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ROLE OF MICROALGAE IN CONDITIONING WATER IN PENAEID LARVAL CULTURE.

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ABSTRACT: Microalgae plays an important role in conditioning water quality for penaeid larval culture. Recently it has been demonstrated that a modification of the 'green water' larval culture system (Ling,1969) for *Macrobrachium* allows the production of postlarvae without any water change, despite extensive use of artificial feeds (Ang and Cheah, 1986). Increase of toxic metabolites such as ammonia and nitrite are also common in penaeid larval culture, especially where excessive amounts of artificial feeds are employed. Present work examines the use of six marine microalgae (*Skeletonema costatum, Chaetoceros calcitrans Rhodomonas baltica Pavlova lutheri, Tetraselmis chuii* and *Chlorella japonica*) at four cell concentrations (500, 1000, 1500, 2000 cells μl^{-1}) as a 'biological filter' system, to control and detoxify levels of ammonia and nitrite in *P. monodon* larval culture water whilst using artificial diet. Preliminary results indicate that amongst the six algal species tested, *C. japonica* at 1000 cell μl^{-1} was most effective in reducing accumulated toxic metabolites from an unchanged culture water environment.

KEY WORDS: Microalgae - ammonia - nitrite - shrimp larval feed trial.

INTRODUCTION

Apart from supplying all the nutrients essential for early larval development it has been suggested that microalgae may play an important role in conditioning water quality for penaeid larval culture.

Recently it has been demonstrated that enhancement of the 'green water' larval culture system (Ling, 1969) for *Macrobrachium rosenbergii* allows the production of postlarvae without any water change, despite extensive use of artificial diets (Ang and Cheah, 1986). Ammonia and its intermediate product nitrite, are common toxicants in shrimp hatcheries. The accumulation of ammonia in culture water is due to protein catabolism, nitrogenous excretion by crustaceans (Hochachka and Somero, 1973) and by the breakdown and nitrification of uneaten organic matter (artificial diets) added as food. Between 40 to 90% of the nitrogen eliminated by crustaceans is ammonia (Parry, 1960), and is generally considered as a major cause of mortality in nonconditioned recirculatory cultrue systems (Spotte, 1970). In a cnoditioned system, ammonia stimulates nitrifying bacteria which first oxidize liberated ammonia and convert it into nitrite (NO_2) by *Nitrosomonas*. Nitrite is oxidized again to relatively non-toxic nitrate (NO_3) by *Nitrobacter*.

Total ammonia nitrogen includes nitrogen in both ionic states: ionised [NH_4^+], and unionised [NH_3]. Unionised ammonia is considered the principal toxic form as the free base [NH_3] has a relatively high solubility because it carries no charge, and is therefore able to diffuse readily across cell membranes in the gills (Fromm and Gillette, 1968). The ionised ammonia on the other hand penetrates these membranes less readily since it is charged and hydrated and consequently has low lipid solubility (Whitfield, 1974). Ionised and unionised ammonia exist in an equilibrium state [$NH_4^+ \rightleftharpoons NH_3$] in water. Factors which influence shifts between these two ionic states include temperature, salinity, and pH (Wickins, 1976). A shift of one unit (7

to 8) will increase NH_3 levels ten fold (Armstrong *et al.*, 1978). It is likely that ammonia interferes with the ability of hemocyanin to carry oxygen in blood. Nitrite oxidises the copper of crustacean hemocyanin and produces a detrimental effect on the growth and development of shrimp and their larvae (Chen *et al.*, 1986). *Penaeus monodon* larvae are commercially reared in static water hatcheries in Taiwan (Liao, 1977). Accumulation of ammonia and nitrite in these static systems adversely affects the survival of larvae. Wickins (1976), and more recently Chen and Chin (1987) and Chin and Chen (1988), have demonstrated the toxicity of both ammonia and nitrite to penaeid larvae such as *P. monodon*, and measured accumulation of these toxicants in hatcheries. Toxicity levels for NH_4^+.N, NH_3.N and NO_2.N affecting prawn culture are listed in Table I.

