Cultivation of *Daphnia similis* Species in Standard Conditions

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***Daphnia* is an organism used for ecotoxicity tests to evaluate acute or chronic toxic effects of substances and effluents on an organism's population. *Daphnia* must be cultivated under standard conditions to reproduce themselves. This article highlight the importance of standard *Daphnia* cultivation since unfavorable conditions may impact or interrupt the cultivation progress. The method was based on the NBR12713 standard, which focused on compliance and validation of established cultivation guidelines. The results revealed the adverse effects and impact that *Daphnia* culture might have if they are not in the required conditions.**

Keywords: *Daphnia*. Ecotoxicity. Cultivation.

Introduction

The application of aquatic organisms for ecotoxicological assays has been increasing because some organisms are more sensitive and have a contact surface that allows an analysis of specific compounds, mainly chemicals [1,2]. So, *Daphnia* (*Cladocera* order) is used to analyze and monitor the quality of aquatic ecosystems. Furthermore, they are increasingly applied to ecotoxicological assays because they are susceptible when exposed to toxic compounds at low concentrations [3].

Daphnia reproduces by parthenogenesis, which results in a female population. From the 3rd to the 7th day, they mature for reproduction (depending on the species) [4]. It has an anatomy (Figure 1) that allows easy locomotion through the antennae and an abdominal region that allows food particles to be filtered and conducted to the mouth [5,6].

The two species most used for testing are *Daphinia magna* and *Daphinia similis*, originating from freshwater. They are small (0.5 to 5.0 nm), easier to handle, and favorable for cultivation in laboratory environments. The difference is their life cycle, in which *D. magna* has a longer life cycle than *D. similis* and is more sensitive [7-9]. However, in Brazil, the species most used for testing is *D. similis* since it is recommended for ecotoxicological evaluation in tropical and subtropical climatic countries [3].

These *Daphnia* species applied in ecotoxicological tests allow a study of acute effects (quick responses for organisms exposed to some compound) and chronic effects (responses with extended periods of exposure, which may lead to deleterious effects to organisms, such as immobility). The ecotoxicological tests occur through neonates obtained by reproducing young or adult *Daphnia* (7 to 21 days of age) [6].

This study aims to highlight the importance of standard cultivation of *Daphnia similis*, signaling unfavorable conditions that may impact or interrupt the progress of organism cultivation.

Materials and Methods

We based this study on ABNT NBR:12713 (Aquatic ecotoxicology — Acute toxicity — Test method with *Daphnia* spp.), which specifies a method for evaluating the acute toxicity of liquid samples and chemical substances soluble or dispersed in water for *Daphnia similis* and *Daphnia magna* [10]. Figure 2 presents the standard method flow.

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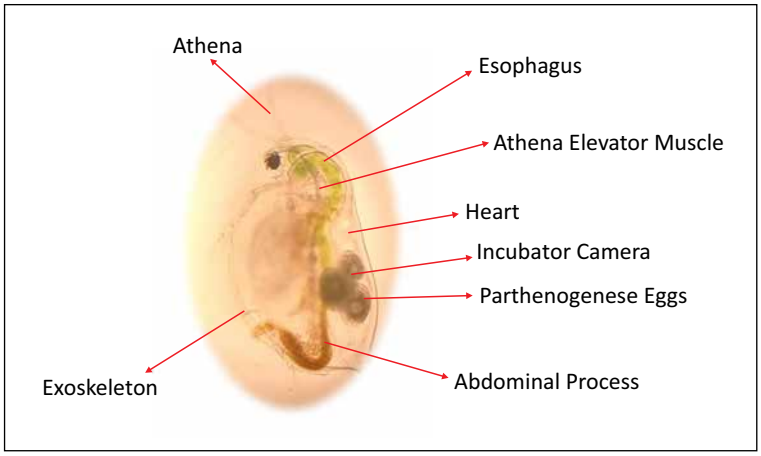
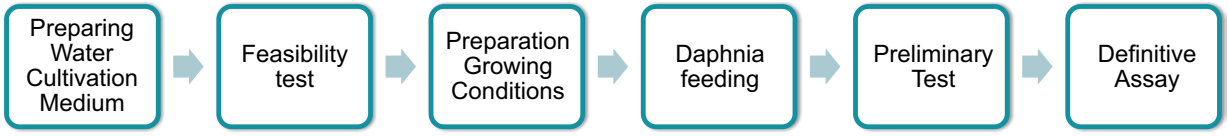
Figure 1. Simplified anatomy of *Daphnia similis*.



