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Chapter 3

The CORALZOO project – preliminary results of the evaluation of different types and concentrations of zooplankton food on the growth of *Pocillopora damicornis* (Linnaeus, 1758) comparing diurnal and nocturnal feeding

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ABSTRACT

The Coralzoo project undertakes, on behalf of the European Association for Zoos and Aquaria (EAZA), scientific and technological research activities, aiming to develop protocols and methodologies to better culture and maintain corals *ex-situ,* minimizing negative impact on the environment. Within Coralzoo, Acquario di Genova is involved especially in nutrition study, testing different types and concentrations of food and establishing whether diurnal or nocturnal feedings could influence the growth and welfare of *Pocillopora damicornis.*

The experimental tank system consists of 24 small aquariums of approximately 40 L each. These aquariums run in the same LSS (Life Support System) and can be individually isolated by valves during feeding time. To test the difference between diurnal and nocturnal feeding 12 aquariums are fed simultaneously at a nocturnal lighting regime, and 12 during a diurnal regime, applying the same protocol.

In each of the two experiments on zooplankton food a total of 1728 *P. damicornis* nubbins are used as samples, 216 for each concentration.

Different parameters are considered to evaluate the growth and welfare of *P. damicornis*: survival, health condition, three-dimensional growth, weight, two-dimensional surface and polyp number.

The results of the experiments on zooplankton food are shown in this paper. In the first experiment the growth of *P. damicornis* at three different concentrations of *Artemia* was tested, and in the second experiment it was tested at three different concentrations of rotifers.

The data of the two experiments leads to the following preliminary husbandry suggestions. First of all, two hours and half is quite enough for the corals to eat most of the tested food available. Light regime feeding doesn't seem relevant. Feeding *P. damicornis* with *Artemia* and high concentration of rotifers results in a faster growth. Feeding *P. damicornis* with *Artemia* is less expensive than feeding with rotifers, while the same result is obtained.

Data elaboration of the other programmed experiments of Coralzoo project hopefully will give a more complete final description of the optimum diet for *P. damicornis* bred *ex-situ.*

INTRODUCTION

Although it has been known for decades that hermatypic corals feed on plankton (Sorokin, 1991; Ferrier-Pagès *et al*., 1998; Houlbrèque *et al*., 2004; Phillips, 2004) the importance of

this process for the growth of the corals has not been fully described and experimentally tested (Clayton *et al.*, 1982).
The aims of this study is to provide some scientifically tested information and further husbandry suggestions on techniques generally used in aquaria to breed and maintain corals *ex-situ*.
The planned studies in Coralzoo project are structured for four years from 2004 through 2009. The whole project program consists of five different experiments: two using zooplankton, *Artemia salina* and *Brachionus plicatilis* (rotifers) (presented in this paper), two using phytoplankton, *Nannochloropsis* sp. and *Tetraselmis suecica*, and one final comprehensive experiment with the simultaneous comparison of the better previous results.
In this first section of the Coralzoo project (2004-2006), presented in this paper, Acquario di Genova has completed two experiments to test the effectiveness of different types of zooplankton food at different concentrations, the timing of food administration and also to establish whether diurnal or nocturnal feeding could influence the growth and welfare of the *P. damicornis*.

MATERIALS AND METHODS

Experimental facility

The experimental facility consists of two fiberglass tanks of 2,500 L each and contains eight PVC tanks of 130 L each, specifically built for this purpose. The latter are further subdivided into three separate identical compartments (a total of 24 small aquariums of approximately 40 L each); these aquariums run in the same main LSS and can be isolated by valves during feeding time. Smaller volume tanks immersed in a single large system allow to be conservative on the number of individual food items to be fed avoiding waste but maintaining the temperature and the water quality of a larger volume.

Samples

In all the experiments samples are *Pocillopora damicornis* nubbins, small coral fragments of 5 -15 polyps as described by Shafir *et al.* (2001) (Figure 1a and 1b) fixed 9 by 9 with cyanacrylate glue on a PVC tile (10 cm x 10 cm) (Bongiorni *et al.*, 2003a). The total number of the samples is 1,728 nubbins for each experiment pruned from around 100 colonies: 72 nubbins for each aquarium, 216 nubbins for each food concentration tested.

