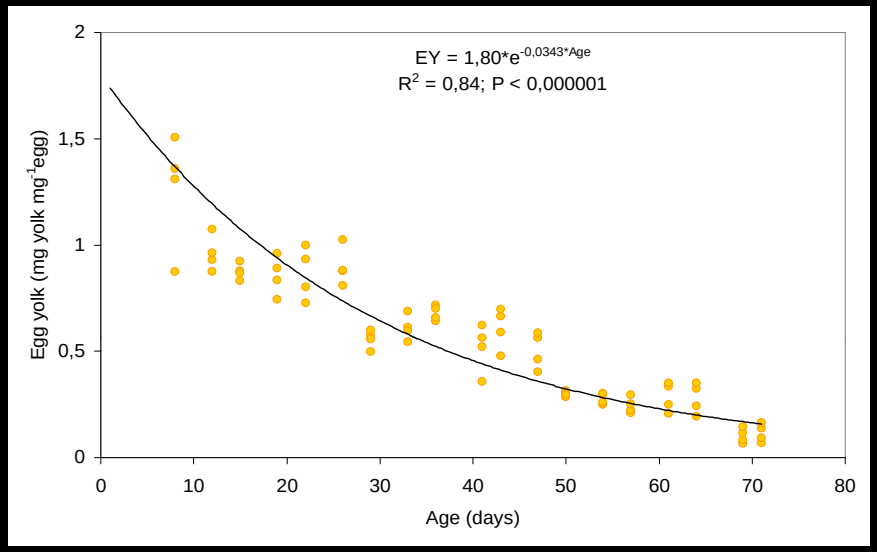


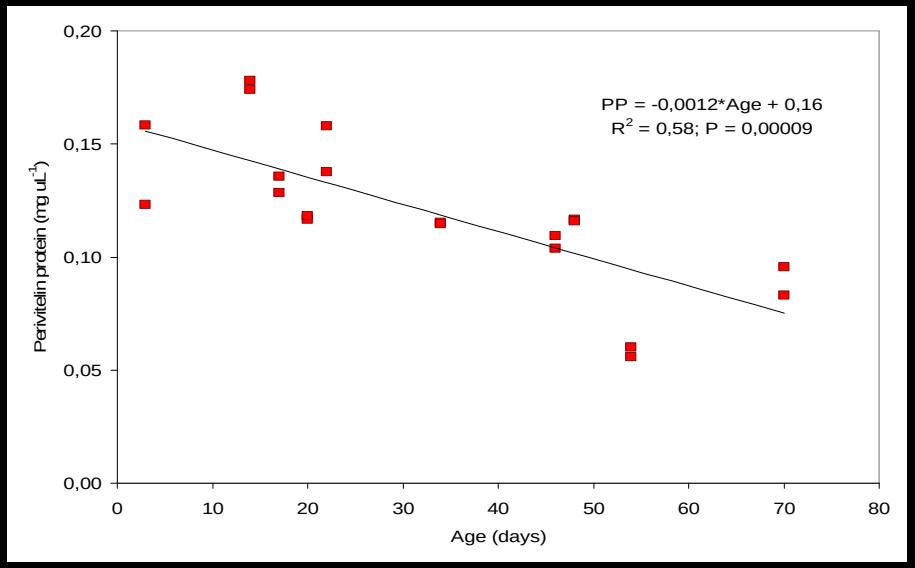


Uriarte et al., 2009 Vie Milieu. *in revision*

The embryo also grows exponentially within the egg, which is related to an exponential growth of the mantle and the arms. The eye growth is quite different, which follows a logarithmic equation.

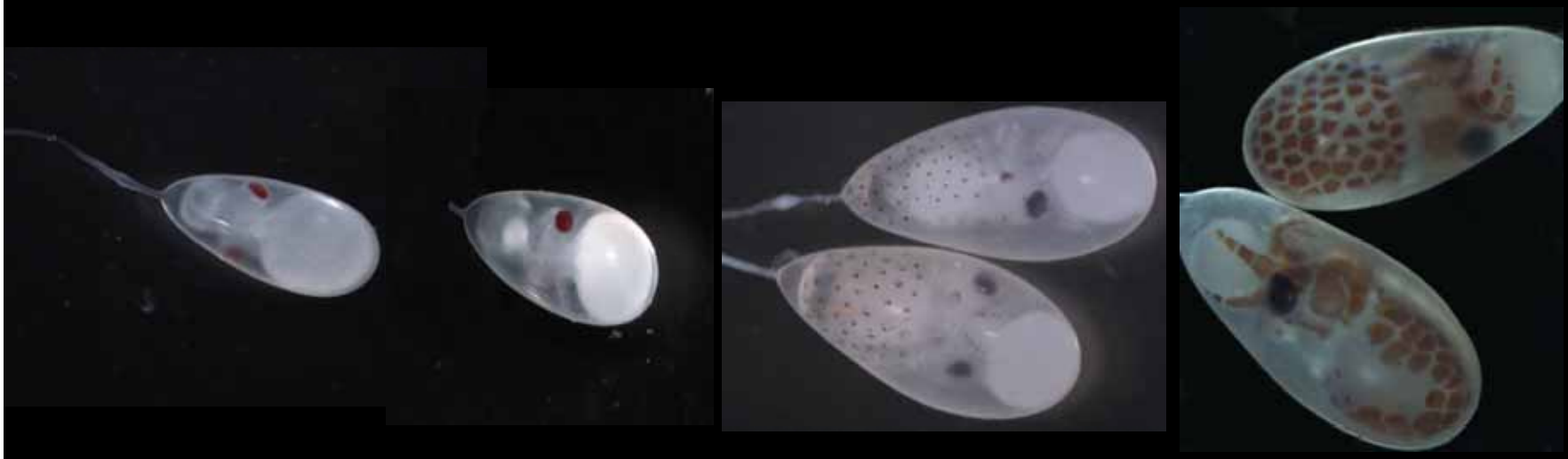
The embryo use not only the yolk, neither also the perivitelline fluid.





