

Macrobrachium rosenbergii (DE MAN) CULTURE IN POLYNESIA:
OBSERVATIONS ON THE WATER CHEMODYNAMISM IN AN INTENSIVE LARVAL REARING

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ABSTRACT

In order to specify the limits of the high density larval rearing method (80-100 larvae per liter) in clear water with daily total exchange and to control the water quality evolution, the nitrite and total ammonia were measured.

Nitrite concentrations in antibiotic treated tanks stayed low ($5 \mu\text{g NO}_2\text{-N/l}$) but can reached $40 \mu\text{g NO}_2\text{-N/l}$ after 41 days in untreated tanks, in spite of daily water exchange.

Maximum concentrations of total ammonia were 1.70 ppm N during a 24-hour cycle. In the rearing conditions (pH 7.85 to 8.20, temperature 25 to 28 C) the un-ionized ammonia concentrations were 0.07 to 0.18 ppm N. At those levels, no specific larval stress was noted.

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The interaction of variations in ammonia and nitrite concentration versus, antibiotics, temperature, light intensity, larval stages, diseases, was studied. Ammonia variations, resulting from the larval metabolism, could be a useful indicator for detecting the beginning of bacterial diseases.

INTRODUCTION

In order to develop production of *M. rosenbergii* in French Polynesia, a larval rearing method in high density (100 larvae per liter), clear water, with a total daily water exchange, is in progress at the "Centre Océanologique du Pacifique" (Aquacop, 1977a). Water quality control is of prime importance for such rearing. In clear water, it is necessary to know the range of variations in nitrous and ammoniacal nitrogen, between two water changes, so a toxicity level could be reached.

Ammoniacal nitrogen in solution is a product of mineralization of organic matter (Spotte, 1970) and a waste product of aquatic invertebrates (Delaunay, 1931). It is found either as un-ionized (NH_3) or as ionized (NH_4^+). The relative proportions of these two forms depend on the pH, the ionic strength and the temperature of the solution. The un-ionized form is the most toxic as it is cellular-permeable. Its toxicity, reported for numerous aquatic animals (Downing and Merckens, 1955; Burrows, 1964; Wicking, 1976; Cohen et al., 1976; Colt and Tchobanoglous, 1976), depends on temperature (Burrows, 1964), pH (Warren, 1962; Warren and Schenker, 1962), salinity (Sousa, 1974), and level of dissolved oxygen (Downing and Merckens, 1955).

Nitrous nitrogen, transitory product of the nitrification of ammonia nitrogen by aerobic chemoautotrophic bacteria (Stanier et al., 1966), is a less violent poison than ammonia. Its toxicity has been checked for numerous marine and fresh water animals (Russo et al., 1974; Wickins, 1976; Colt and Tchobanoglous, 1976; Armstrong, 1976). In solution, nitrite (NO_2) generates nitrous acid (HNO_2). This equilibrium also depends on pH and temperature. Colt and Tchobanoglous (1976) state that nitrite toxicity is probably related to the proportion of nitrous acid in solution.

This study focuses on the monitoring of nitrous and ammoniacal nitrogen concentrations throughout several larval generations. Some biological factors responsible for the evolution of nitrite and ammonia can be correlated with a high density culture method.

MATERIALS AND METHODS

The larvae were reared in 800 liter cylindro-conical polyester tanks with clear water. Larval density was 80-100/liter.



Salinity was kept at 8 or 12 ppt depending on the larval stages. Aeration was supplied by four air-stones set at the center of the tank. The air-flow varied between 1.5 and 2.6 m³/h/tank, keeping the dissolved oxygen concentration between 6.6 and 7.2 ppm (90 to 100% saturation): Nauplii and frozen adult *Artemia* and skipjack meat were given. The proportions of these three foods changed, depending on the larval stages. Larval stages were monitored daily and their evolution was expressed in relation to the LSI (Larval Stage Index) defined by Maddox and Manzi (1976). The LSI was determined from 60 to 80 animals. Larvae were then dried at 105 C for 15 min, cooled in a dessicator for 10 min, and weighed (0.1 mg accuracy). At the beginning of the rearing, water was totally renewed every day at 15.00. For stages 9-10 water was also sometimes renewed at 09.00.

