

ASEXUAL REPRODUCTION OF *NAUSITHOE AUREA* (CNIDARIA, SCYPHOZOA, CORONATAE) INDUCED BY STERILE POLYSTYRENE DISHES

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Some scyphozoan species have a few peculiarities and these are reflected in their life cycles (e.g. type or seasonality of strobilations) (see more in Berrill, 1949 and Silveira *et al.*, 2003). For the scyphozoan order Coronatae, the solitary polyps are hypothesized to strobilate under conditions of energy surplus, while colonial species tend to strobilate in determined periods (Werner, 1970). The strobilation process of the scyphozoans is a relevant morphological event that helps understanding the evolution and relationships of other taxonomic groups (see more in Berrill, 1949 and Collins *et al.*, 2006). The variety of reproductive traits is very interesting and, unfortunately, quite overlooked in Scyphozoa (see more in Arai, 1997).

Strobilation inducing factors of some solitary scyphozoans are widely known, such as iodine, temperature, light regime and abundance of food (see details in Kroiher *et al.*, 2000). These inducers are able to be tested experimentally, by adding controlled amounts of food or concentrations of iodine, or controlling the variation of temperature and light regime. However, some other factors have not been considered, for example the influence of some objects during the experiment (e.g. forceps, pointers or dishes), as pointed out by the results of Herrmann *et al.* (2003). The influence of the culturing dishes in the strobilation of the coronate scyphozoan *Nausithoe aurea* is the focus of this communication.

The species employed in this work was the solitary coronate *Nausithoe aurea* Silveira & Morandini, 1997. It is a rather common scyphopolyp species found in southeastern Brazilian coast and there is detailed information on the life cycle, embryonic development and behavior in Silveira & Morandini, 1997; Morandini & Silveira, 2001; Silveira *et al.*, 2003; Holst & Jarms, 2006; Stampar & Silveira, 2006. In the life cycle of *N. aurea* the strobilation may

produce either ephyrae (which grows to mature medusae) or planuloids (Silveira & Morandini, 1997).

Using the results and the protocol established by Herrmann *et al.* (2003) we created a protocol to verify the possible strobilation induction by sterile polystyrene dishes in the species *Nausithoe aurea*. The dishes used in this work were from Corning®, series (430165), sizing 35 mm x 10 mm.

Fifty four polyps were used in the experiment. All polyps were sampled in São Sebastião Channel, the type locality of *N. aurea*. These polyps were divided into three different groups (eighteen polyps in each group divided in 6 dishes of 4 ml with, 3 polyps per dish), as described below:

Group **A** - Sterile polystyrene dishes, without any previous cleanse.

Group **B** - Sterile polystyrene dishes, but washed with artificial seawater at 70°C by 15 min.

Group **C** - Polystyrene dishes, washed with HCL (1%) and artificial seawater at 22°C by 15 min.

All experiments were conducted at controlled temperature (22°C).

The observations on the polyps were performed daily until the conclusion of the experiments (21 days). The artificial seawater (Tropic Marin®) used in the experiments was changed every week. When a polyp starts to strobilate, it was removed from the experiment to avoid induction of other polyps (Loeb, 1974; Arai, 1997).

The experiments showed two distinct results. In the initial four days, strobilation was not observed (Fig. 1). However, in the fifth day a polyp from experiment **A** began the process of strobilation. These results were observed in the following days with up to six strobilating polyps (Fig. 1) only in group **A** (sterile dishes). The results showed a strobilation ratio of 30% in the polyps from group **A**. The two other experimental groups, **B** and **C**, did not present any strobilating polyps.