Feeding techniques

All the aquariums were fed daily from Monday through Friday. During the feeding time of five hours each day the aquariums were isolated by valves from the main LSS and the water circulation were supported by an airstone to avoid plankton sedimentation.
In the first experiment the growth of corals was tested administering three different concentrations of *Artemia*: 8,000 ind.L^{-1}, 4,000 ind.L^{-1}, 2,000 ind.L^{-1} and 0 ind.L^{-1} as control; in the second experiment the growth was tested administering the following concentrations of *B. plicatilis*: 16,000 ind.L^{-1}, 8,000 ind.L^{-1} and 4,000 ind.L^{-1} and 0 ind.L^{-1} as control.
To test the difference between diurnal and nocturnal feeding simultaneously 12 aquariums were fed at nocturnal regime lighting and 12 applying the same protocol during diurnal regime; they have been separated by a dark curtain so that the feeding will actually take place during working hours for both systems, avoiding night workload.

Preliminary tests

Preliminary tests were carried out to be sure that the environmental conditions of the water in each of the 24 aquariums would be the same and wouldn't change during feeding time: chemical and physical parameters (temperature, oxygen, pH, ammonium and nitrite) have been routinely measured in each aquarium before and after the isolation period for the feeding.
A clearance prey test (Ribes *et al.*, 1998; Hansson *et al.*, 2005; Hansson *et al.*, 2006) has been done in each experiment to check the distribution of the plankton in the aquariums during the whole feeding period and the nubbins efficiency to eat it at different concentrations and lighting regimes.

Biological measurements

Different parameters have been considered to evaluate growth and welfare of *P. damicornis*: survival, health conditions, three-dimensional growth and drip-dry wet weight. Area and polyps number have also been calculated using digital photographs (Bongiorni *et al.* 2003b), and the Image-J program (see Table 2). This software allows to analyze the pixel values associated with images and to convert

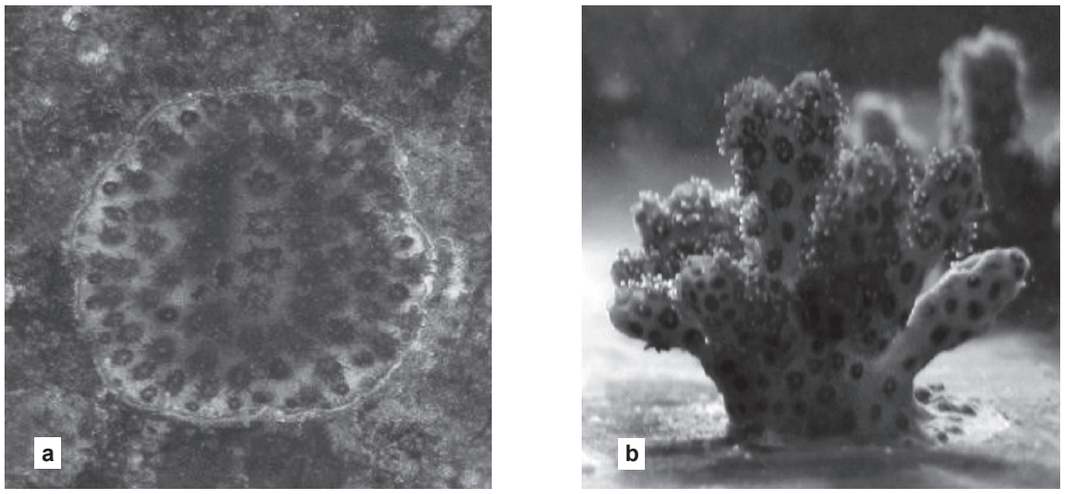

Figure 1: a: Pocillopora damicornis *nubbin with the new growth surface all round, b: Three-dimensional nubbin*