Subsamples for nitrite and ammonia analyses were taken to measure nitrite and total ammonia at 08.00, 12.00 and 15.00. The samples, filtered through 48 μ plankton mesh, were preserved in closed polyethylene falsks, in darkness, at +4 C and analysed daily.

Ammoniacal and nitrous nitrogen were determined colorimetrically using phenol-hypochlorite for ammonia and diazotation with sulfanilamide for nitrites (Strickland and Parsons, 1972). Nitrous nitrogen data are expressed in μg NO₂-N/l. In the observed range of concentrations, with the slightly alkaline pH, the proportion of nitrous acid is low and has not been taken into account.

Ammoniacal nitrogen data are expressed either in ppm of total ammonia nitrogen (NH₃ + NH₄⁺) or in total ammonia enrichment rate of the water (V_{NH₄} mg N/m³/h). The evolution of the concentrations is defined therefore in relation to time and for one cubic meter of water:

$$V_{NH_4}/m^3/h : \frac{\Delta [(NH_3) + (NH_4^+)] \text{ ppmN}}{\Delta t \text{ hours}} \times 10^3$$

Diurnal (08.00 to 15.00) and nocturnal rates (15.00 to 08.00) were calculated.

The molar fraction of un-ionized ammonia was estimated using the following formula :



$$K_{NH_3} = \frac{(OH^-) (NH_4^+)}{(NH_3)} \quad (2) \quad pK_{NH_3} = 4.767 - 0.0027 (T-20)$$

$$F_{NH_3} = \frac{(NH_3)}{(NH_3) + (NH_4^+)} \quad (3) \quad pKw = \frac{4470.99}{T + 273.16} - 6.0875 + 0.01706 (T+273,16)$$

(1) Colt and Tchobanoglous (1976)

(2) Colt and Tchobanoglous (1976) (after Bates and Pinching, 1950)

(3) Harned and Owens (1958)

In our experiments the un-ionized ammonia percentage (0.2-0.5% to the maximum) is not affected by minor fluctuations in salinity.

The monitoring of ammonia and nitrite was done on two rearing series (Table 1). A separate experiment was made to determine the relative proportion of ammonia produced by *Artemia nauplii* excretion and break down of dead food proteins. For these determinations the total ammonia concentrations were monitored over the period between two total water exchanges, in tanks that were maintained as rearing tanks but without larvae (Table 1).

RESULTS

In the tanks treated with antibiotics, the nitrite concentrations remained low. After 40 days they attained a maximum of 5 micrograms of nitrous nitrogen. In the untreated tank, the concentrations also remained low, till the 35th day, then suddenly increased to 39 micrograms $\text{NO}_2\text{-N/l}$ the 41st day, in spite of daily water exchanges (Figure 1).

The maximum concentrations of total ammonia measured on a 24-hour cycle was 1.60 to 1.70 ppm. In the rearing conditions (pH 7.85 to 8.20, temperature 25 to 28.5 C) the proportion of un-ionized ammonia is then 3.9 to 10.4%, equivalent of 0.07 to 0.18 ppm of toxic $\text{NH}_3\text{-N}$ (Figure 2). At this concentration no particular stress was observed that could be related to toxicity. The level of total ammonia changed with the LSI during a 24-hour cycle (Figure 3). The different range of variations of V_{NH_4} mg $\text{N/m}^3/\text{h}$ appeared to depend on the metabolism of the larvae which is dependent on light intensity, temperature, larval stage, amounts of food given and pathological condition.

Ammonia levels in the rearing water were related to the light intensity. The concentration increased more rapidly in the daytime than at night (Table 3). Tank kept in penumbra presented low daytime enrichment rates compared to submitted daylight tanks (Figure 4; Table 2).

The Man and Witney test points out a significant difference at the 5% level between series no. 1 (T = 27.5-28.5 C) and series no. 2 (T = 24.9-26.3 C) for the ammonia enrichment rates relative to the same LSI (Figure 5).

The variations in ammonia enrichment rates have been correlated with the different larval stages (Figure 6). At the 1% significance level, the Man and Witney test does not show difference between the two tanks. From stage 5 an increase of enrichment rate in total ammonia is recorded with a peak between stages 8 and 10.