Table I: Toxicity levels of NH_4^+.N, NH_3.N and NO_2.N for penaeid prawns

Species	Larval stage	Toxin	Exposure		Result	Author
			Concentration $\mu g.l^{-1}$	Duration		
P.japonicus	Juveniles	NH_3.N	0.37	3 weeks	Growth reduced by 50% of that of the control	Wickins, 1976
P.occidentalis	Juveniles	NH_3.N	0.40	3 weeks	Growth reduced by 50% of that of the control	Wickins, 1976
P.schmitti	Juveniles	NH_3.N	0.69	3 weeks	Growth reduced by 50% of that of the control	Wickins, 1976
P.semisulcatus	Juveniles	NH_3.N	0.22	3 weeks	Growth reduced by 50% of that of the control	Wickins, 1976
P.setiferus	Juveniles	NH_3.N	0.59	3 weeks	Growth reduced by 50% of that of the control	Wickins, 1976
P.indicus	Juveniles	NO_2.N	6.4	34 days	Growth reduced by approx. 50%	Wickins, 1976
P.monodon	Larvae	NO_2.N	8.5	96 hours	LC_{50}	Colt and Armstrong, 1981
P.japonicus	Juveniles	NO_2.N	0.1-0.6	9 months	Mortality between 5-33%	Mevel and Chamroux, 1981
P.indicus	Larvae	NH_4^+.N	3.5	24 hours	LC_{50}	Jayasankar and Muthu, 1983a
P.indicus	Larvae	NO_2.N	1.8	9 days	50% larvae did not metamorphise into post larvae	Jayasankar and Muthu, 1983b
P.monodon	Post larvae	NO_2.N	0.11	21 days	4% survival	Chen *et al.*, 1986
P.monodon	Larvae	NH_3.N	0.10	96 hours	LC_{50}	Chin and Chen, 1987
P.monodon	Larvae	NO_2.N	0.11	96 hours	LC_{50}	Chen and Chin, 1988
P.monodon	Larvae	NO_2.N	0.23	13 days	20-40% survival to PL stage	Present study

Present work examines the ability of six algal species, *Skeletonema costatum* (Greville), *Chaetoceros calcitrans* (Paulsen), *Rhodomonas baltica* (Karsten), *Pavlova lutheri* (Droop), *Teteraselmis chuii* (Butcher) and *Chlorella japonica* (Shihara and Krauss) at four levels of cell concentrations, 500, 1000, 1500, and 2000 μl^{-1} to act as "biological filter" system by controlling the levels of ammonia and nitrite in *P. monodon* larval cultures fed with artifical diets.

MATERIALS AND METHODS

All experiments were conducted in 2 litre round bottom flasks containig aerated ultra violet sterilised seawater at 28±1°C at a salinity of 32 °/oo. Continuous illumination of $6.6x10^{15}$ quanta sec^{-1} cm^2 was maintained inside the flask by suspending a light source directly above the culture flask. A portable laboratory Qunatum Scalar Irradiance meter model QSL-100 was used to measure light.

PRODUCTION OF AMMONIA

Ammonia was produced in a round bottom culture flask by adding an artificial diet, at the rate of 4 mg. $litre^{-1}$ day^{-1} (PZ1 to PZ3) for 6 days and 8 mg. $litre^{-1}$ day^{-1} (M1 to PL1) for the next 4 days, with no water change and in the absence of larvae and microalgae. This feed level mimics actual amounts normally fed to *P. monodon* larvae. Ammonia and nitrite accumulate as a result of breakdown of feed components, and levels of each were measured on alternate days. Ammonia (NH_4^+.N) was measured by the phenolhypochlorite methods (Solarzano, 1969) and the unionised ammonia (NH_3.N) calculated from Bower and Bidwell (1978). Nitrite (NO_2.N) was measured from procedures described by Bendschneider and Robinson (1952). pH was measured with a WPA instrument CD 300. On day 11 the total volume of water in the culture flask was filtered through a Whatman filter paper (No.4) to remove remaining fragments of feed. The filtered water was returned to clean flasks and algal species were introduced separately at 4 cell concentrations.

ALGAL CULTURES

Non-axenic algal cultures of *S. costatum, C. calcitrans, P. lutheri, T. chuii, R. baltica* and *C. japonica* maintained in exponential phase in Conway medium (Walne, 1966) were harvested and centrifuged at 2000 rpm. for 3 min. to remove algal metabolites. The supernatant was decanted and algae resuspended in fresh seawater. Cell counts were made using a Coulter Counter model ZB and hemocytometer. Four cell concentrations (500, 1000, 1500 and 2000 μl^{-1}) of each of the 6 algal speceis were introduced separately, into culture flasks containing a known concentration of ammonia and nitrite liberated from the artificial diet.

