J. mar. biol. Ass. India, 2001, **43** (1&2) : 181 - 185

Removal of nitrogenous wastes by seaweeds in closed lobster culture systems

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Abstract

Removal of nitrogenous wastes excreted by lobsters in a closed culture systems by two species of seaweeds, *Gracilaria corticata* and *G.verrucosa* was studied. Both species removed ammonia-N more readily than nitrites and nitrates. Among the two species. *G.verrucosa* was more efficient in uptake of ammonia-N (100%) compared to *G.corticata* (72.3%). The experiment shows that the seaweeds *Gracilaria* sp. can be used in maintaining water quality in closed culture systems by ready uptake of nitrogenous wastes excreted by the cultured organisms. The advantages of water reuse systems incorporating biological agents such as seaweeds for water management in closed culture system is outlined. The potential of developing doubly productive farming system by integrating seaweeds with other marine organisms is discussed.

Accumulation of soluble wastes is a major problem in clsoed aquaculture systems. Ammonia, which is toxic in high concentrations, constitutes nearly 60% of the nitrogenous wastes excreted by crustaceans. The upper limit of tolerance of ammonia-N is usually low for crustaceans, which varies from 286 to 1786 ug-at/l (Pandian, 1975).

Ammonia toxicity could present a major problem in culture systems where a major part of the seawater is recycled. The treatment of effluent waters from culture systems may be practised to remove waste nutrients in the form of harvestable algae (Ryther *et al.*, 1981). Alternatively, algae may be used in closed aquaculture systems to maintain water quality (Harlin *et al.*, 1978).

Experiments were conducted at the Kovalam Field Centre of the Central Marine Fisheries Research Institute, Chennai to evaluate the local seaweeds for their capacity to remove soluble nitrogenous wastes excreted by lobsters in closed culture systems.

Material and methods

The spiny lobster *Panulirus homarus,* is used as a continuous source of soluble nitrogenous wastes for the experiment. The weight of experimental animals ranged between 152 and 222 gm. They were fed on the clam *Meretrix casta* once daily in the evening.

Two species of seaweeds were used of which *Gracilaria corticata* is a marine form, found on the intertidal rocks around Kovalam, and *G. verrucosa,* an estuarine form present in the Kovalam backwaters. *G. verrucosa* was gradually acclimatised to a salinity of 30 ppt in the laboratory. The algae were collected, cleaned and maintianed in the laboratory prior to

experimentation. *G. verrucosa* was pale yellow in colour, presumably due to nitrogen deprivation.

The experimental set-up consisted of nine tanks containing 30 l seawater each. All the tanks contained two lobsters whose combined weight in each tub was an average 359.2 gm. Experimental tanks contained, in addition, 200 gm of either of the two species of algae. Of the six such tanks, three contained *G. corticata* and another three having *G.verrucosa.* The three control tanks had lobster alone. Water was not changed during the course of the experiment. The mean salinity and the temperature in the experimental tanks were 30 ppt and 25.9±1.01°C, respectively. The experiment was conducted indoor but sufficient natural light was available for the algae to grow normally.

Inorganic nitrogen as ammonium, nitrite and nitrate was monitored in all the tanks daily. Ammonia-N was estimated following Solorzano (1969) and nitrite-N and nitrate-N were measured following the methods given in Strickland and Parsons (1972). Statistical treatment of data on uptake of nitrogenous wastes by the two species of algae was carried out following Campbell (1967).

Results and discussion

Table 1 summarizes daily change in different forms of nitrogen in different treatments. Increments in the concentration of N-compounds each day were averaged for seven days and the mean total output per day by the lobsters was obtained. Mean total uptake by algae was obtained from the difference between the daily output from the lobster as estimated above and the daily increment in algal tanks calculated in a similar fashion. Rates of change were calculated as change in concentration per gram wet weight of the lobster and the algae. Table 2 gives the daily concentration in µg-at/l of inorganic nitrogen, as the mean of values for seven days.

Table 1. *Inorganic Nitrogen - Average daily output by Lobster (control tank) and uptake by Seaweed (experimental tank)*

Treatment	Control Tank	Experimental Tank	
		G. corticata	*G. verrucosa*
Ammonia-N			
Total (µg-at/d)	900.0	651.0	900.0
% of control		72.3	100.00
Rate (µg-at/g wet wt/day) of uptake	2.508	3.255	4.695
Nitrite-N			
Total (µg-at/d)	291.60	195.60	231.00
% of control		67.0	79.0
Rate (µg-at/g wet wt/day)	0.812	0.978	1.155
Nitrate-N			
Total (µg-at/d)	234.6	187.5	235.2
% of control		80.0	100.0
Rate (µg-at/g wet wt/ day)	0.653	0.937	1.176

Ammonia-N was seen to comprise the major output of the animals. It was excreted at the rate of 2.508 µg-at/gm wet wt/day, which constituted 63% of the total nitrogenous output as measured in the control tanks (Table 1). Correspond-

Table2. *Inorganic Nitrogen-Average daily concentration in tanks (µg-at/l) ±S.D*

Treatment	Control Tank	Experimental Tank	
		G. corticata	*G. verrucosa*
Ammonia-N	188.88±66.85	46.45±19.54	11.60±11.03
Nitrate-N	83.34±19.34	69.53±11.85	57.71±15.32
Nitrite-N	41.02±25.47	19.48±11.73	14.01±5.26

ingly, there was a significant uptake of this waste product by the algae. Thus, *G. verrucosa* removed on an average 4.695 µg-at/g wet wt/day, the total quantity it removed at this rate being greater than the output from the lobster. *G.corticata* was somewhat less efficient under the same conditions, removing 3.255 µg-at/g wet wt/day representing 72.3% of the total output from lobster every day (Table 1).