The larvae were fed such that the amount of food depended on the biomass (Aquacop, 1977a). There is correlation at the 0.001%

significance level between the daily variations of total ammonia concentration and food given (Figure 7). The enrichment rates produced only by *Artemia* nauplii and proteins breakdown from the food, in control tanks. In these cases the enrichment rates (2.6 to 6.6 mg NH₄ - N/m³/h) were lower than those noted in the standard larval rearing tanks (Table 4). The ammonia variations are thus more in direct relation with consumption than with food protein breakdown or *Artemia* nauplii excretion.

Synchronised moltings, detected by a sudden increase of the larval mean weight, seemed related to a decrease of the enrichment rates (Figure 8).

The evolution of total ammonia was different in tanks where bacterial disease occurred (Figure 9). The level was quite similar up to 6.8-7.0 LSI in the two tanks. At this point it leveled out, and suddenly decreased on the day before the death of the whole batch. Bacteria were seen under microscope only on May 1st, whereas the decrease of total ammonia had already occurred.

DISCUSSION

The nitrite concentrations monitored in the high density clear water culture with a daily total water exchange were low and do not seem to be a cause of stress or mortality. The lethal amounts are much higher than the measured concentrations. For 14 day old larvae, Armstrong et al. (1976) mentioned LC50 for 24 hours, with about 70 ppm NO₂-N, and Wickins (1976), experimenting with juveniles, recorded LT50 of 880 min for concentrations of 204 ppm NO₂-N. In the tank without antibiotics, the sudden increase of nitrites after 35 days could have been provoked by the establishment of a nitrifying bacterial population. Srna (1975) recorded a maximum of activity of a bacterial population in a sand filter after 40 days. In the treated tanks the nitrifying bacteria may have been inhibited by antibiotics. Lees (1951) mentioned concentrations of 20 µg/ml NO₂-N after 11 days in a *Nitrosomonas* culture treated with 0.4 ppm of streptomycin while in the nontreated culture, the concentration is 280 µg/ml NO₂-N. The source of nitrifying bacteria remains to be determined since the water was totally exchanged daily. They could have attached to the tank walls or on the larvae themselves.

Under the experimental conditions, the maximum concentrations of total ammonia recorded at the end of a 24-hour cycle (1.6 to 1.7 ppm N maximum) corresponded to 0.18 ppm toxic NH₃-N. Wickins (1976) indicated for *M. rosenbergii* juveniles a significant decrease of growth, after 6 weeks, for concentrations above 0.10 ppm NH₃-N. In our larval rearing tanks, the enrichment rate in total ammonia rose to a maximum value of 114 mg N/m³/h during the day and 66 mg N/m³/h during the night. Thus, the animals were submitted only during 7 or

8 hours a day to un-ionized ammonia concentrations above 0.10 ppm $\text{NH}_3\text{-N}$. During the molt, when water absorption is high, the larvae are the most sensitive to toxicity. Since molting occurs during the night, the water was changed in the evening; and thus the larvae molted when the ammonia concentrations were low. Under these conditions no delays were reported in the progression of stages. However, it is possible that, these sublethal concentrations increase the sensitivity of larvae to mechanical and thermal stresses and bacterial infestations.

The amount of total ammonia produced by the *Artemia* nauplii excretion was low compared to reported values (Cohen et al., 1976). Similarly the breakdown of dead food protein results was only 6 to 7 mg of total ammonia per m^3 per hour. The aeration intensity in the rearing tanks did not significantly change the ammonia concentrations. From Colt and Tchobanoglous (1976), 5,000 hours of aeration at the rate of 2 m^3 /hour will be necessary to eliminate ammonia from the 800-liter larval rearing tank. For these reasons the variations of total ammonia represent the larval metabolism.

Without light energy the larvae presented a reduced metabolism and the excretion rate was low. Sick and Beaty (1974) reported a decrease in oxygen consumption for *Macrobrachium* larvae kept in the darkness. It is also likely that animals had some problems catching food particles (Aquacop, 1977a). On the other hand, it is possible that the increase of the photoperiod could shorten the larval rearing duration.

The concentrations of ammoniacal nitrogen were certainly related to the influence of temperature upon metabolism. Botsford et al. (1975) have defined a metabolite production for the lobster as $\text{dM}/\text{dt} = \text{KM W}^6 \text{A}$. A is a factor related to temperature. Similarly Guerin-Ancey (1976) recorded for a teleostean fish (*Dicentrarchus labrax*) an increase of more than 100% of ammonia excretion for a temperature rise of 2 C. Whether the higher temperature of the warm season (29.5-30 C) will induce higher ammonia levels, thus becoming a limiting factor for a high density method should be determined.