In another set of experiment, cells of *Chlorella* (500, 1000, 1500, 2000 μl^{-1}) were retained in a hollow transparent cylinder measuring 100 mm in length and 20 mm in diameter, the top and bottom ends were covered by a 5 micron mesh cloth to prevent cells escaping into the culture medium. The cylinder containing algae was suspended vertically in the culture flask, so that water could flow through the cylinder driven by aeration to encourage water circulation. All other experimental conditions were similar.

Reduction in the levels of ammonia and nitrite concentrations were measured for each of the 6 algal species at 4 concentrations after 24 and 48 hours respectively. All experiments were replicated.

RESULTS

AMMONIA PRODUCTION FROM THE ARTIFICIAL DIET

Total ammonia liberated from rehydrated artificial larval diet, together with nitrite levels, measured under culture conditions at 2 day intervals for 11 days are shown in Fig.1 along with control values. Ammonia (NH_4^+.N) levels steadily increased from 222 $\mu g.l^{-1}$ on day 3 to 2116 $\mu g.l^{-1}$ on day 11. Unionised ammonia (NH_3.N) also increased from 11.81 $\mu g.l^{-1}$ to 139.86 $\mu g.l^{-1}$. Nitrite (NO_2.N) levels ranged from 21.62 to 314.7 $\mu g.l^{-1}$ and pH values remained between 7.96 to 8.1. In the blank controls, levels of NH_4^+.N were 15 to 18.4 $\mu g.l^{-1}$, NH_3.N ranged from 0.61 $\mu g.l^{-1}$ to 1.2 $\mu g.l^{-1}$, NO_2.N measured between 7.3 to 8.55 $\mu g.l^{-1}$ and pH values were 7.3 to 8.1.

ASSIMILATION OF AMMONIA AND NITRITE BY ALGAE

Six algal species; *S. costatum, C. calcitrans, P. lutheri, T. chuii, R. baltica, C. japonica* (free floating) and *C. japonica* (trapped) were added to the water containing ammonia and nitrite from the above experiment at 500, 1000, 1500 and 2000 cells μl^{-1} in a separate experiment to ascertain their affect upon ammonia and nitrite. Algae reduced toxicant levels to within recommended safe limits (Table I) by assimilating total ammonia and nitrite from culture medium after 24 and 48 hours. The effect of the 4 algal cell densities 500 μl^{-1}, 1000 μl^{-1}, 1500 μl^{-1}, 2000 μl^{-1} upon ionised and unionised ammonia and nitrite concentrations in culture water was ascertained and is given in Tables II, III, IV and V.

PENAEUS MONODON LARVAL FEED TRIAL ON ARTIFICIAL DIET WITH *C. JAPONICA*

Results showed that *C. japonica* at 1000 cells μl^{-1} was most effective in reducing the toxic levels of ammonia and nitrite in the culture medium. An experimental feed trial was set up using *P. monodon* larvae (PZ1-PL1) fed on an artificial diet in the presence of free floating and trapped *C. japonica* at 1000 cells μl^{-1}. Culture water was not changed during the experiment. *Penaeus monodon* larvae fed on the artificial diet mixed with free floating *C. japonica* collapsed at larval stages PZ2/PZ3 on day 7 of the feed trial. Ammonia and nitrite levels in culture water were 81.6 and 18.4 $\mu g.l^{-1}$. Larval mortality resulted from ingestion of free floating *C. japonica* cells by the protozoeal stage larvae. *Chlorella jcponica* is considered to be a poor food for crustacean larvae due to its thick cell wall. *Penaeus monodon* larvae on the artificial diet in the presence of trapped *C. japonica* reached the intermediate postlarval stage with larval survival ranging between 20-40% on day 13, (Fig.3). Ammonia and nitrite levels in the culture water were 1200 $\mu g.l^{-1}$ and 230 $\mu g.l^{-1}$ respectively. Low survival and larval growth was due to inadequate nutrition for larval development provided by the artificial diet in the absence of an algal (10 cell μl^{-1}) supplement (Amjad and Jones, 1992). Larval development on a control diet containing microalgae at 20 cells μl^{-1} of