Uriarte et al., 2009 Vie Milieu. *in revision*

During the embryonic development the yolk is used as a source of energy (triglycerids) and to produce new tissues (proteins + phospholipids). By day 25, part of the yolk has been introduced into the embryo and by day 60 the whole external yolk has disappeared and the embryo is close to hatch.



Uriarte et al., 2009 Vie Milieu. *in revision*

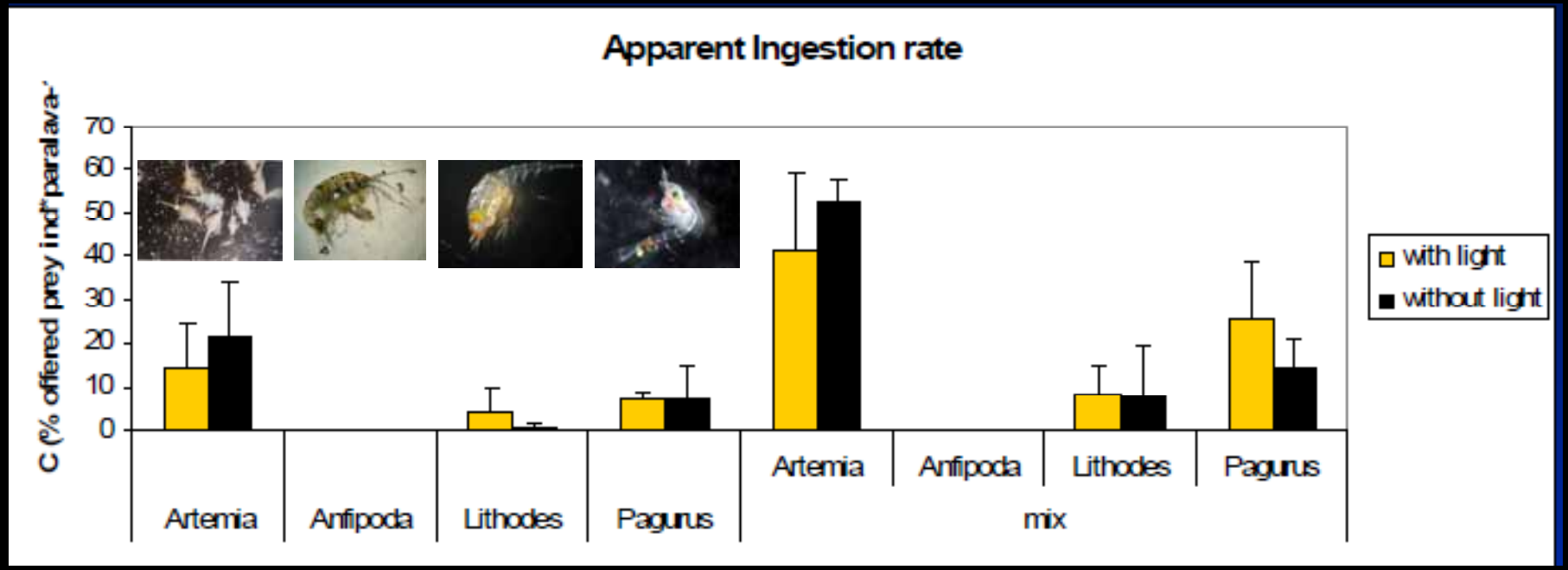
The embryo rotates twice during the development.
The first two are after the first rotation.
The last embryo is after the second rotation.

Not only the yolk disappears during this stage but also the protein available in the perivitelline fluid decreases, so this is another source of material for the embryo tissues.

Once the paralarvae hatched, they were transferred to culture systems in acrylic tanks and glass vials.