The low concentrations of ammonia nitrogen recorded up to stage 5 prove that during this period larval density could be increase (Figure 6). Furthermore it was noted in the tanks submitted to low light conditions, where the food was not well consumed, that growth is the same as the others until stage 5 and then stopped (Aquacop, 1977a). For stage 5 to stage 10 the enrichment rates of total ammonia were proportional to biomass increase. For stages 11 and post-larvae the recorded decrease did not appeared related with metabolic change but could be explained by the mortality at metamorphosis.

The sudden fall in ammonia concentrations recorded in Figure 9 had started three days before the bacterial necrosis disease (Aquacop,

1977b) was noticed. Recent data from the pilot hatchery indicate that an abnormal decreasing in ammonia concentration is rapidly followed by a stagnation of the LSI and by the appearance of dead animals.

CONCLUSION

The monitoring of nitrous and ammoniacal nitrogen concentrations throughout several larval rearings of Macrobrachium rosenbergii with high densities and in clear water, has pointed out that only total ammonia concentrations are important. Nitrite produced does not appear to be a limiting factor for this method. The variations of total ammonia concentrations are during a 24-hour cycle in close relation with the metabolism of the larvae and could be a good diagnosis of the larval development in the next days.

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Table 1. - Experimental conditions for the two larval rearing series and the three controls
(*Macrobrachium rosenbergii*, 800 liter-tank)

Tanks	Series 1 April-May 1976				Series 2 June-August 1976				Controls		
	1	2	3	4 5	A	B	C	D	C ₁	C ₂	C ₃
Daylight	+	penumbra	+	+	+	+	+	+	+	+	+
Antibiotics	+(1)	+	+	+	-	cur.(3)	prev.(2)	-	cur.	-	-
Chlorinated (4) water	-	-	-	-	-	+	+	+	-	-	-
Filtered water (1 μ)	+	+	+	+	+	-	-	-	-	-	-
Temperature (C)	— 27.5 - 28.5 —				— 24.9 - 26.3 —				26-27	25.5-26.6	27-28
pH	— 7.90 - 8.20 —				— 7.85 - 8.15 —				8.05	8.15	8.20
Salinity (ppt)	— 8 - 12 —				— 8 - 12 —				12	12	12
<i>Artemia</i> nauplii (n/ml)	— 5 —				— 5 —				0	5.0	3.0
Frozen adult <i>Artemia</i> wet weight (g)	— 20 to 150 —				— 20 to 150 —				250	0	0
Initial densities (larvae/l)	— 80 to 100 —				— 80 to 100 —				0	0	0

- (1) Furanace, Erythromycine phosphate, Streptomycine-bipenicilline: 1.00 to 2.50 ppm
 (2) preventive: Streptomycine-bipenicilline: 1.25 to 2.50 ppm every two days
 (3) curative: Streptomycine-bipenicilline: 5.0 ppm
 (4) Chlorination with sodium hypochlorite: 1.5 ppm total chlorine (0.30 ppm HClO at pH 8.20)
 (Residual chlorine elimination before utilisation)

Table 2. - Daily changes during 7 days in diurnal enrichment rate of total ammonia mg N/m³/h:
Larval Macrobrachium rosenbergii rearing, series 1

Tanks Daylight	1 +	2 penumbra	3 +	4 +	5 +
Date 04/27	76.1	8.6	86.2	97.8	82.0
28	64.2	7.6	110.4	136.0	97.9
29	86.0	16.0	88.1	100.2	82.3
30	58.4	6.8	94.0	109.3	58.4
05/01	34.5	6.8	61.9	79.0	67.8
02	42.1	6.4	81.8	92.5	76.2
03	10.2	6.0	88.1	87.3	60.1

Table 3. - V_{NH₄} values for the tank A during 27 days from 03 PM to 08 AM and from 08 AM to 03 PM

