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Ammonia-nitrogen production by the bivalve mollusc *Tapes japonica* and its recovery by the red seaweed *Hypnea musciformis* in a tropical mariculture system

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ABSTRACT: Production and recovery of ammonia-N was studied in the second and third trophic levels of a mariculture system on St. Croix, US Virgin Islands. The diatom *Chaetoceros curvisetus*, grown on nutrients in artificially upwelled deep water from 870 m depth, was the food source for *Tapes japonica*. Consumption of the phytoplankton by *T. japonica* increased throughout the day and decreased at night, and was related to corresponding changes in algal culture density. Feeding efficiency was highest at night. Ammonia-N production by the clams fluctuated over a typical 24 h period; dropping during the day and increasing at night. The ammonia-N concentration in the shellfish tank effluent was inversely related to the quantity of phytoplankton consumed by the clams. At all ration levels the small clams produced ammonia-N at a greater rate than large clams. *H. musciformis* fragmented and washed out of some tanks; the fragmentation was related to high ammonia-N concentrations in the inflowing water. High light intensity and temperature alone do not appear to cause fragmentation, but may have induced a trace nutrient deficiency in *H. musciformis* grown in the ammonia-rich seawater. Where fragmentation was not obvious, ammonia-N uptake per g *H. musciformis* was highly correlated with the average ammonia-N concentration of the inflowing seawater both day and night. Percent uptake of ammonia-N increased with increasing concentration of the nutrient in the inflowing seawater, reaching a plateau of about 70 % uptake of the available ammonia-N at concentrations above 4 μg-at/l.

INTRODUCTION

In the past few years the interest in controlled marine ecosystems has increased markedly due to the concern over recycling of waste nutrients and developing new food and energy sources (Othmer & Roels, 1973; Goldman et al., 1974; Ryther et al., 1975; Roels et al., 1976). In these systems there are a limited number of trophic levels, each of which is interrelated in such a manner that, starting with a dissolved nutrient source, each level utilizes one or more of the nutrients that is not available to, or has been regenerated by, the previous level. At the artificial upwelling project on St. Croix (US Virgin Islands) the nutrient source is seawater that is pumped from a depth of 870 meters and has high concentrations of nitrate, phosphate and silicate (Othmer & Roels, 1973; Roels et al., 1976). These nutrients are used for the production of

phytoplankton which is then fed to bivalve molluscs. Recovery of the soluble waste produced by the shellfish is accomplished by growing seaweed in the effluent from the shellfish tanks.

This paper deals with the generation of ammonia-N by the bivalve mollusc *Tapes japonica* Dehayes feeding on the diatom *Chaetoceros curvisetus* Cleve and the recovery of the ammonia-N by the red seaweed *Hypnea musciformis* (Wulfen) Lamouroux.

MATERIALS AND METHODS

Experimental design

The experimental system consisted of three trophic levels connected in series so that seawater flowed continuously from one trophic level to the next, as follows: phytoplankton→bivalve molluscs↦seaweed. The methods employed with each trophic level are given in the following sections.

Phytoplankton

The diatom *Chaetoceros curvisetus* (clone STX-167, isolated from St. Croix deep water by KCH) was grown in an outdoor 2000 liter epoxy-coated concrete tank (reactor) (Malone et al., 1975). The growth medium used was Antarctic Intermediate Water, pumped from a depth of 870 m, hereafter referred to as deep water. Deep water was continuously pumped into the reactor to give a dilution rate of one reactor volume per day. The overflow from the reactor was fed by gravity to the tanks containing the shellfish.