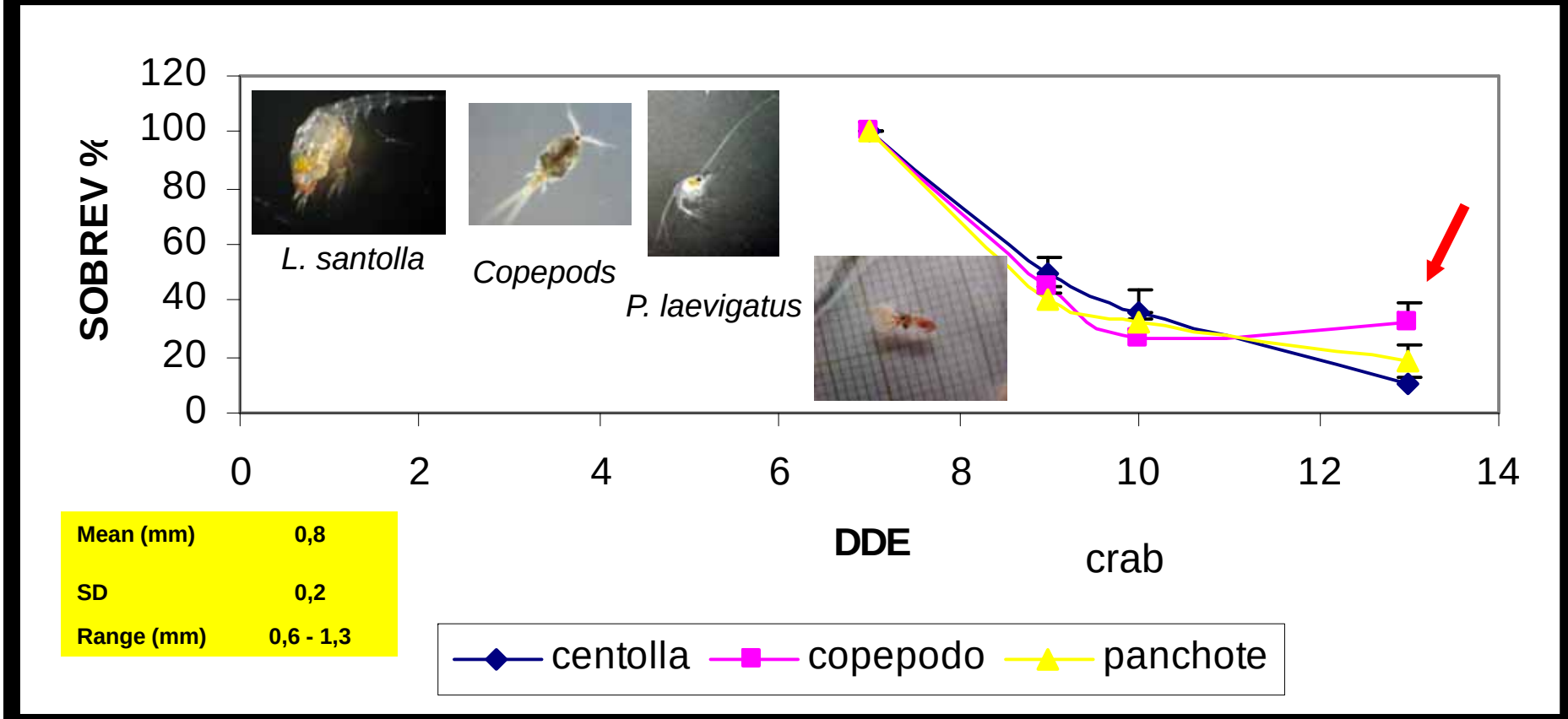
12 experiments were conducted that tested different conditions of diet, temperature, density, management and light intensity.

Ingestion rate (%) (number of pray/paralarva with and without light)



We found that *Artemia*, *Pagurus* and *Lithodes* zoeae were the most consumed with and without light conditions.
Artemia was easy to rear and *Lithodes* was a better diet for *R. fontaniana* than *Pagurus.*

Survival with different diets: zoeae frozen and live adult copepods.

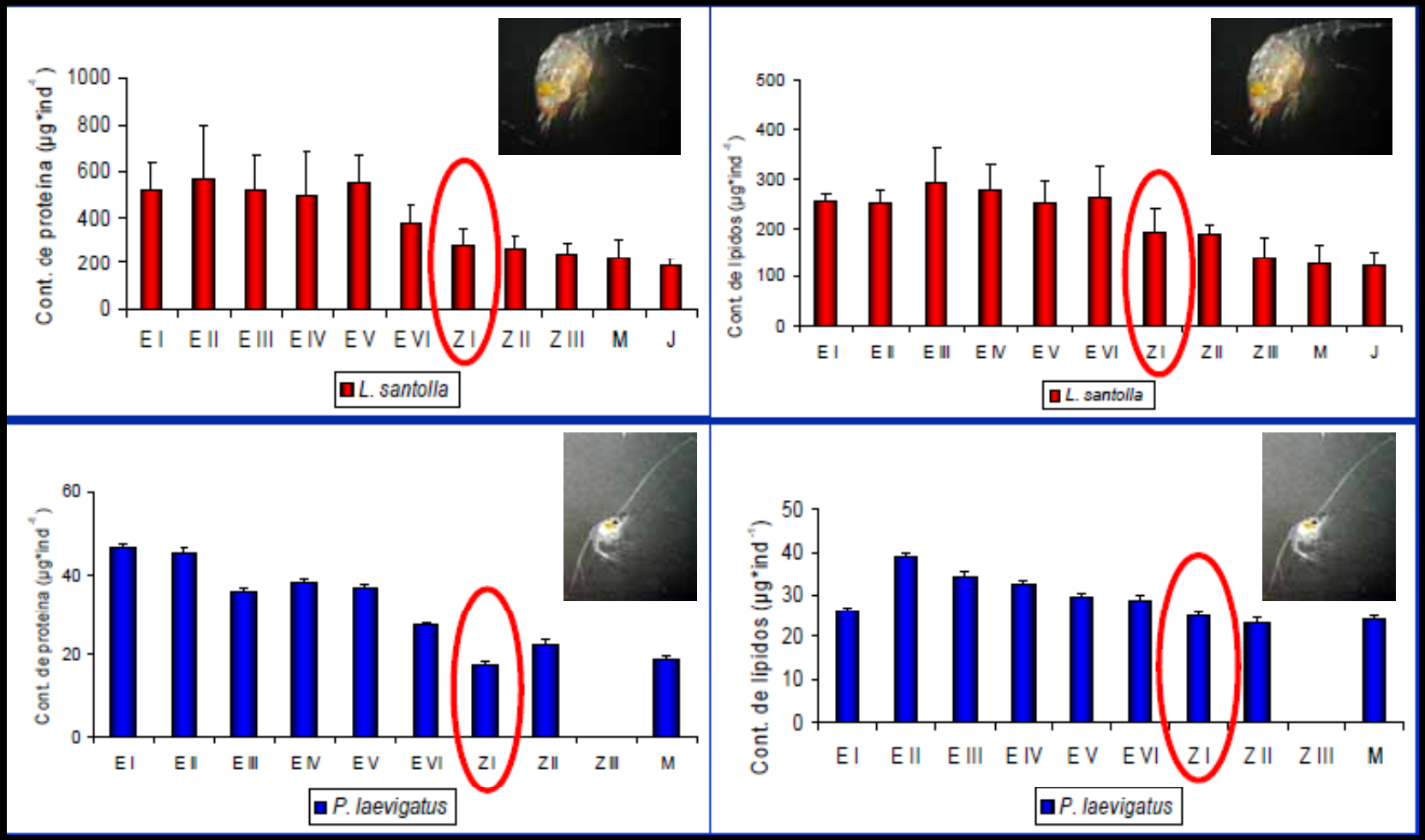


In comparison with *Lithodes* and *Petrolisthes* frozen zoeae, live copepods showed better results: 32% survival after 13 days of rearing.