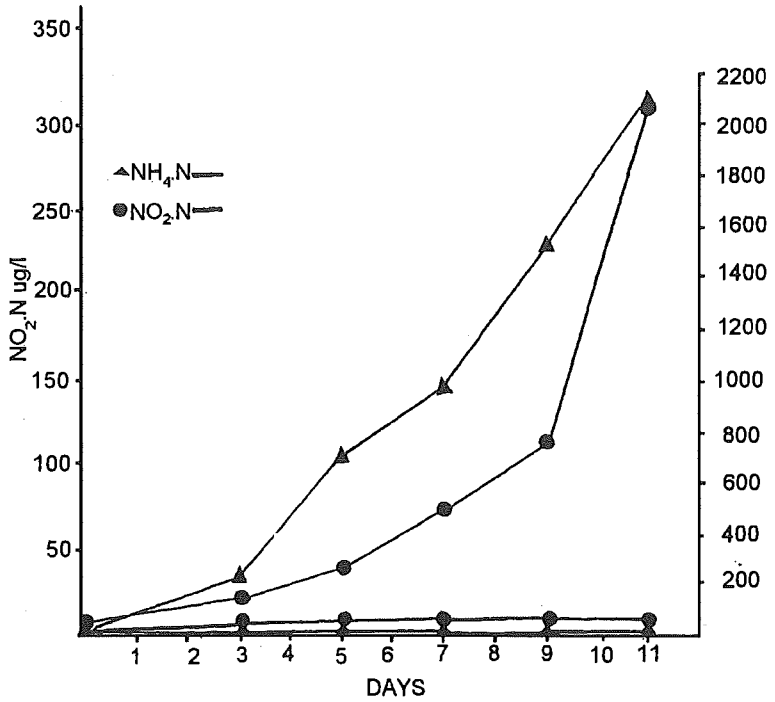

Fig.1. Accumulation of total ammonia and nitrite level upon breakdown of artificial diet measured in culture water at 2 days interval with control values.

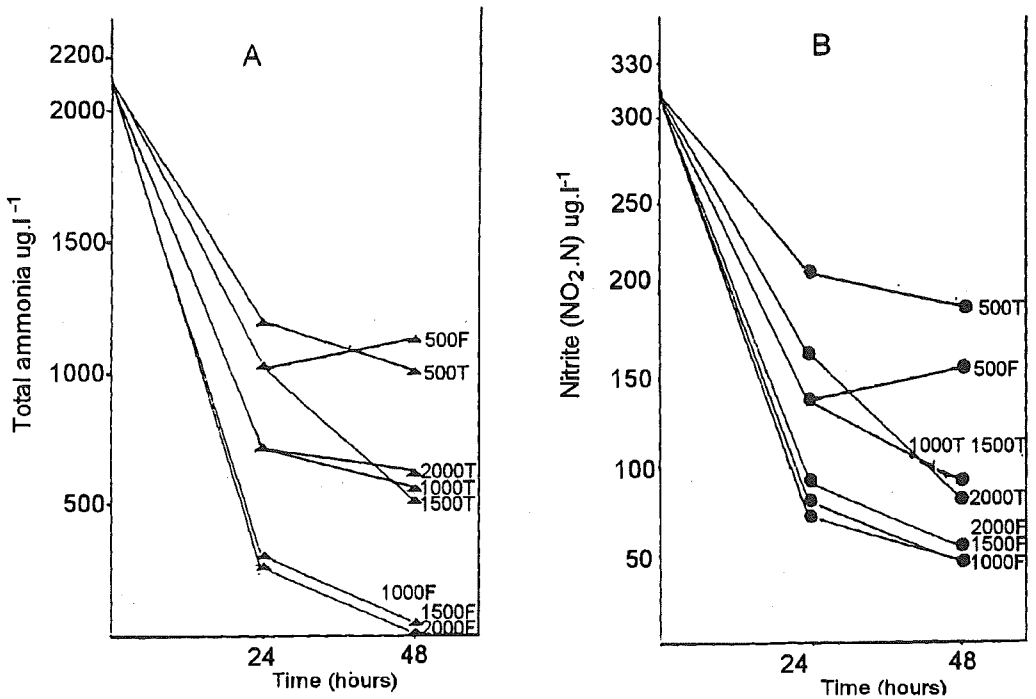

Fig.2.(A) Reduction of total ammonia by *C. japonica* (T=trapped, F=free floating) in culture water at cell densities of 500, 1000, 1500 and 2000 μl^{-1}. (B) Reduction of nitrite by *C. japonica* (T=trapped, F=free floating) in culture water at cell densities of 500, 1000, 1500 and 2000 μl^{-1}