Table 1: Chemical and physical parameters monitored during the experiments

Parameter	Range t_0-t_1	Frequency
pH	8.1-8.4	5 x week
salinity (‰)	36.0-37.5	5 x week
ammonium ($mg.L^{-1}$)	0.01-0.02	3 x week
nitrite ($mg.L^{-1}$)	0.008	3 x week
nitrate ($mg.L^{-1}$)	1.0-6.5	3 x week
phosphate ($mg.L^{-1}$)	< 0.05	3 x week
KH	8	3 x week
calcium ($mg.L^{-1}$)	400-420	3 x week
alkalinity ($mEq.L^{-1}$)	1.9-2.3	2 x month
oxygen ($mg.L^{-1}$)	6.7-7.0	1 x month
light PAR ($\mu E.m^{-2}.s^{-1}$)	200	1 x month
temperature (°C)	25.6-26.0	daily

Table 2: Biological measurements considered to evaluate the growth and welfare of P. damicornis

Measurements	Means	Frequency	Period (days)
% survival	visual + photo	weekly	1-134
% suffering	visual + photo	weekly	1-134
% 3-D growth	visual + photo	weekly	1-134
area	photo + image J	weekly	1-56
nr. of polyps	photo + image J	weekly	1-56
weight	scale	monthly	1-134

them to centimeters, thus obtaining the surface measurement of the object studied (in this case the nubbin area).

Samples at the beginning and at the end of the experiments have been frozen and preserved to allow future analysis of other parameters such as zooxanthellae number, protein, lipid and carbohydrate contents.

RESULTS

In the clearance tests carried out during the experiments a constant concentration of plankton in the control (an aquarium with no nubbins prepared for the test) was found, indicating no problems of plankton sedimentation during the feeding time. In all the aquariums with the

different concentrations tested, two and a half hours after the moment of food administration, most of the zooplankton had disappeared.
A general high percentage of survival for all the nubbins in the two experiments has been noted (Shafir *et al.*, 2001): from higher than 97 %, up to 100 % in the aquariums with high *Artemia* concentrations, slightly less in the control but not significantly different.
Nubbins that were bleached, eaten or damaged (especially the external border) have been considered "suffering". They have been few: less than 17 % in rotifers experiment and absent with high *Artemia* concentrations; generally control fasting nubbins have been more suffering than fed nubbins (χ^2 – test $p < 0.05$).
Nubbins have reached three-dimension faster with *Artemia* food (till 80-100 % at the end of the experiment) (Figure 3) and with the highest rotifers concentration tested in diurnal regime feeding (till 60 % at the end of the experiment) (χ^2 test $p<0.01$).
Area and polyps number have been considered only for rotifers experiment because nubbins fed with artemia become three-dimensional very quickly and with photographs and Image-J program it is not possible to measure exactly the area and polyps of three-dimensional nubbins.
After 56 days nubbins fed diurnally with 16,000 rot.L^{-1} have grown more than fasting ones (Kruskal-Wallis – multiple comparisons with rank-sums Tukey's HSD $p < 0.05$) (Figure 4).
After four months experiment, all nubbins fed with *Artemia* weighed more than fasting ones (Tukey's HSD $p<0.05$) (Figure 5); there haven't been significant differences between nubbins weight of the different rotifers concentrations tested (Kruskal-Wallis – multiple comparisons with rank-sums Tukey's HSD $p>0.05$), even if nubbins fed diurnally with 16,000 rot.L^{-1} tended to weigh more than all the others (Figure 6). *Artemia* fed nubbins weighed more than rotifers fed nubbins.
There is no apparent difference between diurnal and nocturnal feeding regime.

