

# Bacterial Flora of Water and Rotifers in Outdoor Mass Culture Tanks Fed with Different Microalgal Diets

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**Abstract:** Investigations were carried out on the bacterial flora of water and the quality of rotifers in outdoor mass culture tanks. *Brachionus plicatilis* and *B. rotundiformis* were cultured in 10 numbers of 1 tonne tanks and the physico-chemical parameters and microbial loads of water, and total bacterial and *Vibrio* loads of microalgae and rotifers, during the period 2014-15, were studied. The study revealed a significant variation of the ammonia levels and total bacterial loads with different diets ( $P < 0.05$ ). A positive correlation was observed between the total bacterial loads of algal concentrations and loads of rotifers, but no correlation was observed between *Vibrio* loads of water and rotifers. The prevalence of *Vibrio* loads of rotifers was low in tanks fed with *Nannochloropsis oculata* and maximum *Vibrio* loads were recorded in *Chaetoceros calcitrans* fed rotifers. The *Vibrio* loads significantly varied with days of culture and also with the algal diets. A positive correlation was observed between the total *Vibrio* loads of water and rotifers.

**Key words:** *Brachionus plicatilis*, *B. rotundiformis*, marine microalgae, bacterial flora.

## 1. Introduction

For several decades, *Brachionus plicatilis* is one of the most important starter feeders used in the initial stages of fish larval stages and has been used in fish hatcheries. Due to their smaller size and faster rate of reproduction, these rotifers are being cultured on a mass scale as a feed for fish larviculture. Significant research contributions were made on the nutritional aspects of these rotifers in association with density [1]. The success of rotifer mass cultures is determined not only by the higher density, but also by their nutritional composition and their associated microbiota [2-4]. Health and survival of fish larvae depend on the quality of feed given and hence it is essential to understand the health status of rotifers before they are fed to fish fry. Although few studies in this aspect have been carried out worldwide [3-6], studies in India were found meagre. The present paper deals with the association of microbial flora of live feed in relation to the microalgal diets and water quality in

outdoor mass culture tanks.

## 2. Materials and Methods

A study was carried out on the quality of rotifers in marine hatchery during 2014-15. Rotifers were grown semi-continuously on *Chlorella vulgaris* in 1,000 L tanks with a density of 100 rotifers ml<sup>-1</sup>. Rotifers were fed with *Nannochloropsis oculata*, *Isochrysis galbana*, *Chlorella vulgaris*, *Chaetoceros calcitrans* and combination of *Nannochloropsis* and *Isochrysis*. For each diet, duplicates were maintained. For every three days, total bacterial and total *Vibrio* loads were estimated from water and rotifer samples following standard protocols. In brief, water samples were serially diluted and pour plated on Zobell Marine agar and TCBS agar, following standard procedures. Rotifers and microalgae were rinsed with sterile seawater and 0.1 g of sample was suspended and homogenised in 1 mL of sterile seawater. Serial dilutions were made and pour plated on Zobell Marine agar and TCBS agars and the loads were estimated after 24 h incubation. Water quality parameters (Temperature, Salinity, pH, Dissolved oxygen and

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Ammonia) were also recorded at the time of sampling. The data was analysed using one-way and two-way ANOVA for significant occurrence of *Vibrio* between the treatments.

### 3. Results and Discussion

#### 3.1 Microbial Loads of Water in Rotifer Tanks

Total bacterial loads were observed to be very high

in the water and also rotifers fed with *C. calcitrans* (Fig. 1). Maximum total bacterial loads and total *Vibrio* loads in tank water fed with *C. calcitrans* were  $0.22 \times 10^7$  cfu/ml and  $0.15 \times 10^6$  cfu/ml respectively and minimum values were observed in tank water with *I. galbana*. Total bacterial counts of water were low in tanks fed with *I. galbana* and varied between  $0.79 \times 10^2$ - $0.225 \times 10^3$  cfu/ml. The total bacterial