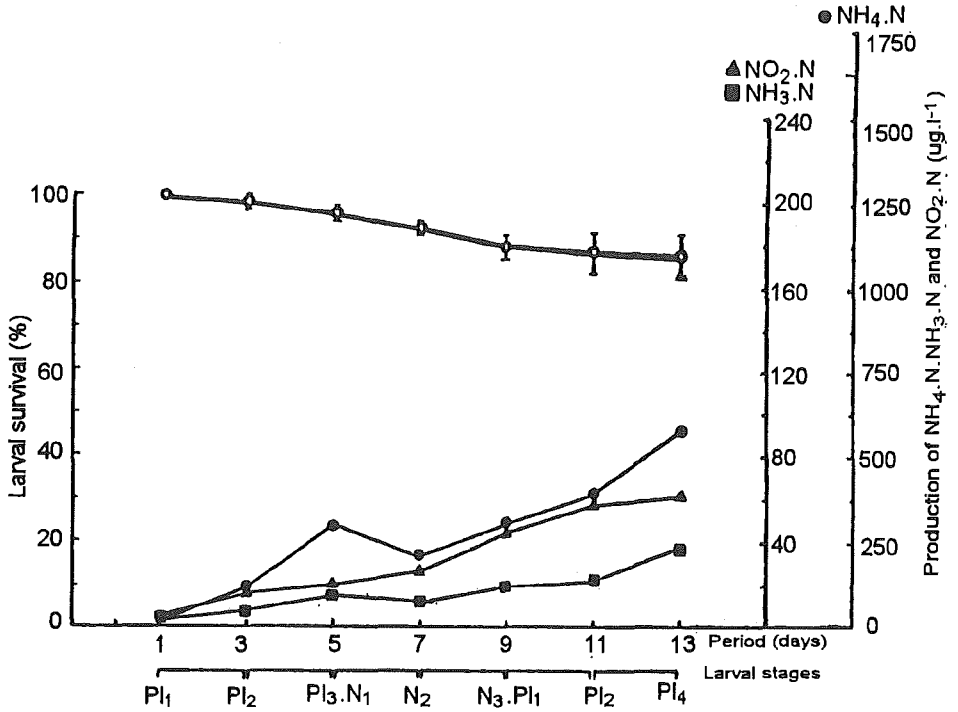

Fig.3. *P. monodon* larval survival (o) on artificial larval diet in the presence of trapped *Chlorella*, plotted together with NO_2.N (▲), NH_3.N (■) and NH_4.N (●) levels in replicated feed trial.

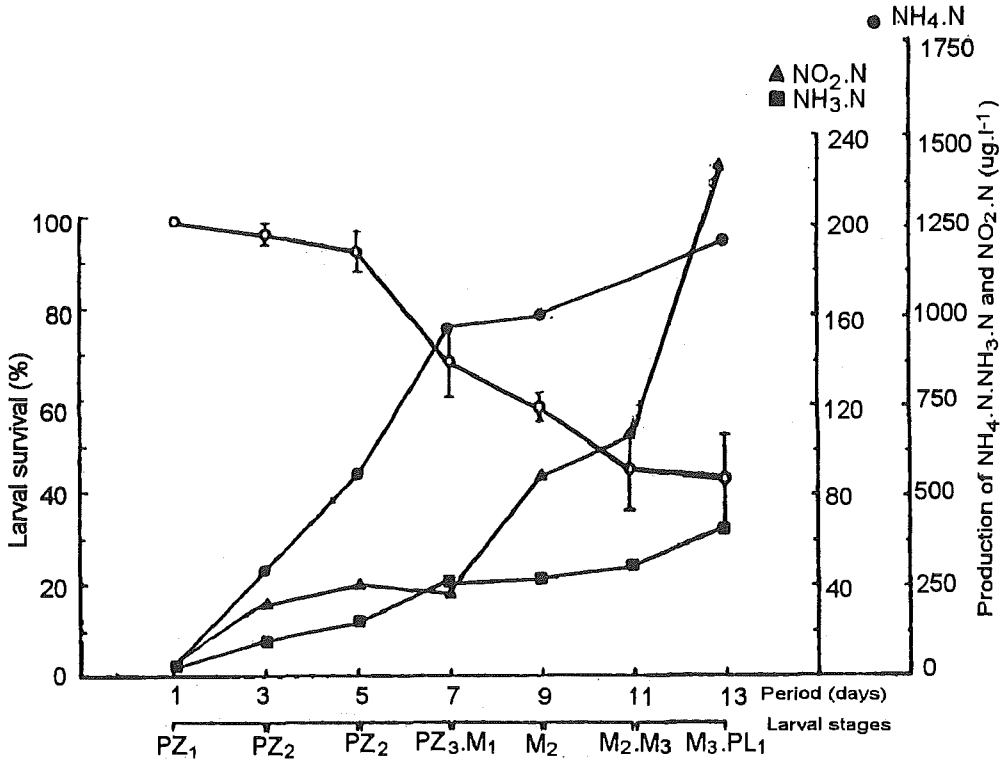

Fig.4. *P. monodon* larval survival (o) on control diet of live feeds, plotted together with NO_2.N (▲), NH_3.N (■) and NH_4.N (●) levels in replicated feed trial.