DISCUSSION

Comparison between A. salina *and* B. plicatilis *food experiments*

Hermatipic scleractinian corals function as phototrophs, through their association with zooxanthellae, as well as heterotrophs (Antony, 2000): they receive photosynthate from zooxanthellae and also utilize a variety of food sources for heterotrophic feeding including zooplankton (Clayton, 1982). In these experiments the growth of *Pocillopora damicornis* in three different energy source conditions has been evaluated: only phototrophy (controls), phototrophy contemporary with heterotrophy (diurnal regime feeding with *Artemia* or rotifers) and heterotrophy not contemporary with phototrophy (nocturnal regime feeding with *Artemia* or rotifers). The results of the two experiments shows that in both experiments there is no evident difference between diurnal and nocturnal feeding regime, probably because *P. damicornis* has his polyps extended both day and night (Anthony, 2000). The suitable timing of food administration found is two hours and half, much less than the total time tested in the experiments.
In *Artemia* experiment the growth of corals has been evaluated taking into consideration two parameters: percentage of three dimensional nubbins and nubbins weight; corals welfare has been estimate by percentage of suffering and survival nubbins. The elaboration of the data collected in this experiment shows that all the nubbins fed with *Artemia* grow quicker and healthier than fasting ones. This result is in agreement with Houlbrèque *et al*. (2004), who observed that both light and dark calcification rate in *Stylophora pistillata* is greatly enhanced by feeding. Besides there isn't a significant growth difference between the various food concentrations administered because nubbins fed with the smallest concentration grow as nubbins fed with the biggest concentration of *Artemia*.
In rotifers experiment it has been possible to consider also area and polyps number to evaluate nubbins growth. The data show that only nubbins fed diurnally with the highest concentration grow a little bit more than fasting ones. It's possible that nubbins need more rotifers than 16,000 ind.L^{-1} to increase their growth but it could be useful to evaluate the cost of plankton management to guarantee an high food concentration.
For this reason, in addition a cost analysis has been done between *Artemia* and rotifers food for corals. Table three shows an example of feeding a 1,000 L coral tank with 2,000 *Artemia*.L^{-1} (the minimum suitable concentration found in experiment one) and with 16,000 rotifers.L^{-1} (the minimum suitable concentration found in experiment two). This suggests that feeding with *Artemia* is generally less expensive than feeding with rotifers.

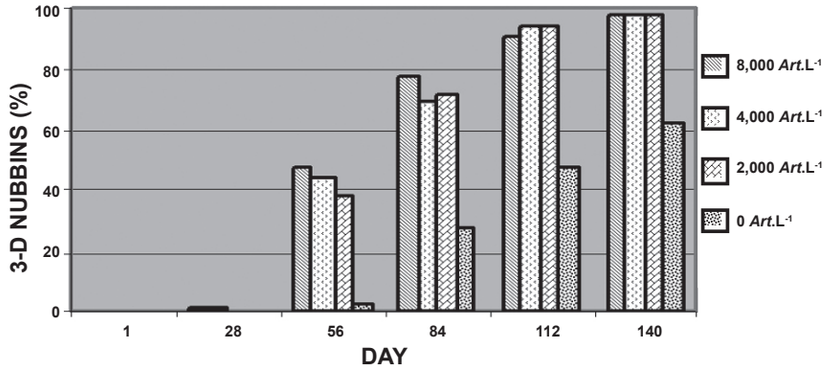

Figure 3: Percentage of three-dimensional nubbins with Artemia *diurnal feeding*

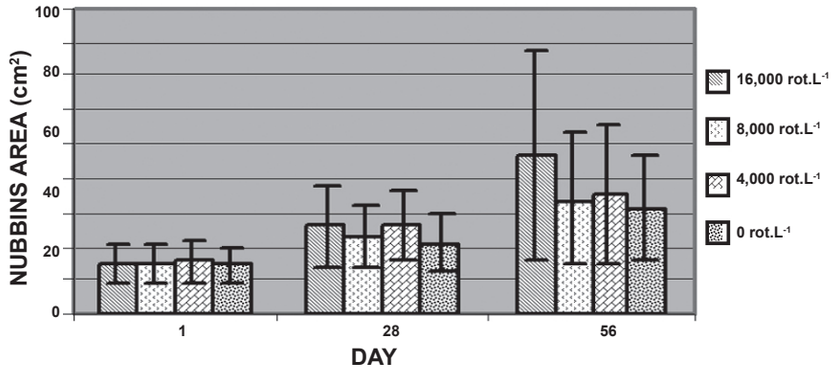

Figure 4: Nubbins area with rotifers diurnal feeding from the beginning to day 56 where three-dimensional percentage is less than 20 %