Date	V _{NH₄} mg N/m ³ /h		Date	V _{NH₄} mg N/m ³ /h		Date	V _{NH₄} mg N/m ³ /h	
	3PM-08AM	08AM-3PM		3PM-08AM	08AM-3PM		3PM-08AM	08AM-3PM
07/5	23.2	41.2	14	35.2	-	23	48.4	80.1
6	15.1	50.1	15	27.1	80.4	24	58.2	90.1
7	22.6	20.1	16	16.1	61.5	25	24.6	82.4
8	15.4	30.3	17	23.3	53.8	26	30.7	114.5
9	17.2	29.5	18	25.3	75.2	27	20.3	71.0
10	19.2	30.6	19	27.5	50.1	28	20.5	70.3
11	20.1	30.4	20	23.4	55.4	29	25.8	31.3
12	10.5	64.7	21	24.1	68.6	30	66.1	62.1
13	17.8	47.2	22	21.2	91.2	31	58.2	70.4

Table 4. - Changes in total ammonia in the three control tanks

Time (hours)	Control 1 Frozen <i>Artemia</i> 205 g		Control 2 <i>Artemia</i> nauplii (5/ml)		Control 3 <i>Artemia</i> nauplii (3/ml)	
	Total ammonia	V _{NH₄}	Total ammonia	V _{NH₄}	Total ammonia	V _{NH₄}
	ppm N	mg N/m ³ /h	ppm N	mg N/m ³ /h	ppm N	mg N/m ³ /h
0	0.000		0.000		0.000	
6			0.024			
24	0.160	6.6		4.0	0.066	2.6
30	0.184	6.1	0.120	4.0	0.097	3.2

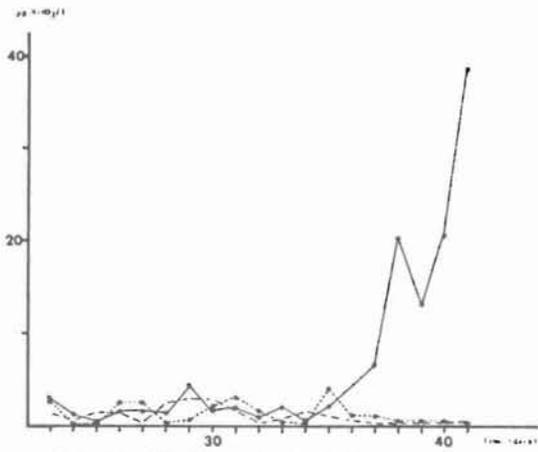


Fig. 1 - Ammonia evolution (200 - 40) in day in 2 rearing tanks. Tank 1 (solid line) and tank 2 (dashed line) treated with antibiotics. Tank 1 (solid line) non-treated.

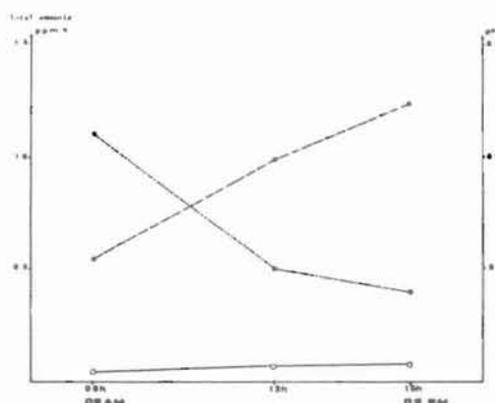


Fig. 2 - Changes in total ammonia (ppm N), pH and un-ionized ammonia (ppm) in a larval rearing tank, during one day (tank 4 section 1 L21 - T2) T = 28°C

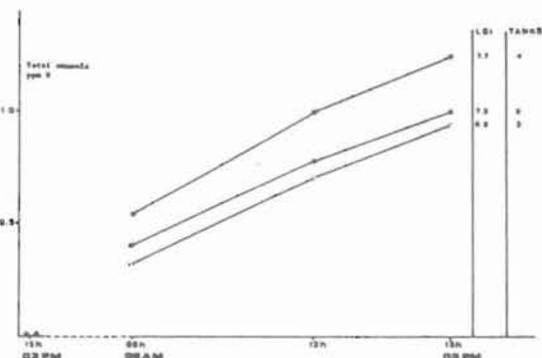


Fig. 3 - The change in total ammonia concentration during 1 day in 3 rearing tanks with same density but different LEI

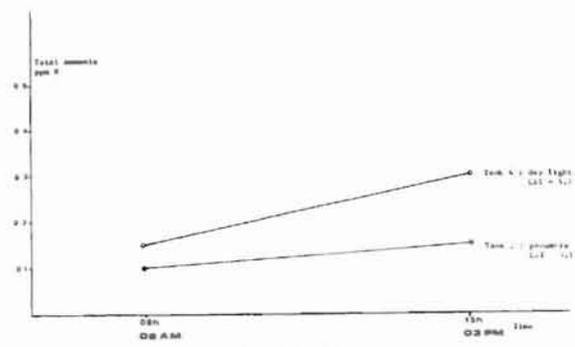


Fig. 4 - Total ammonia evolution during one day in two rearing tanks with the same densities but different lighting conditions.