In other words, feeding paralarvae with frozen preys is useless.

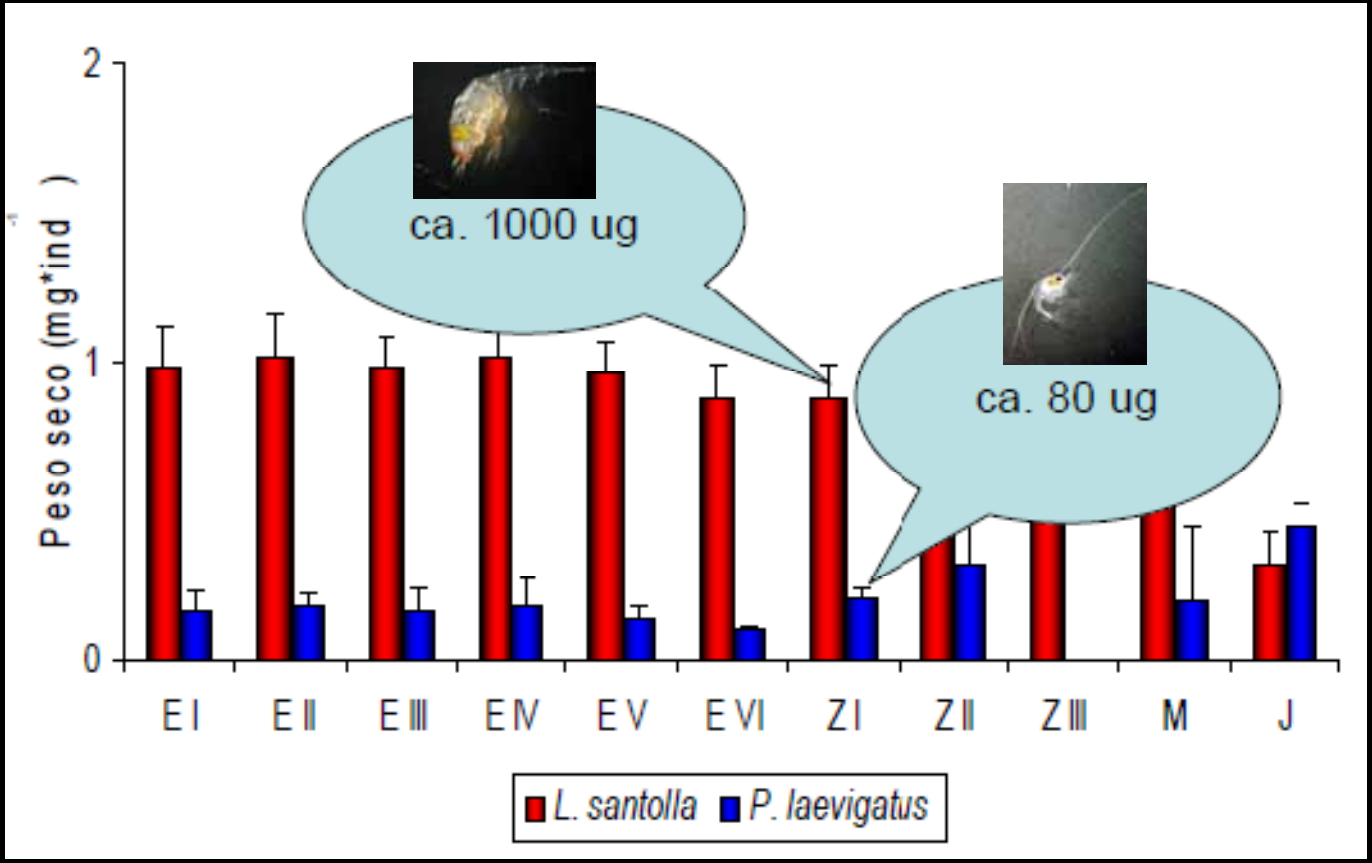
Protein

Lípids



Petrolisthes zoeae were well consumed by the paralarvae. Between both types of zoeae, *Lithodes* zoeae offer higher protein and lipid contents.

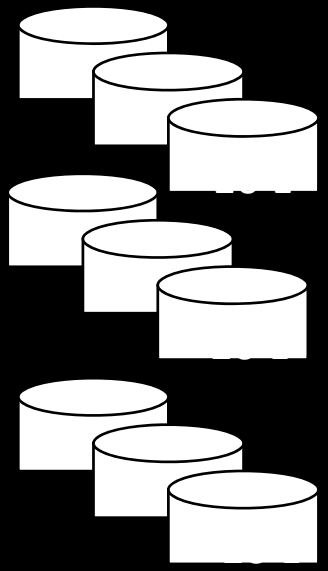
Dry Weight



Lithodes zoeae had almost 10 times more dry matter per zoea than *Petrolisthes.* This may indicate the reason why *Lithodes* zoeae were more beneficial for paralarval growth and survival. King crab (*L. santolla*) zoeas are lecitotrophic, meaning that they do not need food to complete their larval development (Anger et al., 2004).

This characteristic gives *L. santolla* zoeas a particular nutritional condition.

Enzymatic activities



Culture conditions:

Temperature: 12-13ºC

Density: 5 larvas/L

Ration: *ad libitum*

Flow-through system UV-FSW (5 µm)

ST (starvation)

Negative control

FA (fed *Artemia* sp.)