Table II: Reduction of NH_4^+.N, NH_3.N and NO_2.N levels by algal species (500 cells μl^{-1}) in the culture medium, after 24 and 48hours. Values are means of replicates with ± S.D.

		24 hours				48 hours			
Microalagal species	Cell size µm	NH_4^+.N $\mu g.l^{-1}$	NH_3.N $\mu g.l^{-1}$	NO_2.N $\mu g.l^{-1}$	pH	NH_4^+.N $\mu g.l^{-1}$	NH_3.N $\mu g.l^{-1}$	NO_2.N $\mu g.l^{-1}$	pH
C.japonica	5	1020.0 ±219.20	54.26 ±4.24	138.0 ±49.49	8.0 ±0.0	1122.0 ±141.42	59.69 ±14.14	156.4 ±35.35	8.0 ±0.0
C.calcitrans	5	1275.0 +156.97	67.83 ±15.55	202.4 ±63.63	8.0 ±0.0	1122.0 ±173.94	59.69 ±8.48	165.6 ±46.66	8.0 ±0.0
C.japonica (Trapped)	5	1190.0 ±155.56	63.30 ±18.38	211.6 ±127.27	8.0 ±0.0	999.6 ±77.78	53.17 ±11.31	192 ±84.85	8.0 ±0.0
T.chuii	10	1275.0 ±226.27	67.83 ±14.14	184.0 ±63.63	8.0 ±0.0	1173.0 ±79.19	62.40 ±5.65	192 ±42.42	8.0 ±0.0
P.lutheri	5	1530.0 ±197.98	65.331 ±12.72	230.0 ±96.16	7.9 ±0.14	1200.0 ±155.56	63.84 ±12.72	202.4 ±79.19	8.0 ±0.0
R.baltica	10	1785.0 ±254.55	76.219 ±7.07	276.0 ±73.53	7.9 ±0.0	1836.0 ±212.13	97.67 ±31.11	230 ±73.53	8.0 ±0.0
S.costatum	7	1836.0 ±169.70	78.397 ±21.21	184.0 ±35.35	7.9 ±0.14	204.0 ±169.70	108.52 ±46.66	255 ±89.09	8.0 ±0.0

Table III: Reduction of NH_4^+.N, NH_3.N and NO_2.N levels by algal species (1000 $\mu g.l^{-1}$) in culture medium, after 24 and 48hours. Values are means of replicates with ± S.D.

		24 hours				48 hours			
Microalagal species	Cell size µm	NH_4^+.N $\mu g.l^{-1}$	NH_3.N $\mu g.l^{-1}$	NO_2.N $\mu g.l^{-1}$	pH	NH_4^+.N $\mu g.l^{-1}$	NH_3.N $\mu g.l^{-1}$	NO_2.N $\mu g.l^{-1}$	pH
C.japonica	5	295.8 ±56.56	19.49 ±4.24	73.6 ±9.89	8.1 ±0.14	36.0 ±11.31	2.37 ±0.84	46.0 ±8.48	8.1 ±0.14
C.japonica (Trapped)	5	714.0 ±70.71	30.48 ±4.24	138 ±28.28	7.9 ±0.14	561 ±89.09	23.95 ±2.82	92 ±8.48	7.9 ±0.14
T.chuii	10	1326.0 ±124.45	56.62 ±2.82	276 ±73.53	7.9 ±0.0	765 ±84.85	32.66 ±7.02	255 ±46.66	7.9 ±0.28
P.lutheri	5	918.0 ±79.19	48.83 ±11.31	303.6 ±93.33	8.0 ±0.14	1020 ±70.71	54.26 ±12.72	322 ±79.19	8.0 ±0.14
C.calcitrans	5	1346.4 ±120.20	57.49 ±5.65	184 ±46.66	7.9 ±0.0	1071 ±120.20	45.73 ±5.65	147.2 ±30.97	7.9 ±0.0
S.costatum	7	1632.0 ±108.89	69.68 ±9.89	230 ±62.22	7.9 ±0.0	1836 ±127.27	97.67 ±28.28	184 ±29.69	8.0 ±0.0
R.baltica	10	1836.0 ±169.70	78.39 ±7.70	322 ±35.35	7.9 ±0.14	2040 ±28.28	108.52 ±22.62	303.6 ±84.85	8.0 ±0.0

Table IV: Reduction of NH_4^+N, $NH_3.N$ and $NO_2.N$ by algal species (1500 cells μl^{-1}) in culture medium, after 24 and 48h. Values are means of replicates with ± S.D.