Shellfish

Four different populations, comprised of two different size classes, of the bivalve mollusc *Tapes japonica* were maintained in the flow through system. Either 70 or 140 gms, total wet weight, of clams of a particular size class were placed in 4.6 l tanks (28 cm × 16.4 cm × 10 cm deep) and the algal culture was metered into each tank at the rate of 1 ml sec^{-1}. The inflow into the tanks was adjusted by restricting the flow with an appropriate length of capillary tubing. The water in the shellfish tanks was continuously mixed by aeration from a horizontal air manifold on one short side of each tank, and the tanks were shaded from direct sunlight. The clams were suspended off the bottom of the tank on an epoxy-coated wire mesh. A single population of clams consisted of two tanks containing an equal biomass of the same size clams. The choice of flow rate and clam biomass was based on unpublished data which indicated that these flow rate/biomass combinations approached the maximum conversion rate of phytoplankton protein to shellfish meat protein.

Every fourth day, at the conclusion of a 24 h experiment, the clams were removed from each tank, blotted dry with a paper towel and the total wet weight determined.

Enough clams were removed to return the weight to the the initial 70 or 140 g. Samples of clams were taken from both the large (mean shell length = 18.9 mm, mean dry meat weight = 84.0 mg) and small (mean shell length = 10.1 mm; mean dry meat weight = 9.7 mg) size classes at the beginning and end of the experiment and the dry meat weight was found to be 6.02 ∓ 0.91 % of the total wet weight. This factor (.0602) was used to convert total wet weight to dry meat weight for the entire experiment.

A number of taxonomic synonyms exist in the English and Japanese literature for the Japanese little neck clam, *Tapes japonica*. The more common names being *Venerupis semidecussata*, *Tapes semidecussata*, *T. japonica* and *Paphia philippinarum*. Throughout this paper we refer to the clam as *Tapes japonica* Dehayes, 1853 (see Cahn, 1951; Ohba, 1959).

Seaweed

The effluent from each of the four shellfish populations flowed into a 4.6 l tank containing the red seaweed *Hypnea musciformis*. The inflow was maintained at the rate of 2 ml sec^{-1}, or 37.6 tank volumes per day. A second *Hypnea* tank received the effluent from the first in an attempt to maximize nutrient removal. The tanks had the same dimensions as the clam tanks, and the seaweed was kept constantly revolving by aeration from a manifold located along the long axis on one side of the tank. The tanks were exposed to full sunlight throughout the day. The *Hypnea musciformis* used for these experiments was collected from a single population on a local reef (0–0.5 m depth) and none of the seaweed was sexually reproductive. The plants were picked free of epiphytic seaweeds and only actively growing branches were used in the experiment. An initial wet weight of 20 g of seaweed was placed in each tank. Every four days, at the conclusion of a 24 h experiment, the seaweed was removed from each tank, blotted dry on a paper towel and the wet weight determined. 20 g of *H. musciformis* was replaced in the appropriate tank. The total wet weight at the end of each experiment was used to calculate the ammonia-N uptake per gram of seaweed for the entire 24 h experiment. In some of the shellfish-seaweed combinations the seaweed fragmented and washed out of the system, resulting in a decrease in biomass. Data collected from these tanks was not used for calculating ammonia-N uptake. When fragmentation did occur, *H. musciformis* was replaced with newly collected plants. Control *Hypnea* tanks were set up as described above, except that instead of receiving the effluent from the clam tanks they received the phytoplankton culture directly at the rate of 2 ml sec^{-1}. In a separate experiment an additional control was run to test for ammonia-N uptake by residual phytoplankton in the effluent from the shellfish tanks by omitting the *H. musciformis* from the system.

Sampling program

A series of five experiments, each covering a 24 h period were carried out on July 9, 13, 17, 21 and 26, 1976. Samples of the phytoplankton culture flowing into

the shellfish tanks, the effluent from each of the shellfish populations and the effluent from the seaweed tanks were collected at four hour intervals beginning at 06:00 h (except on July 26, when sampling began at 02:00 h). The water temperature in the tanks from one of the shellfish-seaweed combinations was measured at each sampling time. The means and ranges of temperature are given in Table I.