FL (fed *L. santolla*)

Sampling time :

DAH: 0, 3, 6, 13, 21, 27

Samples (n=5) taken for:

- Enzyme activity
- Protein content
- Mortality

1. Digestive enzyme screening*:*

semi-quantitative micromethod api© ZYM (bioMerieux, France)

2. Determination of digestive enzyme activity:

a) Total proteolytic activity

b) Total acid phosphatase activity

c) Trypsin and chymotrypsin activities

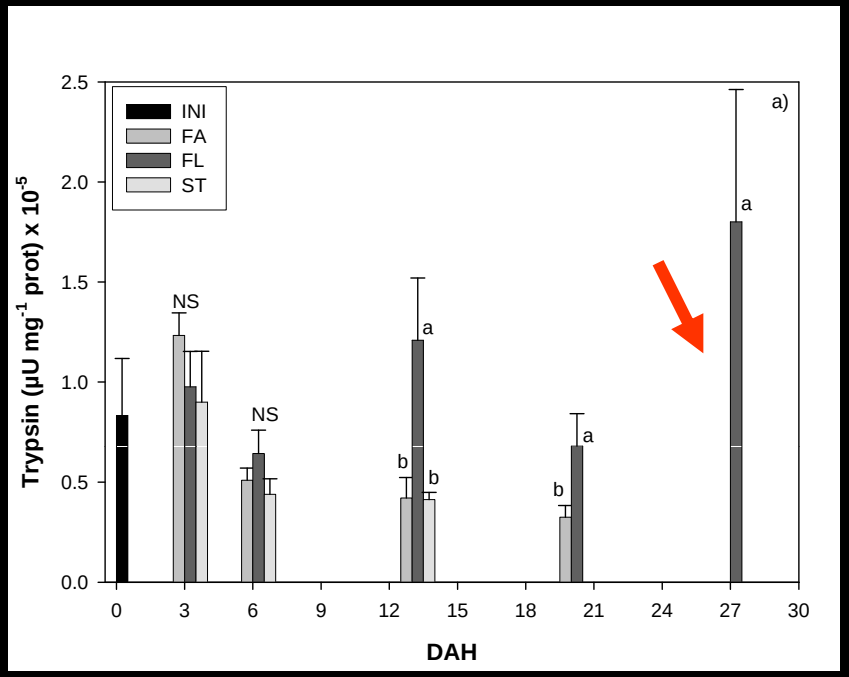
Pereda et al., 2009 Mar. Biol. *in press*

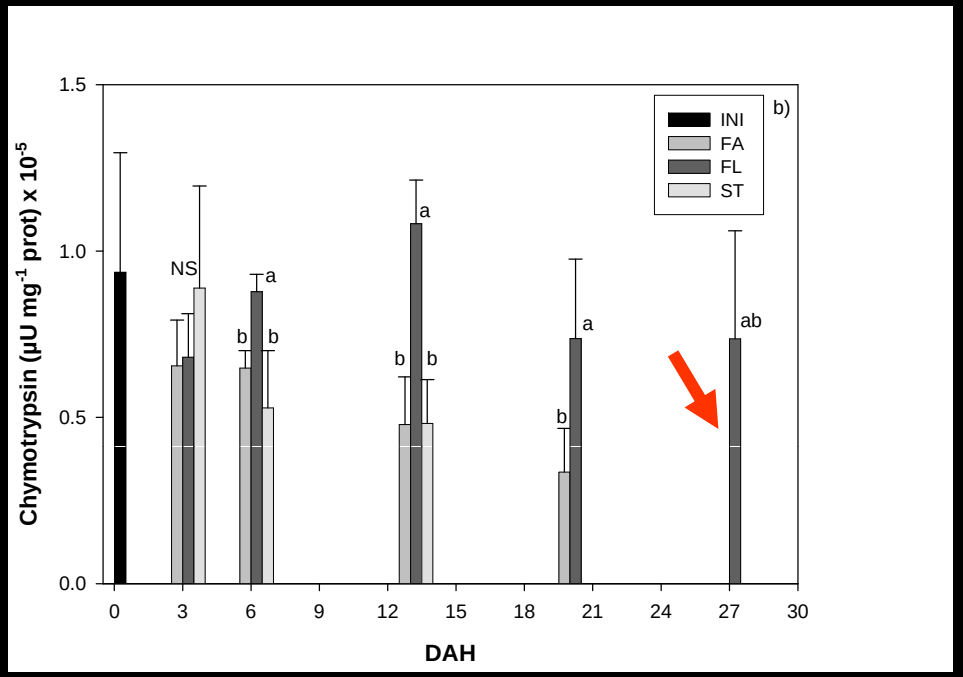
3. Protein content

This experiment consisted in feeding paralarvae with *Artemia* (FA) and *Lithodes* (FL) zoeae (ST), following the development of digestive enzymes during the first month after hatching in the culture conditions best for paralarvae: 12º C, 5 paralarvae/l, *ad libitum*, sampling at days 0, 3, 6, 13, 21 and 27 after hatching.

The activity of trypsin and chymotrypsin 10 days after hatching, depended on diet ($p<0.05$).