		24 hours				48 hours			
Microalagal species	Cell size µm	$NH_4^+.N$ $\mu g.l^{-1}$	$NH_3.N$ $\mu g.l^{-1}$	$NO_2.N$ $\mu g.l^{-1}$	pH	NH_4^+N $\mu g.l^{-1}$	$NH_3.N$ $\mu g.l^{-1}$	$NO_2.N$ $\mu g.l^{-1}$	pH
C.japonica	5	255 ±39.59	13.56 ±7.07	82.8 ±14.14	8.0 ±0.14	18.4 ±4.24	1.216 ±0.707	46.0 ±2.0	8.1 ±0.14
C.japonica (Trapped)	5	1020 ±138.59	43.55 ±8.48	138 ±16.97	7.9 ±0.0	510.0 ±91.92	21.78 ±5.65	9.2 ±32.52	7.9 ±0.14
C.calcitrans	5	1009.8 ±325.27	43.12 ±7.07	276 ±15.515	7.9 ±0.0	969.0 ±156.97	51.55 ±4.24	340 ±59.39	8.0 ±0.14
T.chuii	10	1122 ±213.54	59.69 ±19.79	294.4 ±32.52	8.0 ±0.0	1020.0 ±155.56	54.26 ±11.31	230 ±72.12	8.0 ±0.0
P.lutheri	5	1326 ±301.22	56.62 ±9.89	184 ±22.62	7.9 ±0.28	1428.0 ±299.8	75.96 ±15.55	138 ±43.84	8.0 ±0.0
S.costatum	7	1428 ±224.85	75.97 ±21.21	202.4 ±18.36	8.0 ±0.14	1530 ±195.16	81.39 ±8.48	230 ±114.55	8.0 ±0.0
R.baltica	10	2040 ±70.71	134.84 ±16.97	340 ±11.31	8.1 ±0.14	1836 ±176.77	97.67 ±12.72	276 ±73.53	8.0 ±0.0

Table 5: Reduction of $NH_4^+.N$, $NH_3.N$ and $NO_2.N$ levels by algal species (2000 cells μl^{-1}) in culture medium, after 24 and 48h. Values are means of replicates with ± S.D.

		24 hours				48 hours			
Microalagal species	Cell size µm	$NH_4^+.N$ $\mu g.l^{-1}$	$NH_3.N$ $\mu g.l^{-1}$	$NO_2.N$ $\mu g.l^{-1}$	pH	NH_4^+N $\mu g.l^{-1}$	$NH_3.N$ $\mu g.l^{-1}$	$NO_2.N$ $\mu g.l^{-1}$	pH
C.japonica	5	265.2 ±57.27	11.32 ±2.82	92.0 ±7.07	7.9 ±0.0	20.40 ±2.82	0.87 ±0.70	55.2 ±7.07	7.9 ±0.0
C.japonica (Trapped)	5	714 ±138.59	30.48 ±9.89	174.8 ±50.34	7.9 ±0.0	612.0 ±63.63	26.13 ±8.48	82.8 ±11.31	7.9 ±0.0
T.chuii	10	1020 ±141.42	54.26 ±11.31	276 ±73.53	8.0 ±0.0	816.0 ±169.70	34.84 ±21.21	230.0 ±36.76	7.9 ±0.14
C.calcitrans	5	1122 ±144.24	59.69 ±7.07	257.6 ±53.74	8.0 ±0.0	1020.0 ±173.94	54.26 ±16.97	276.0 ±91.92	8.0 ±0.0
P.lutheri	5	1224 ±173.94	65.11 ±12.72	193.2 ±77.78	8.0 ±0.14	1122.0 ±205.06	59.69 ±16.97	202.0 ±70.71	8.0 ±0.0
S.costatum	7	1275 ±247.48	67.83 ±15.55	165.6 ±32.52	8.0 ±0.0	1326.0 ±173.94	87.64 ±18.38	185.0 ±35.35	8.1 ±0.0
R.baltica	10	2550 ±226.27	135.66 ±21.21	294.4 ±79.19	8.0 ±0.0	2040.0 ±212.13	134.84 ±34.76	280.0 ±63.63	8.1 ±0.14

