The production of ammonia in mass cultures of the rotifer, *Brachionus plicatilis* O.F. Müller, feeding on bread yeast.

by

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**SUMMARY**

The production of ammonia in mass cultures of the rotifer, *B. plicatilis*, fed with different concentrations of bread yeast and temperatures are compared with that of the rotifer initially inoculated with algae culture. The levels of ammonia remained low in the last ones when the algae cells were still present. They increased when the cells had been ingested by the rotifers. Nevertheless, the rotifer population initially inoculated in algae culture always show a better growth than the ones fed only with bread yeast. This fact seems to be independent of the ammonia concentration in the culture water.

**RéSUMÉ**

On compare la production d'ammonium dans la production en masse du rotifère *B. plicatilis* nourris avec des différentes concentrations de levure de panification avec celle de rotifères initialment inoculées dans une culture algale. Les niveaux d'ammonium se maintiennent bas dans ceux derniers seulement pendant qu'il y a des cellules algales dans le milieu, augmentant quand celles-ci sont ingérées par le rotifère. Pourtant les populations de rotifère installés initialement dans des cultures algales ont toujours un plus grand nombre d'individus que celles qui furent nourris exclusivement avec de la levure, n'importe quelle soit la concentration d'ammonium au milieu.
INTRODUCTION

The mass culture of the rotifer, Brachionus plicatilis, fed with bread yeast (Y-rotifer) was pioneered by Japanese workers (Hirata and Mori, 1967).

Although the yeast does not form part of the natural diet of Brachionus, the advantages of its use are obvious from a commercial point of view. It reduces considerably the requirements of space, energy and manpower on making the production of rotifer independent of the production of large volumes of algal cultures.

Yufera and Fasqual (1980) have obtained yields of 63,733 rot. 1⁻¹ day⁻¹ in mass cultures of rotifer fed with bread yeast and initially inoculated in algae cultures on plastic bags of 70 l, using a daily ratio of 0.5 g. 1⁻¹ of yeast.

In previous studies we have obtained yields of 64,972 rot 1⁻¹ day⁻¹ in mass cultures of rotifer fed only with bread yeast in fiberglass tanks of 1000 l using a daily ratio of 1 g. 10⁶ --rot. (Reguera et al., in press).

Y-rotifers, as opposite to the rotifer fed with algae (A-rotifer), have low nutritional value when used as food for crustaceans and fish larvae (Kitajima and Koda, 1975). This is due to its low content in ω3-HUFA (ω3-highly unsaturated fatty acids) which are essential components on the diet of marine fishes -- (Watanabe et al., 1978, 1979). Nevertheless the nutritional value of the Y-rotifer can be improved by subculturing it on algae culture several hours before being given to the larvae (Kitajima and Koda, 1976; Kitajima et al., 1979), or by feeding the rotifer with bread yeast produced with a complemented culture medium (Imada et al., 1979).

The main problem to face in mass cultures of Y-rotifer in closed-systems is the fast deterioration of the culture water.
The bread yeast is an inert food that decomposes quickly when remains uneaten or settles down in the bottom of the container. Among the potentially life-endangering substances accumulating in the culture water, ammonia is of primary importance. The tolerance limits depend on both the species and the environmental conditions.

This communication briefly considers the production of ammonia in mass cultures of *B. plicatilis* fed with bread yeast in closed-systems. The possible influence of ammonia in the poisoning of the culture water and the collapse of the population is also considered. The levels of ammonia reached are compared between rotifer cultures initially introduced in algae culture and rotifer cultures fed only with bread yeast. In this way, the better growth showed by the A-rotifer population might be explained.

**MATERIAL AND METHODS**

Two series of experiments were carried out in 3 pairs of containers, each pair being a duplicate under identical treatment.