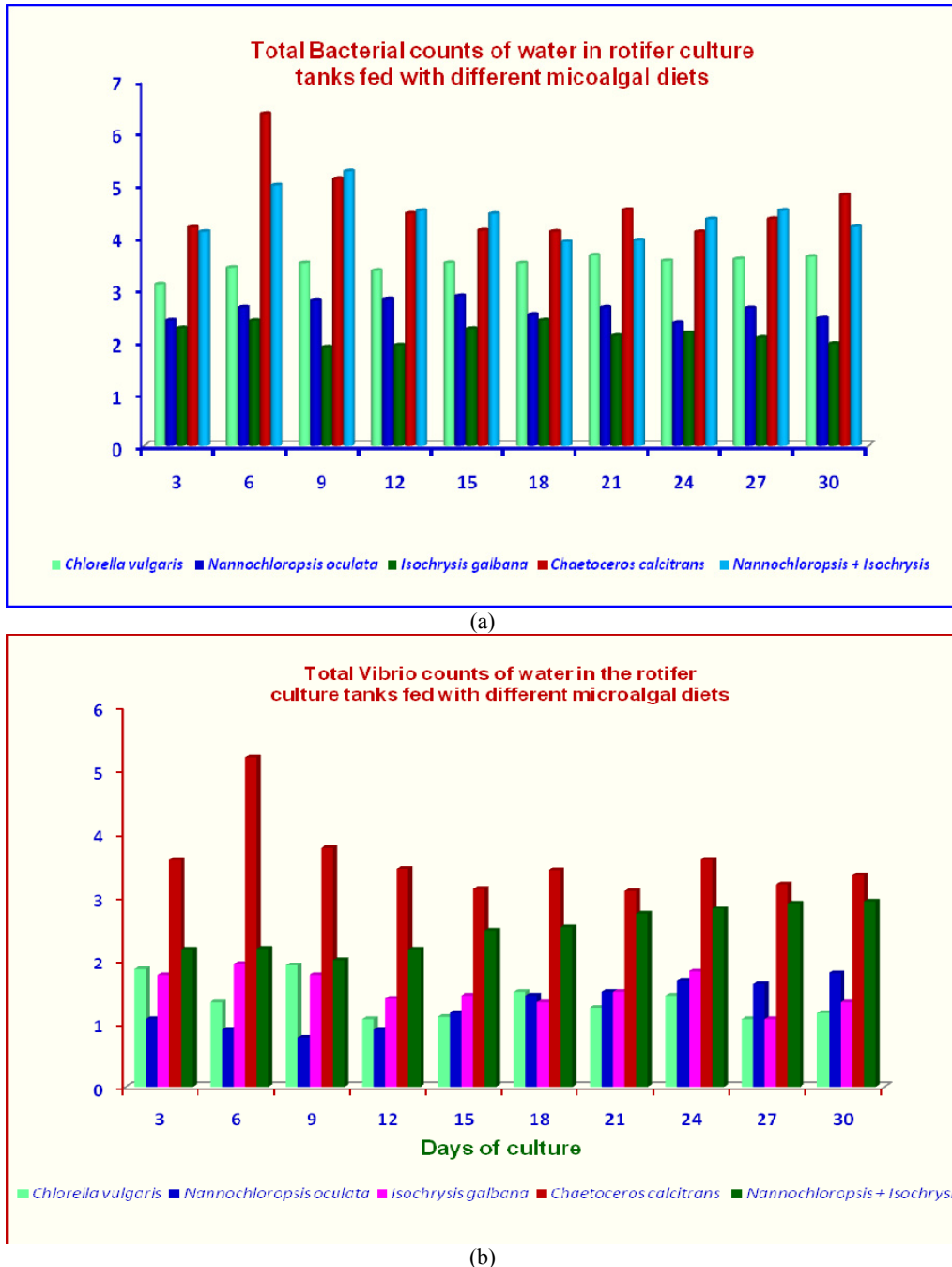


Fig. 1 Total Bacterial Loads and total *Vibrio* loads of water in rotifer tanks fed with different microalgal diets.

loads were observed to be low initially in the tanks fed with algal diets other than *C. calcitrans*, but increased from 10th day onwards with partial water exchange. Maximum *Vibrio* loads of water in rotifer tanks fed with *C. calcitrans* were recorded with a density of  $0.15 \times 10^6$  cfu/ml, whereas, minimum of 6 colonies were recorded from tanks fed with *N. oculata*. It was observed that lower bacterial loads were recorded in tanks fed with *I. galbana* but the *Vibrio* loads of water were less in tanks fed with *N. oculata*. A positive correlation was observed between the total bacterial loads and *Vibrio* loads of water of all the tanks ( $r = +0.52$ ), where as a significant variation was observed between the *Vibrio* loads of water between the algal diets ( $P < 0.05$ ).