Source: Adapted from Oliveira TMN, Kleine T, Vaz C [6]. Photo taken under a microscope.

Figure 2. Applied method flow.



We focused on the tests to achieve standard *Daphnia* (*D. similis*) cultivation, donated by the Environmental Company of the State of São Paulo (ECSSP). The tests were carried out at the Laboratory of Applied Research in Chemistry (LIPAQ), which followed the cultivation method (Figure 3).

Results and Discussion

This article brings the results of cultivating *Daphnia similis* under unfavorable conditions, highlighting the importance of their standard cultivation. We need to adjust some parameters of the culture medium (pH, hardness, dissolved oxygen, temperature, and photoperiod of 12 h to 16 h of light) to maintain the increase and constancy of new cultures. Monitoring these variables in the culture is essential to guarantee that these indicators' alteration will not impact toxicity test performance.

For the maintenance and control of *Daphnia similis* cultures, they must be conditioned in suitable environments, such as temperature (18°C to 22°C) and humidity control at a photoperiod incubator (Figure 4), which simulates day and night (12h to 16h of light) by the ABNT NBR 12713 normative.

However, due to the absence of an incubator to maintain the cultures, since the organism was not cultivated in the laboratory where the present study was conducted, it was necessary to set up a structure to adapt the *Daphnia* to the required conditions. Figure 5 shows a comparative scenario of the conditioned cultures in the incubator of the ECSSP ecotoxicity laboratory and the cultures carried out in LARC.

This condition becomes unfavorable since the organisms are subject to temperature changes by the external environment, which can lead them to death or immobility.

For instance, factors such as temperature, photoperiod, overpopulation of organisms, and

Figure 3. Flowchart method.

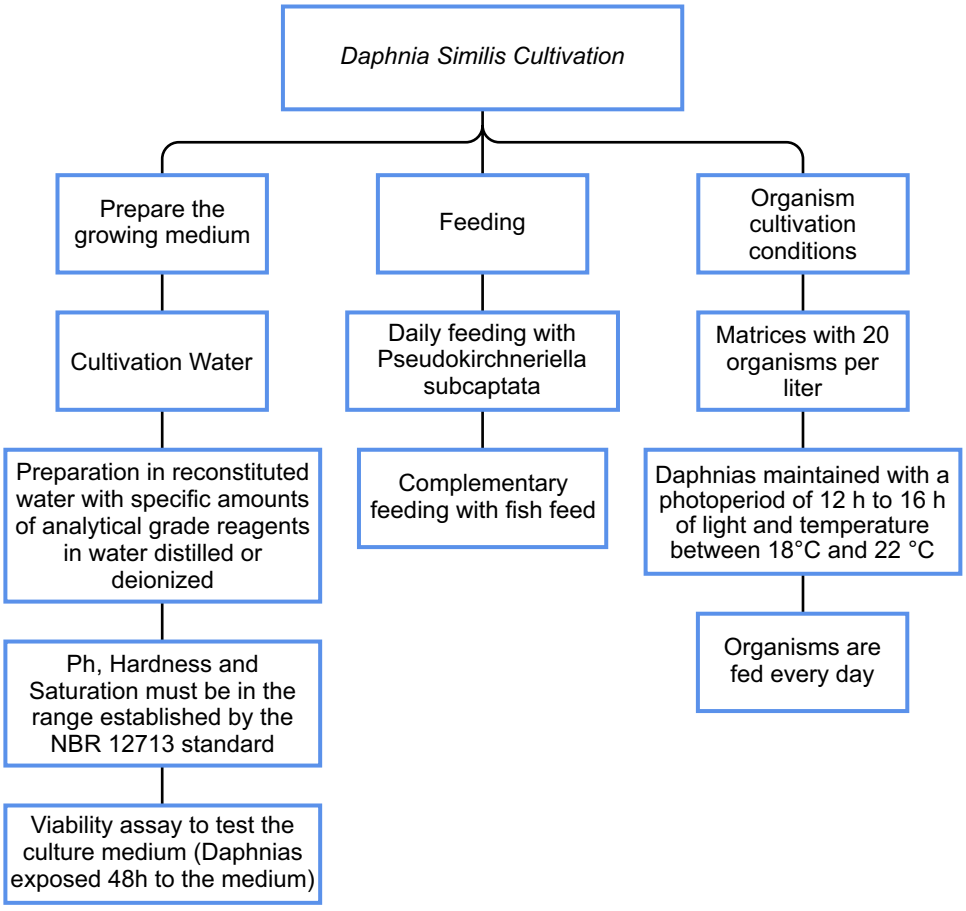


Figure 4. Photoperiod incubator image.