In the first experiment (A), the cultures were grown in plastic cylindrical containers of 20 l and the algae employed was *Tetraselmis suecica* from 50 l cultures in plastic bags. The mean test temperature was 18±0.5°C. In the second experiment (B), the cultures were grown in 5 l erlenmeyers and the algae employed was *Dunaliella tertiolecta* from 5 l cultures in erlenmeyers. The mean test temperature was 20±1°C.

In the first and second pair of containers (1&2, 3&4) the rotifers were inoculated in filtered and sterilized seawater and fed with a daily ratio of 1 g and 2 g per million of rotifer respectively. In the third pair (5&6) the rotifers were
inoculated in algae culture and fed with a daily ratio of 1g yeast per million of rotifer.

Daily measurements of pH, temperature, concentration of ammonia and nitrite (only in experiment A) and rotifer concentration were taken. The concentration of ammonia was determined by the method of Solórzano (1969) and the nitrite concentration by the method of Strickland and Parsons (1968).

All the algae cultures were carried out in a culture chamber under constant illumination and temperature with filtered and sterilized seawater enriched with Walne medium. Rotifer concentration was estimated from five 1 ml samples from each culture.

To estimate the dry weight of B. plicatilis samples of rotifer cultures of determined density of population were filtered on a 63 µm aperture nylon mesh and washed with destilled water. These samples were dried overnight in a desiccation oven until a constant weight was reached. The average dry weight was estimated as 174 ng.

RESULTS

The changes in ammonia concentration over a period of 9 days are shown in figure 1.

Fig. 1A shows the result of the first experiment in 20 l containers at 18 ± 0.5°C and 1B, the second experiment in 5 l beakers at 20 ± 1°C.

All the cultures showed a great increase in the concentration of NH₄⁺ at the third or fourth day coinciding this with the first peak on the exponential growth of the rotifer population.

The levels of NH₄⁺ concentration reached in 1&2 and 3&4 are similar in each experiment although 3&4 received a double ratio of yeast everyday. The values are almost the same or slightly higher in the containers 1&2 of experiment A and a bit higher in beakers 3&4 of experiment B. The absolute values of NH₄⁺ concentration
were greater in the beakers of experiment B.

In both experiments the initial concentration of ammonia was greater in cultures 5 and 6, inoculated with algae, than in the other cultures inoculated in sea water. The concentrations of ammonia in the cultures inoculated in sea water were 1.04 and 1.63 μg.at.NH₄⁺ l⁻¹ respectively and the concentration in the cultures inoculated with algae were 1.96 and 2.28 μg.at.NH₄⁺ l⁻¹.

The containers 5 and 6 reached an unexpectedly high level of NH₄⁺ in experiment A (fig. 1A). This is accounted for by a much denser population of rotifer developed in these two containers. When a relative production of ammonia is employed (fig. 2) there is no difference between 5 and 6 and the others (fig. 2A).

Fig.1B shows that the NH₄⁺ concentration remained low in beakers 5 and 6 for the first 3 days increasing afterwards when the algae cells were removed by the rotifer population.

The relative production of ammonia was greater in experiment B than in A. The relative production of ammonia in beaker 1 and 4 from experiment B, fig. 2B, is not presented because their rotifer population collapsed. This collapse was due to the excess of air introduced into the beakers as a result of a break down in the air system. Rotifers were trapped at the edges of the beaker and suffered from desiccation. The relative production of ammonia showed a maximum value in number 4 of 1270 μg.at.NH₄⁺ per mg.dried weight of rotifer.

For some unknown reason, the rotifer population of beaker number 5 in experiment B showed a very poor growth compared with its duplicate number 6. This low growth rate accompanied a higher production of ammonia.

The concentration of nitrite, determined in experiment A, was harmless, levels ranging from 0.06 to 0.12 g.at.l⁻¹ during the experiment.

The pH value decreased from 8.3 to 7.4.
DISCUSSION AND CONCLUSIONS

The rotifer populations feeding on unicellular algae show higher growth and reproduction rates than that of the cultures feeding on bread yeast. This fact is well known by all the aquaculturists concerned with mass production of *D. plicatilis*.