T. chuii and 20 cells μl^{-1} of *R. baltica* produced good growth and percentage larval survival (80-90%), (Fig.4).

DISCUSSION

Results show that the toxic range of ammonia and nitrite levels in the culture water can be reduced effectively to safer limits by adding microalgae cells at densities of 1000 to 1500 μl^{-1}, at an illumination of $6.6x10^{15}$ quanta sec^{-1} cm^2. Amongst the 6 algal species tested both free floating and trapped *C. japonica* were effective in removing 99.1% ammonia from the culture water after 48 hours (Fig.2a). *Rhodomonas baltica* tested at 4 cell densities (500,1000,1500 and 2000 cells μl^{-1}) was least effective in assimilating and reducing initial ammonia levels (13.2%) after 48 hours from the culture water. The diatom *S. costatum* was also less effective in removing ammonia (37.3%) from the culture water.

Nitrite levels in the culture water were also effectively reduced by both free floating and trapped *C. japonica* (70.7% to 85.3%) in 48 hours (Fig.2b). *Skeletonema costatum* and *R. baltica* were less effective in reducing nitrite levels (Table II) from culture waters. These species periodically collapse and decomposition of dead algal cells may increase toxic ammonia and nitrite levels in the water. The effectiveness of algal species in assimilating and reducing ammonia levels in the culture water, demonstrates that ammonia was the preferred nitrogen source of nutrient for live algae in the present culture water environment. Molly and Syrett (1988) also showed that algae in the presence of ammonia and urea assimilated ammonia first, and only when this disappeared from the culture medium were other nitrogen sources utilized.

Since ammonia and nitrite levels reduced by *C. japonica* at cell densities of 1000, 1500 and 2000 μl^{-1} were not significantly different from each other, a minimum cell concentration of 1000 μl^{-1} of free floating *C. japonica* was tested in a feed trial with *P. monodon* larvae fed on artificial diet. Although ammonia (81.6 $\mu g.l^{-1}$) and nitrite (18.4 $\mu g.l^{-1}$) levels were well below safe limits (1500 and 110 $\mu g.l^{-1}$ respectively) larval growth did not proceed beyond the PZ2/PZ3 stage, and larval mortality occurred on day 7. The reason for this is that larvae consumed *C. japonica* as food. Cells of *C. japonica* are nutritionally inferior for penaeid and other molluscan larvae due to their thick cell wall (Web and Chu, 1982). Growth and survival of *P. monodon* larvae in repeat feed trials on artificial diets in unchanged culture water in the presence of *C. japonica* at 1000 cells μl^{-1} trapped in a flowthrough transparent cylinder were significantly better (20-40%). Levels of NH_4^+.N (1200 $\mu g.l^{-1}$) and NH_3.N (65 $\mu g.l^{-1}$) were within safe limits, but NO_2.N (230 $\mu g.l^{-1}$) exceeded the recommended safe limits (110 $\mu g.l^{-1}$) and may have caused physiological stress resulting in low survival.

It is well documented that accumulated ammonia and nitrite adversely effects growth and survival of penaeid prawns (Wickins, 1976; Mevel and Chamroux, 1981; Jayasankar and Muthu, 1983a, 1983b; Chen *et al.*, 1986 and Chen and Chin, 1988). This study indicates that the algal species of *C. japonica* is effective as a "biological filter" and can efficiently reduce accumulated toxic metabolites from an unchanged culture water environment.

The only alternative method of reducing toxic levels of both ammonia and nitrite in culture water, is to undertake frequent water exchanges. However, most commercial hatcheries rarely exchange culture water (Liao, 1977). Accumulation of toxicants in these static systems adversely affects the survival and development of larvae (Chin and Chen, 1987; Chen and Chin, 1988). Most static water hatcheries include algae during protozoeal culture as feed and only incidentally, as demonstrated in present experiments, as a biofilter. However, during later mysis and postlarval culture algal growth is usually discouraged as live feeds such as *Artemia* may utilise the algae and compete with the shrimp larvae. Today, even the algae used in protozoeal culture is increasingly being replaced with artificial larval foods. In both situations there are clear benefits in the use of algae such as *Chlorella* as biofilter, alternatively, frequent water changes must be undertaken.

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