Source: Ethik.

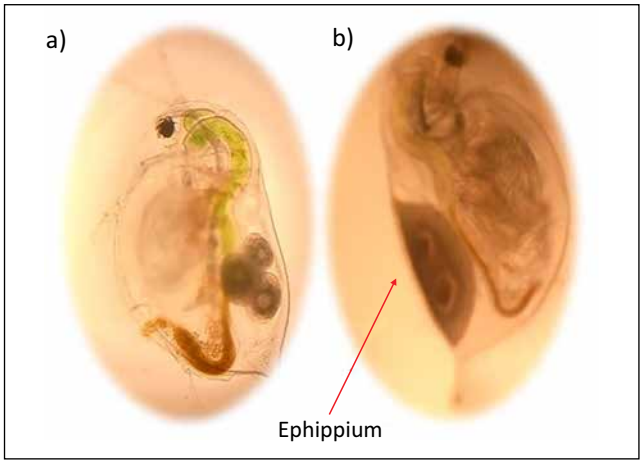
lack or excess of food can influence the cultivation of *Daphnias similis*. This fact can lead to the emergence of males and females with two resistant eggs. These resistant eggs, fertilized by males, are surrounded by a thickening dark color in the incubation chambers of adult females, called ephippium (Figure 6) [4-6]. Therefore, if one or more cases of ephippium occur in a culture, the neonates produced in this culture should not be used, having to discard and reassess the cultivation procedures. For example, we have had a case of ephippium in culture due to the overpopulation of *Daphnia* while testing new growing conditions, such as growing organisms in a container to increase the number of spikes (cultures) [10].

During the *Daphnia* cultivation supervision tests in the laboratory, we observed that not all of them were set when observing dead or immobile

Figure 5. A comparative scenario of crop structure.

Incubator cultivation - ECSSP Cultivation on The Table - LARC Source: Authors.

Figure 6. Comparison between a) *D. similis* with ephippium and b) with parthenogenesis egg.



Source: Authors - Photo taken under a microscope.

Daphnia. Therewith, it was possible to evaluate a change in the pH of the culture medium since it was outside the range established by the standard (7-7.6). We tested this parameter in different scenarios with *Daphnia*:

- Culture containing only reconstituted water;
- Culture containing just algae;
- Culture containing exclusively feed.

These tests were intended to investigate whether the food's pH interfered with the culture's maintenance or whether the problem involved the reconstituted water. However, after 24 hours of observation, the organisms died in all tested cultures. Thereby, we produced a new culture medium to identify if there was any contamination because the organisms continued to die, notwithstanding the pH variations. These tests are still in progress. Therefore, all variables must be in the conditions established for the standard cultivation of *D. similis* since not following may cause negative impacts on the cultivation. Also, it can discontinue the ecotoxicity tests since it needs neonates for their performance and effectiveness. One way to prove this fact is through a study carried out in Germany, which investigated the toxicity of algae organic matter in water reservoirs through bioassays using *D. similis*. Thus, with good cultivation and the emergence of neonates for application in tests, it is possible to determine that the organic matter of algae did not present toxic effects for the species tested under typical environmental concentrations. On the other hand, in non-ideal cultures, it would not be possible to obtain the results with the efficiency and reliability expected from an ecotoxicity assay [11].

Conclusion

This article focused on highlighting the main factors that can interfere in the cultivation of *D. similis*. We observed how sensitive these organisms can be to variations in used parameters (pH, temperature, photoperiod) that must be at ideal intervals to maintain the cultures and allow their reproducibility.

In addition, unfavorable growing conditions, such as overpopulation or temperature variation, can cause ephippium to discard cultures in these conditions, delaying the growth of organisms. Finally, the continuity of the cultures must allow access to neonates to carry out the ecotoxicity tests and, consequently, the studies of toxic compounds.

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