Alderson and Howell (1973) have studied the beneficial effect of the algae *D. tertiolecta* in rearing tanks of juvenile sole. The tanks containing the algae do not accumulate ammonia as the ones without algae do. The algae uptake the ammonia for their nitrogen metabolism keeping the culture water in good conditions.

In our study, the ammonia levels in the containers with algae at the beginning of the experiment remained quite low during the first 3 or 4 days when the algae were still present. Nevertheless, the rapid removal of the algae cells due to the filtering activity of the rotifers prevents the long lasting beneficial effect of the algae. Even if we set a permanent source of light and supplied a daily amount of nutritional medium for the algae, the filtering rate of the rotifer (at the commercial density employed in mass cultures) would exceed the growth rate of the algae.

It was surprising that the initial algae cultures contained a high concentration of ammonia. This $\text{NH}_4^+$ might be due to the metabolic transformation of the nitrate of the Walne medium under light-limited conditions.

There is no information available about the tolerance limits of *Brachionus* for ammonia. The origin of ammonia in the mass cultures of rotifer fed with bread yeast is due to the bacterial decomposition of uneaten yeast, dead rotifers and the own excretion of the rotifer. For this reason its concentration varies rapidly under changing environmental conditions (temperature, pH...).

In a 1000 l container where the rotifers had been grown -
and periodically cropped over 40 days, the NH$_4^+$ concentration changed from 123 to 232 μg.at.NH$_4^+$ l$^{-1}$ after a very hot day with an increase of the culture water temperature of 3°C.

The values of ammonia concentration found on aged cultures of rotifer are quite similar to the values found in the previous experiments after nine days. This suggests that after a period of increasing production of ammonia a equilibrium is established.

The concentration of nitrite found in the tanks the same days on which the NH$_4^+$ was determined are below 0.2 μg.at.NO$_2^-$.l$^{-1}$. Therefore, a conversion of the NH$_4^+$ into dangerous levels of nitrite had not taken place.

Bacterial fermentations in the sediments of the container may have decreased the pH and as well may have been responsible of the bad odours observed.

One way to prevent fermentations could be to increase the aeration to provide enough oxygen concentration for the aerobic bacterial oxidation of the sediments. However, in Brachionus cultures strong aeration can be very harmful. It enhances the detachment of the eggs carried by fertile females and the subsequent collapse of the culture in a short period. At the same time, it disturbs the sediments and because of this the culture water deteriorates in a shorter time. (Reguera et al., in press).

The beneficial effect of the algae on rotifer cultures must be explained for other reasons than their possible control of the ammonia levels.

In rotifer cultures fed with bread yeast the fertile females carry usually one egg or very rarely two. In rotifer cultures fed with algae it is normal to find females carrying 4, 5 and even 7 eggs. Therefore, unicellular algae must have some nutritional compound that the yeast has not got, which allows a more intense reproduction and growth rate of B. plicatilis.

High levels of ammonia fermentations and bacterial proliferation can be avoided if temperatures are maintained low though acceptable for rotifer growth (18-19°C).
REFERENCES


Hirata, H. and Y., Mori (1967). Mass culture of the rotifer Brachionus plicatilis fed the bread yeast. Saibai Kyoryu (Kobe) 5:36-40.


Fig. 1: Changes in the concentration of NH$_4^+$ in rotifer cultures fed with bread yeast. 1&2=1 g. 10$^6$ rot. day$^{-1}$; 3&4=2 g. 10$^6$ rot. day$^{-1}$; 5&6=1 g. 10$^6$ rot. day$^{-1}$, inoculated in algae culture. A) T=18±0.5°C, algae=T. suecica. B) 20°C±1°C, D. tertiolecta.
Fig. 2: Relative production of ammonia ($\mu$g ret. NH$_4^+$ / mg dry weight of rotifer) in the same experiments plotted in figure 1.