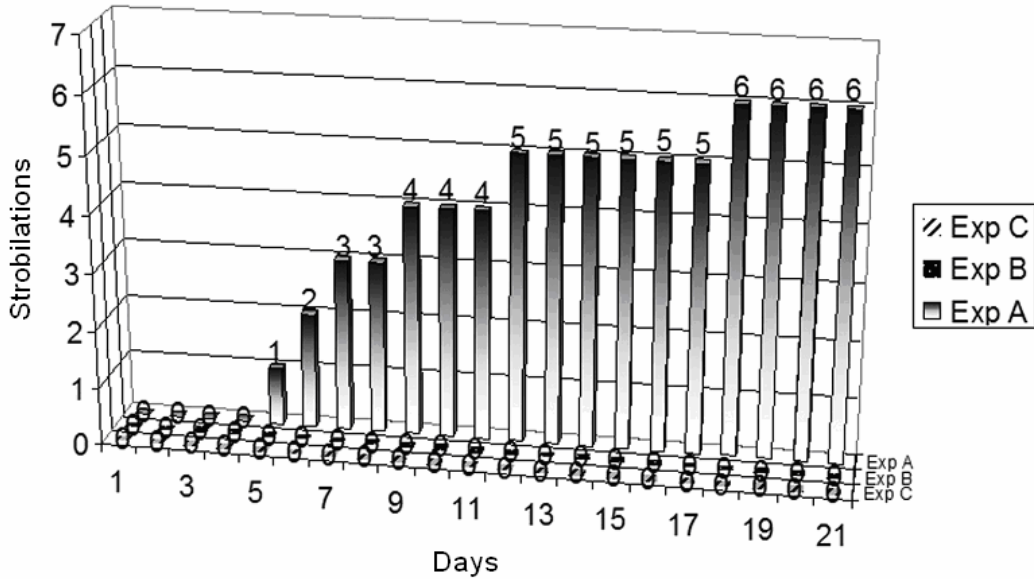


Fig. 1. Strobilation results in 21 days of experiment by group. Legends: Group A – sterile polystyrene dishes; Group B - sterile polystyrene dishes, washed with water at 70°C and Group C – Old dishes (previously used) and washed with HCL (hydrochloric acid 5%).

The strobilation results observed by Herrmann *et al.* (2003) in the scyphozoan *Aurelia aurita* (Linnaeus, 1758) were greater than ours. However, our results showed the same kind of influence from the sterile polystyrene dishes in the Coronatae species *Nausithoe aurea*. When we compare the results of the other two groups, B and C, with those of group A the differences are clearer. The data were analyzed using the method of inference for stratified categorical data – The Mantel-Haenszel Test (X^2_{MH}). The X^2_{MH} value of the experiments is 14.60. Since $X^2_{1,999} = 10.83 < 14.60 = X^2_{MH}$, it follows that $p < .001$ concluding that we reject the null hypothesis (see more in Rosner, 2000).

The induction of strobilation has been attributed to the use of sterile polystyrene dishes. The cause is probably the formation of Reactive Oxygen Species (ROS) by substances that were used to turn the polystyrene dishes sterile, as pointed out by Herrmann *et al.* (2003) and Berking *et al.* (2005).

The fleeting intermediates (chemical compound formed by one reaction and then used in another) produced by radiation and respiration are called free radicals. The use of this term is only a convenience. Not all of these fleeting intermediates are free radicals within the usual definition of the term. Applying the correct terminology, however, is cumbersome. Another umbrella term to these fleeting intermediates, ROS, is even more cumbersome and also untrue – not all are especially reactive and some, such as nitric oxide (NO) are technically reactive

nitrogen species (Lane, 2002). However, ROS is a broader expression than free radicals or oxidizing free radicals (Gilbert & Colton, 1999). ROS is a collective term often used to include not only the oxygen radicals (O_2^- and OH) but also some non-radical derivatives of O_2 (Halliwell & Gutteridge, 1999). ROS (as an umbrella term) includes the superoxide radical anion, hydrogen peroxide, the hydroxyl radical, lipid peroxides, the peroxy radicals, the alkoxy radicals, the radicals of nitric oxide and nitrogen dioxide, ozone and possibly singlet oxygen, either in its low energy form or in its high energy form (Gilbert & Colton, 1999). In the way of confusion in the terminology we prefer to use ROS, because it is more widespread within academic publications as a broader term.

Constantly, the ROS are built by cellular metabolism from oxidize of NADPH in the major part of cell types so far known (Gretzer *et al.*, 2002). Berking *et al.* (2005) showed that the addition of 1nM of Hydrogen Peroxide (H_2O_2) causes in *A. aurita* up to 50% of strobilation. Nevertheless, high concentrations of H_2O_2 can result in cell injuries observed as DNA damages, disabling dehydrogenases (Spragg *et al.*, 1987). In addition, the extra-cellular H_2O_2 can induce apoptosis processes (Laochumroonvorapong *et al.*, 1996; see more in Gretzer *et al.*, 2002).

When the metabolic demand is low (few or no food is offered), mitochondria come into resting stage (Alberts *et al.*, 2002; Blackstone, 2001). In this period, the phosphorylation is minimal and the electrons cart is much reduced, what can induce the

ROS production. Subsequently, this way can be an alternative form of reproduction in this species in conditions of low food availability. On the other hand, when a lot of food is available the phosphorylation is greater and the electron cart is very high and the ROS production is low (Blackstone, 2001).

These aspects are still unexplored in cnidarians; a few works referring to this issue and only one concerning a hydrozoan (Blackstone, 2001), a closer group to the scyphozoans. The induction of asexual reproduction (strobilation) by laboratory stuff is a significant issue and this is once more demonstrated in a different cnidarian species. It is very important to pay attention to other modifications that can be caused by use of sterile polystyrene dishes.

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