### 3.2 Microbial Loads of Rotifers and Microalgae

Total *Vibrio* loads of microalgae varied between a maximum of  $0.1 \times 10^6$  cfu/g in rotifer tanks with *C. calcitrans* and minimum of  $1.2 \times 10^2$  cfu/g in tanks with *I. galbana*. Whereas, the *Vibrio* loads of rotifers varied from  $0.5 \times 10^1$  cfu/g and  $0.24 \times 10^6$  cfu/g in tanks fed with *N. oculata* and *C. calcitrans* respectively. A strong positive correlation was observed between the total bacterial loads and *Vibrio* loads of algal concentrations and loads of rotifers (Table 1). Analysis of Variance revealed a significant variation between the bacterial loads of rotifers and also microalgal diets (Figs. 2 & 3). The study indicated that the occurrence of *Vibrio* was high in the rotifer tanks fed with algal diet of *C. calcitrans* and minimum in *N. oculata*, indicating a direct impact of algal diet on the quality of rotifers. Ammonia levels in the tank waters also varied significantly ( $P > 0.05$ ) between the diets and highest ammonia levels were recorded in *C. calcitrans* tanks. A direct positive

correlation was also recorded between ammonia levels and *Vibrio* loads of water in tanks with *C. calcitrans*. ( $r = 0.58$ ). Maximum Density of rotifers was found in tanks fed with *N. oculata*, followed by the tanks fed with combined diet of *N. oculata* and *I. galbana*. A positive correlation was observed between rotifer density and *Vibrio* loads of algal diets ( $r = 0.68$ ) indicating that the water quality plays a major role in rotifer density.

### 3.3 Application of Probiotics

Preliminary experiment was carried out, in the hatchery, on the effect of commercially available probiotics on the occurrence *Vibrio* loads. The experiment was conducted with algal diet, *C. calcitrans* as more *Vibrio* loads were recorded in those tanks, Four 1 tonne tanks were inoculated with rotifers with a density of 200 numbers/ml and were fed with *C. calcitrans* and *N. oculata* in two tanks each. Commercial water probiotic, with a concentration of 10 ml in 1 tonne water, was applied daily. The study revealed a significant improvement in the quality of rotifer, in terms of *Vibrio* loads of the rotifer. *Vibrio* loads of probiotic fed tank was  $0.18 \times 10^2$  cfu/g and in control load was  $1.8 \times 10^1$  cfu/g in *C. calcitrans* tanks. No *Vibrios* were detected in the tank fed with *N. oculata* along with probiotics. The role of *Vibrios* in live feed culture plays a significant role in terms of quality as well as quantity of rotifers.

The study also revealed that the total vibrio loads of water and rotifers were very high in *C. calcitrans* fed tanks, followed by tanks fed with combined diet *N. oculata* and *I. galbana*. It was observed that the diversity of the bacterial flora of rotifers is dependent on the flora of the rearing water [7] and *V. alginolyticus* was recorded as the most dominant

**Table 1 Correlation between Total Vibrio loads of water, microalgae and rotifers.**

	<i>C. calcitrans</i>	<i>N. oculata</i>	<i>I. galbana</i>	<i>C. vularis</i>	<i>N. oculata</i> + <i>I. galbana</i>
Rotifers	0.532905	0.925928044	0.640333	0.58132	0.7347786
Water	0.617241	0.520991353	0.81993	0.571306	0.507238