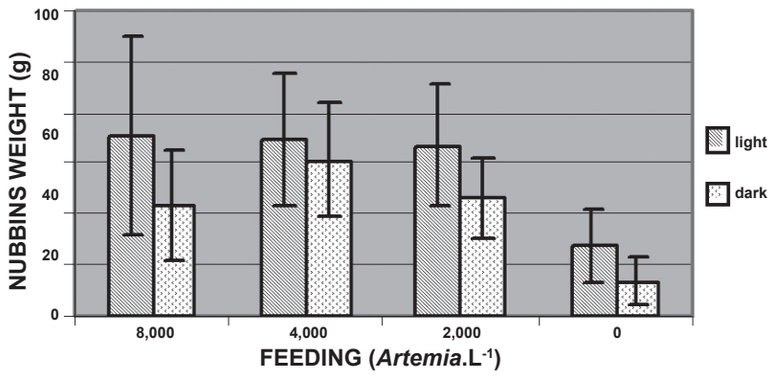

Figure 5: Nubbins weight in the Artemia *experiment after 126 days*

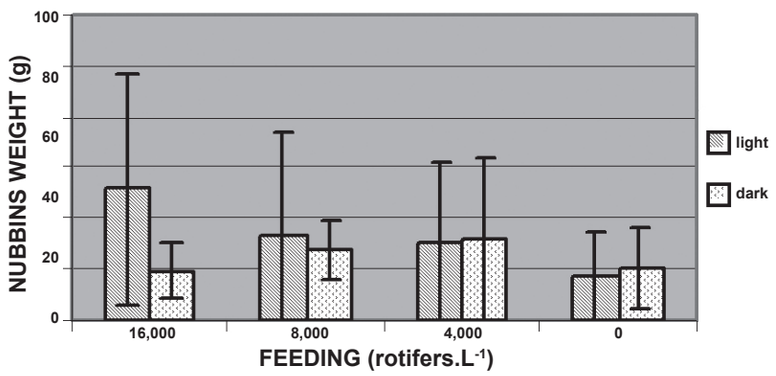

Figure 6: Nubbins weight in the rotifers experiment after 134 days

Table 3: Cost analysis: example of a coral tank of 1,000 L feeding

	2,000 *Artemia*.L^{-1}	**16,000 rotifers.L^{-1}**
Culture volume	9 L [500 *Art*.ml^{-1}]	109 L [500 rot.ml^{-1}]
Lab. culture time	30 min.d^{-1}	1 h.d^{-1}
Cost (only food, enrichment and cysts)	€ 0,07 x day	€ 0,19 x day

CONCLUSIONS

Preliminary husbandry suggestion for* Pocillopora damicornis *nutrition

The present study shows preliminary data of two experiments on nutrition of *Pocillopora damicornis* with zooplankton (*A. salina* and *B. plicatilis*) carried out in a closed system. Attention has also been paid in optimizing husbandry resources and avoiding food waste by administering the tested food concentrations non continuously, but for a period of five hours daily.

The results suggest that light regime during feeding doesn't influence the growth of the corals according to the parameters analyzed in the present study, even if diurnal feeding is often better, as well as more practical.

A feeding time of two hours and half is enough for the colonies to ingest most of the zooplankton available.

Feeding the colonies with *Artemia* results in a faster growth (Houlbrèque *et al.*, 2003) and a concentration of 2,000 art.L^{-1} is enough to see good results; also diurnal feeding with more than 16,000 rot.L^{-1} results in a fast growth.

Much less *Artemia* than rotifers is enough to achieve similar results and it's to be considered that *Artemia* is easier and less expensive to culture than rotifers, although ecologically limiting since rotifers are bred whilst *Artemia* comes from wild cysts.

Evaluation of complete data sets still must be carried out and will establish whether the preliminary results can be confirmed. At present, with the concentrations tested in these experiments, nubbins of *P. damicornis* fed with *Artemia* grow better than nubbins of the same species fed with rotifers and than fasted ones.

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