Pereda et al., 2009 Mar. Biol. *in press*

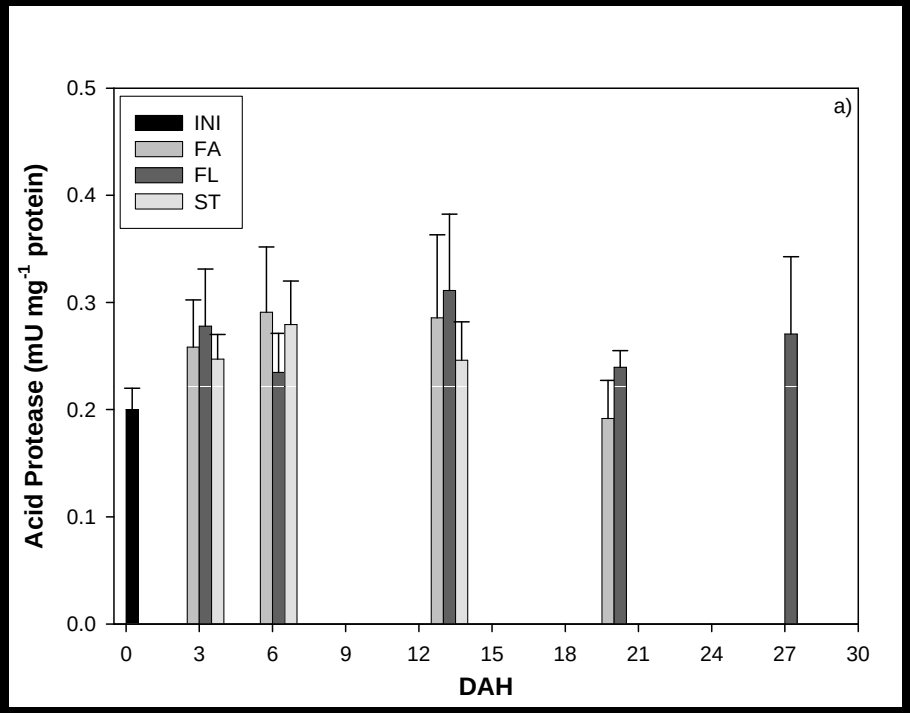


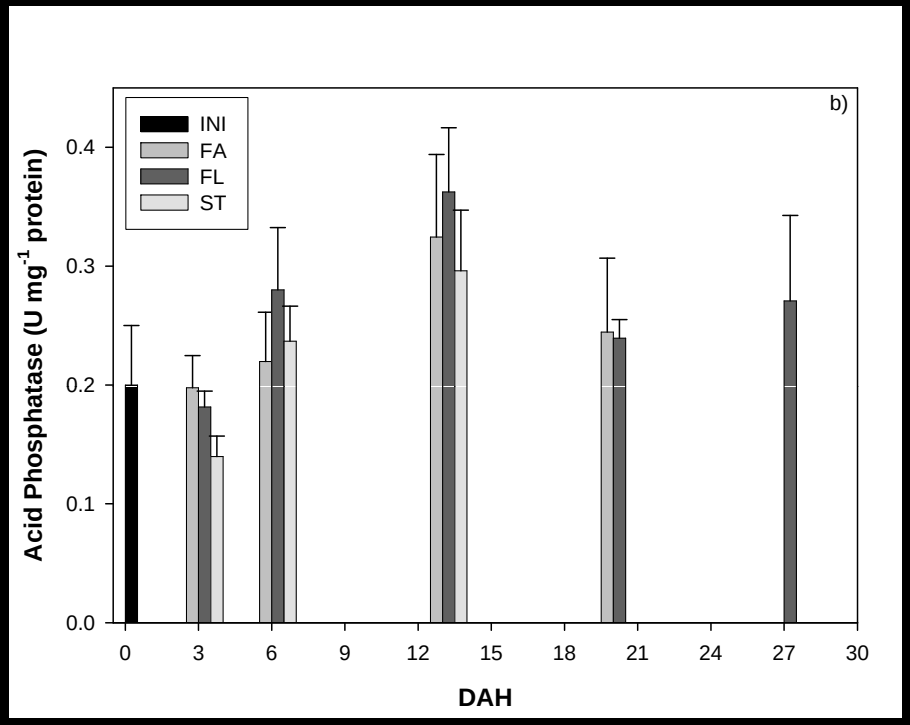


Trypsin and Chimotrypsin activities:

- These specific digestive enzymes are strongly affected by diet and paralarval age.
- *R. fontaniana* paralarvae fed on *Lithodes* zoea perform better (low mortality rates) and have better digestive capacities (higher enzyme activities).

Total protease and acid phosphatase activity was not related to either time of development or diet ($p<0.05$).





Pereda et al., 2009 Mar. Biol. *in press*

Acid Protease and Acid Phosphatase activities:

- These enzymes are not affected either by the diets used in our study or by paralarval age.
- These enzymes work independently of ontogeny and nutritional status and we believe they are related mainly to the degradation and use of paralarval yolk.