The influence of algae on nitrite and nitrate-N levels is also summarized in Table 1. An upward trend prevailed in all the tanks but levels were always lower in the algal tanks. From the total increment of 234.6 µg-at/day of nitrate-N, *G. corticata* removed 187.5 µg/day or 80% while *G. verrucosa* removed 235.2 µg-at/day or 100% of the daily increment in nitrate-N, showing significant difference in removal by these two species ($P < 0.05$). Nitrite-N, on the otherhand, was reduced to a smaller extent of 67% in *G.corticata* tanks and 79% in *G.verrucosa* tanks (Table 1).

Although the bulk of nitrate-N and much of nitrite-N was removed by the algae, the actual quantities removed were much lower than ammonia-N. *G.verrucosa* for instance took up 4.695 µg-at/g wet wt. of ammonia-N, but only 1.176 µg-at/g wet wt/day of nitrate-N and 1.155 µg-at/g wet wt/day of nitrite-N. Although *G. verrucosa* removed all the nitrate added by the lobster, the nitrate-N present at the start of the experiment (56.8 µg-at/l) was left more or less intact. *G. corticata* too showed similar behaviour, although it was less efficient in removal of nitrogenous compounds. The pH during the course of the experiment remained relatively constant between 7.5 and 8.0.

Significant growth was observed in *G.corticata.* resulting in an increase of 26 g representing a growth rate of 1.8 % per day. On the other hand, *G.verrucosa* showed no growth at all during the same period. By the end of the experiment, thalli of *G.verrucosa* had lost the pale green colour present originally and had assumed a healthy, deep red-brown colour. Thalli of both species remained firm and healthy throughout.

The experimental results demonstrate that algae like *Gracilaria* sp. can be used in closed culture systems to remove ammonia-N from the water. However, nitrite-N and nitrate-N are not taken up to the same extent. *G. verrucosa* is more efficient in removing these forms of nitrogen. Similar studies conducted in *G.tikvahiae* demonstrated the highly efficient removal of ammonia-N, while nitrate was not so efficiently removed from system (Topinka and Robbins, 1959).

Several studies on phytoplankton and seaweeds show that in media containing a mixture of ammonium and nitrate, the

former is the first to be absorbed (Spotte, 1970). Harlin *et al.* (1978) has mentioned the inhibition of nitrate uptake in the presence of ammonium in *Gracilaria* sp. Similar behaviour appears to be true also for the species of *Gracilaria* sp. studied in the present experiments. Both the species studied show greater removal of ammonia than of nitrate or nitrite.

Marine animals tolerate concentrations of nitrate many times higher than that of ammonia and recycled water can be reused almost indefinitely if the nitrification process remains stable (Chapman and Craigie, 1977). Since ammonia is the major and most toxic component of the waste of aquatic animals, it appears that the seaweeds could serve very well as controller of water quality in aquaculture systems.

An interesting observation was the lack of growth of *G.verrucosa* under the conditions of this study. This may in part be accounted for by the fact that no supplementary growth stimulants were added to the medium. However, *G.corticata* appeared to grow fairly well under the same conditions. Nitrogenous compounds, especially ammonia-N, disappear to a significant extent from the system, and may be assumed to have been absorbed by the algae. What, then, happens to the nitrogen that is absorbed in such large quantities? If there is no net growth, the nitrogen must have been stored within the tissues of *G.verrucosa*. Accumulation of ammonia and nitrate has been shown in *G.tikvhiae* (Topinka and Robbins, 1976). From the presnt study it may be inferred that *G.verrucosa* has the capacity to absorb and store nitrogen compounds, either as nitrogen pools, or after conversion into pigment biliproteins, as shown by the change in colour of the thallus.

Once the precise factors controlling and promoting the growth of these agarophytes in closed systems is well established and standardised, it will become possible to culture harvestable quantities of these algae from integrated farming systems incorporating shrimps and seaweed. This would lead to the development of doubly productive closed culture systems.

The use of seaweeds as a nitrifying agent also has several advantages over conventional biological filters. Since the seaweed may be placed on the water surface of the culture tank itself, no special air-lifting mechanism is required for recirculation of the water. This is a necessary condition for the normal functioning of biological filters. In addition, the photosynthetic avtivity of the seaweed would contribute to the oxygenation of the system. Furthermore, maintenance of the seaweed is minimal unlike biological filters which require periodic cleaning.

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