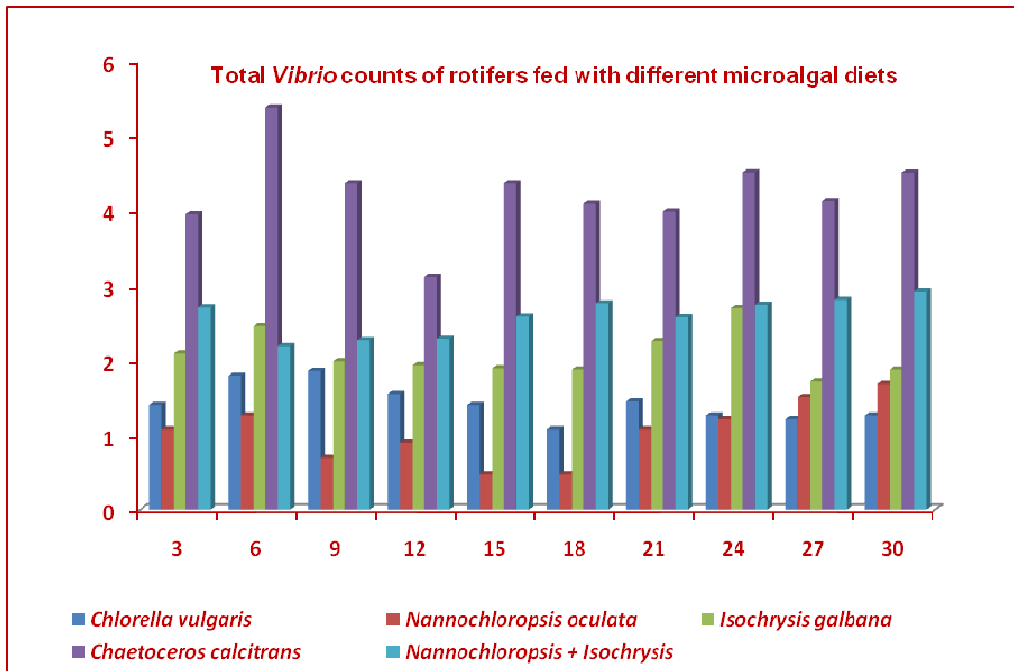


Fig. 2 Total *Vibrio* loads of rotifers fed with different microalgal diets.

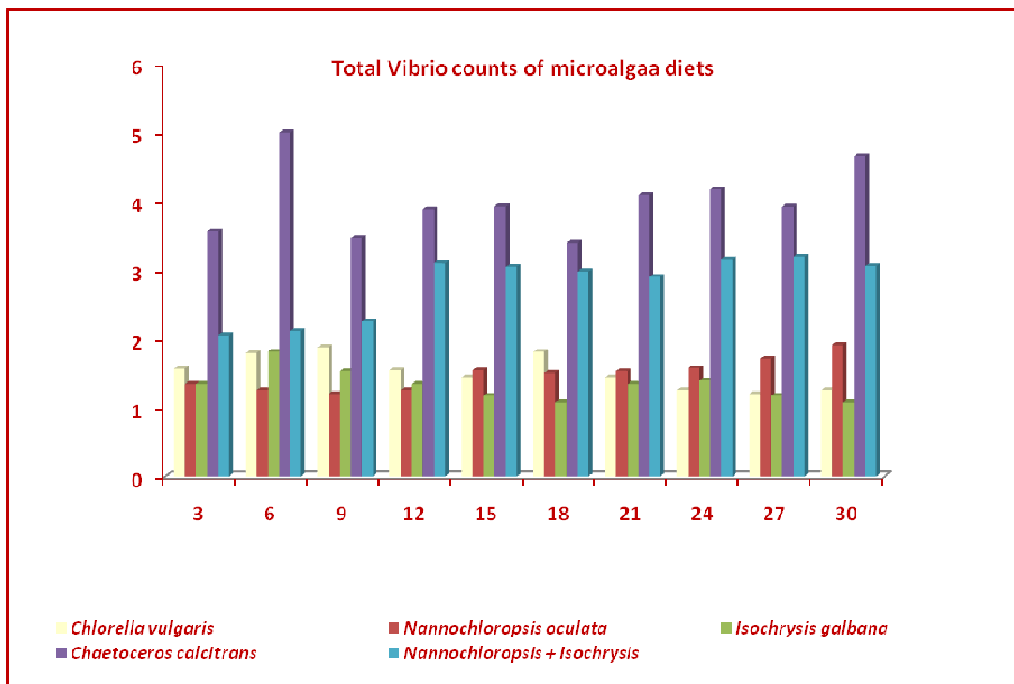


Fig. 3 Total *Vibrio* loads of microalgal diets fed to the rotifers in mass culture tanks.

microflora of rotifers, which produce a fatal exotoxin for *B. plicatilis* [8]. In contrary, *V. anguillarum* isolated and identified as the major dominant species in rotifer cultures but there was no record of mortalities due to this species [8]. High loads of total bacteria ranging between  $0.9 \times 10^8$  and  $56.6 \times 10^8$

cfu/g were recorded [4] from rotifers in marine hatchery. It is hypothesized that the bacteria can be transferred either directly through seawater [10] or by ingestion [10-12]. Although majority of earlier studies indicated that bacteria are not pathogenic, the occurrence of fish larvae could be related to the

accumulation of large quantities of these non pathogenic microbes. Many reports suggested that reduction in microbial loads can increase the larval survival in marine finfish hatcheries [13]. In the present investigation, it was clearly observed a reduction in the density of rotifers in those tanks with *Vibrio* loads, which may be a possible cause of algal crash and increase of ammonia levels in water, which in turn triggers the reduction of rotifer densities. Preliminary study on the effect of probiotics on the elimination of *Vibrio* loads, in the present investigation, also indicated a need to develop an alternate useful live organism for the elimination of contamination in mass culture systems.

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