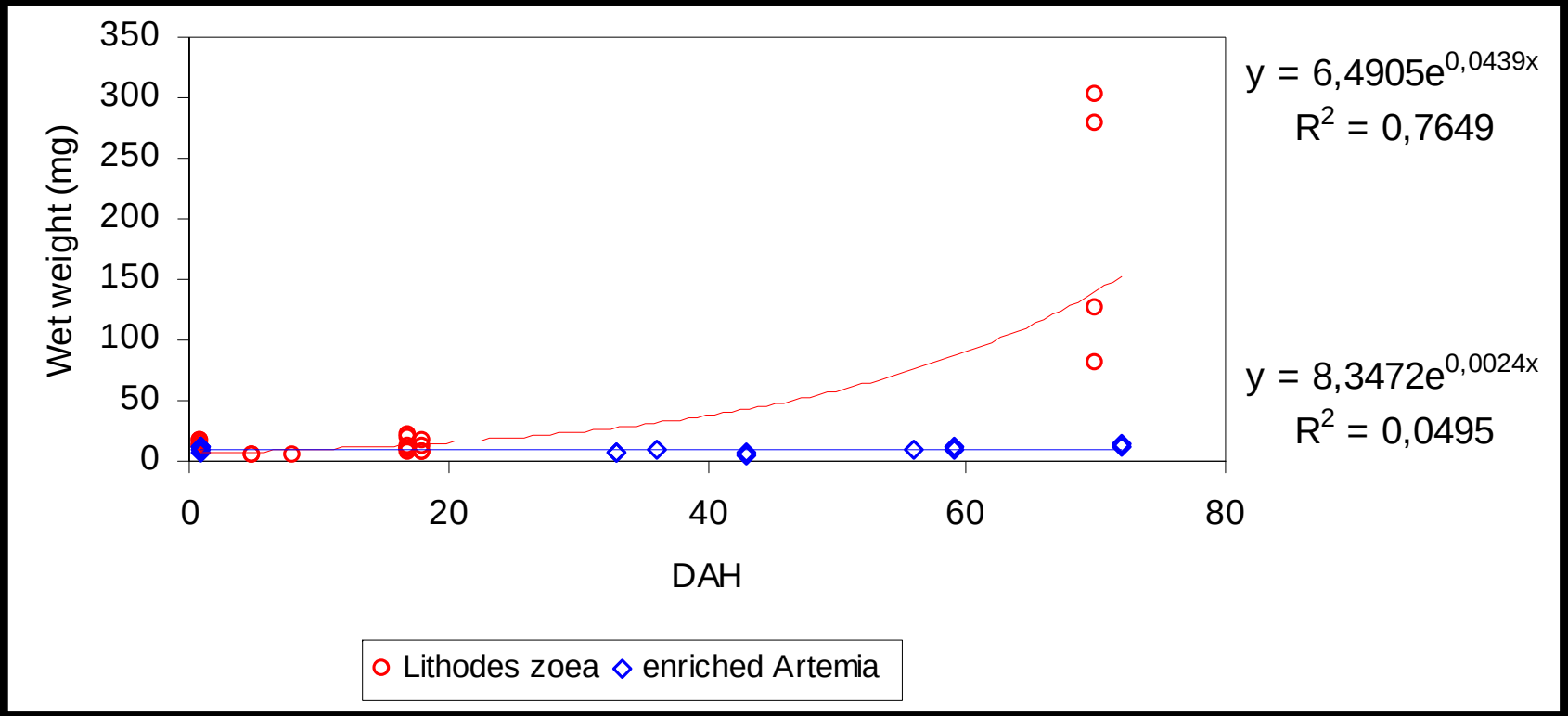
Paralarvae 25 days fed on *Lithodes* and on *Artemia*

Mean (mm)	4,1
SD	0,1
Range (mm)	4,0 - 4,2

Mean (mm)	4,5
SD	0,8
Range (mm)	3,1 - 5,5

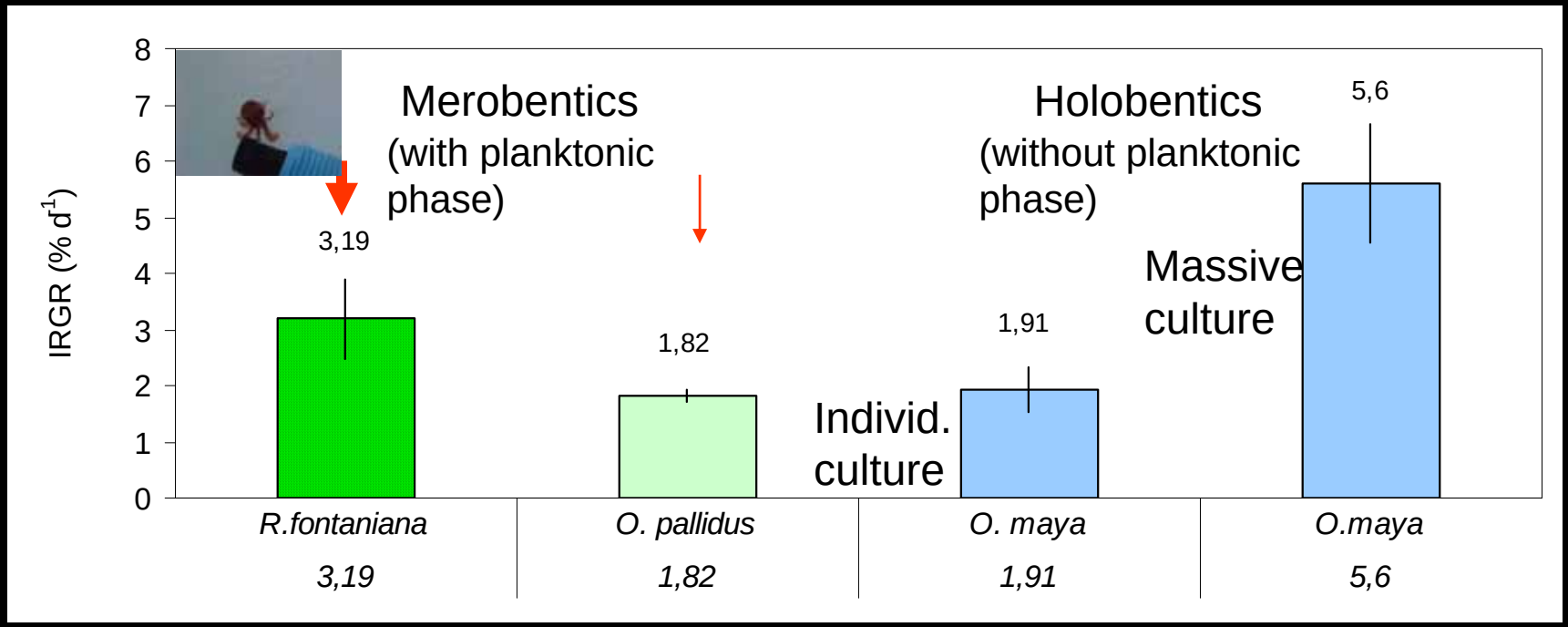
We proved the method by José Iglesias, according to which 2-3 mm *Artemia* enriched with Nannochloropsis are used to obtain the complete paralarval development.