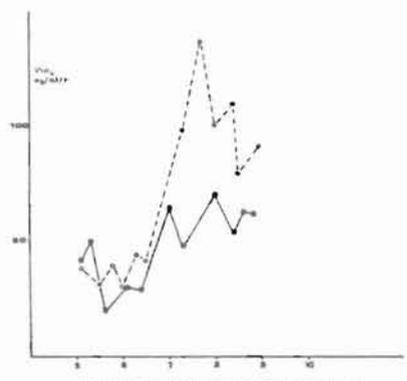


Fig. 5 - NH₃ (ppm) (one test) change for tank 1 (solid line) section 2 (T = 24°C - 26°C) and tank 2 (dashed line) section 1 (T = 27°C - 28°C).

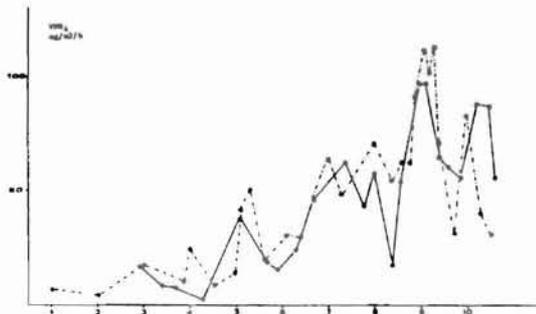


Fig. 6 - VPM₁ (mg/24h) changes during 2 larval rearing under identical conditions. Tank 1 (n=40), Tank 2 (n=40)

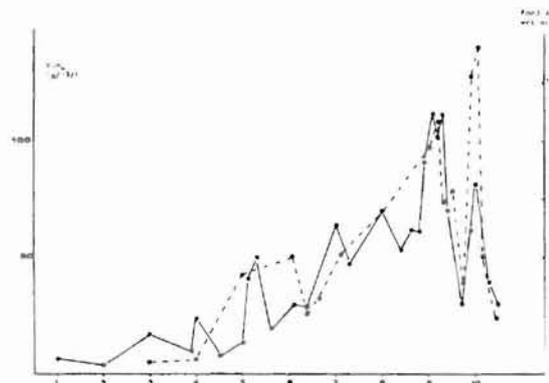


Fig. 7 - Daily food amount changes (mg/24h) and VPM₁ (mg/24h) vestations during a larval rearing (n=40) (Tank 3)

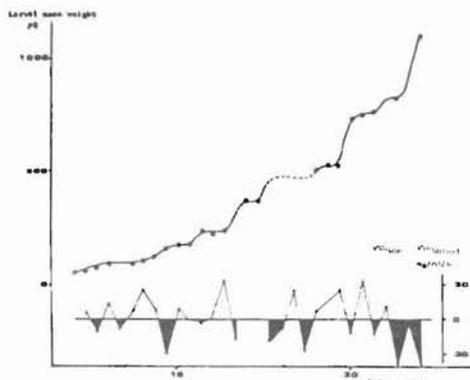


Fig. 8 - Larval mean weight gain and VPM₁ (mg/24h) vestations from day 10 to 30th day (Tank 1)

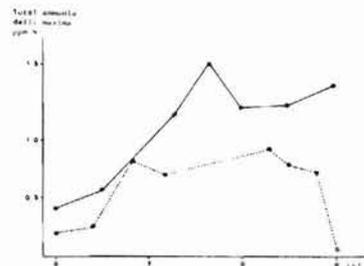


Fig. 9 - Daily changes in total immune concerning 2 rearing tanks. Tank 4 (n=40) with normal evolution. Tank 3 (n=40) where bacterial diseases observed (Increase revealed by microscopical observations only at day 8-9)