Paralarvae fed on *Lithodes* and on *Artemia*

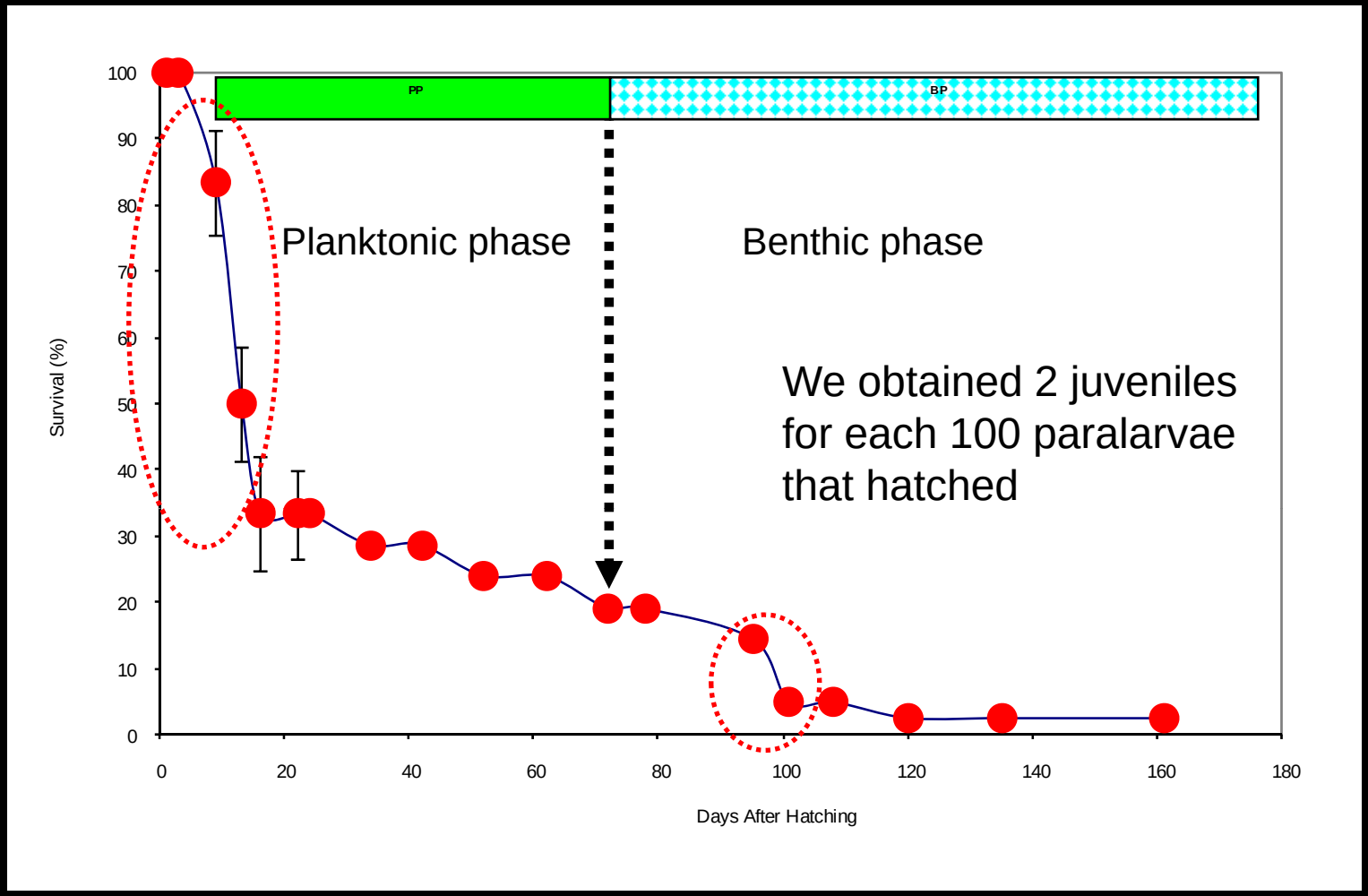


These were two different experiments (*Lithodes* and enriched *Artemia*), in both we obtained final paralarvae with settlement behavior at 70 and 72 days after hatching.

However, the increase in weight of paralarvae had very different results. Paralarvae fed with *Lithodes* zoeae increased in size and weight and reached a SGR of 4.4%/day. While of the paralarvae fed with Nannochloropsis-enriched on *Artemia* did not grow and had a SGR of 0.



The specific growth rate (SGR) was of 3.19%/day, in *R. fontaniana*, which is similar to that obtained for *O. pallidus* and *O. maya* juveniles, as in our case. In a pilot culture with 1000L, Rosas et al. obtained higher rates: 5.6%/day.



Two critical moments were observed: one during the Planktonic phase (9-16) and another during the Benthic phase (90-100).

Only 10 juveniles were obtained. And only 1 survived. This represents a 3% success.

Embryonic phase

70 days

4 days old

21 days old

72 days old

Plancktonic phase

72 days

Benthic phase

1,8 g

2,5 cm

120 days

CONCLUSIONS

Paralarvae fed with *Lithodes* zoea performed better than paralarvae fed on *Artemia*, exhibiting significantly ($p<0.05$):

1.- Higher protein content and the highest proteolytic activity for trypsin and chymotrypsin.

2.- Higher growth rate.

The results indicate that *Lithodes* zoea are a better diet for *R. fontaniana* paralarvae than *Artemia sp* and also when *Nannochloropsis*-enriched *Artemia* are used. However we found two periods with the highest mortalities. And we would like use this model for applying to other species.

Thank you for your attention!!

persecuting/hunting its prey

attempt to capture a prey

entering its hiding place