Table 1

Mean and range of temperatures (°C) in one serial combination of a *Tapes japonica* population and two *Hypnea musciformis* tanks. Times of high and low temperatures are given in parentheses

Temperature range	*Tapes japonica*	*Hypnea musciformis* 1st Tank	*Hypnea musciformis* 2nd Tank
High	30.0° (18:00 h)	30.5° (14:00 h)	31.0° (14:00 h)
Low	25.0° (06:00 h)	25.0° (06:00 h)	24.8° (02:00 h)
Mean ± S.D. (n = 24)	27.1 ± 1.4°	27.2 ± 1.8°	27.1 ± 2.0°

The turbidity of the influent and effluent samples from the shellfish tanks was measured using a model 250 Monitek laboratory turbidimeter (Monitor Technology, Inc., 303 Convention Way, Redwood City, Calif. 94063). All samples were filtered through Gelman Type A–E glass fiber filters and the filtrate was analysed for ammonia-N and nitrate + nitrite-N concentrations using a Technicon Auto-Analyser II. The analytical methods used are described in the standard Technicon Auto-Analyser methodology handbook and are based on the procedures given by Strickland & Parsons (1972). For the influent samples, into the shellfish tanks, a known sample volume was filtered and a protein determination carried out on the filter according to the method of Dorsey et al. (1977).

RESULTS

Feeding activity of *Tapes japonica*

Figure 1 shows the diel periodicity in the average phytoplankton consumption, over five 24 h experiments, for the four populations of *Tapes japonica*. The quantity of *Chaetoceros curvisetus* consumed by *T. japonica* was based on the difference in the turbidity between the influent and effluent water from the shellfish tanks. Although there were some differences in the amount of phytoplankton consumed by the four clam populations the diel periodicity in the rate of consumption was similar for all groups. The actual amount of phytoplankton consumed increased throughout the day, reached a peak around sunset, and then decreased throughout the night.

The periodicity in the amount of phytoplankton consumed by the clams may be related to the periodicity in the algal cell density, measured as turbidity, of the influent phytoplankton culture (Fig. 1). It can be seen that the turbidity of the influent water increased throughout the day, peaking near sunset, and then dropped during the night.

The change in the rate of consumption by the clams reflects this change in algal cell density.

If the average amount of phytoplankton consumed by the four clam populations is expressed as a percentage of the available phytoplankton it is clear that a higher percentage of the phytoplankton was removed at night (Fig. 1). This suggests that the clams increased their pumping activity or altered their filtration efficiency; this resulted in a greater percentage of the available phytoplankton being consumed.

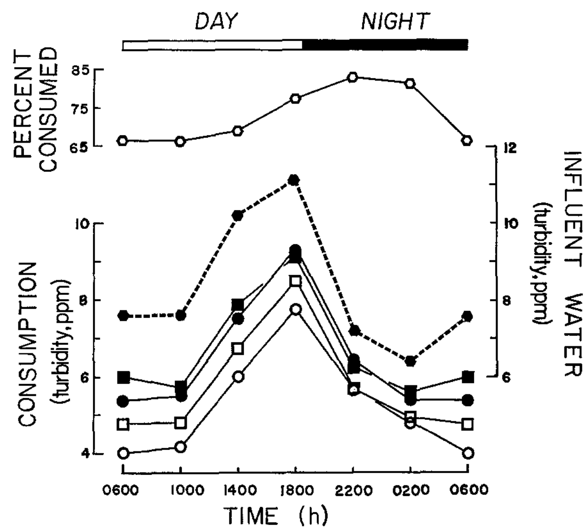

Fig. 1: Diel perodicity in the average phytoplankton consumption by 4 populations of *Tapes japonica* over five 24 h experiments. The 06:00 h values are repeated on the right-hand side of the graph. Open circles = 70 g (total wet weight) large clams; open squares = 140 g large clams; closed circles = 70 g small clams; closed squares = 140 g small clams; closed hexagons = turbidity of water entering clam tanks; open hexagons = average percent consumption of phytoplankton for all 4 clam populations

Ammonia-N production by *Tapes japonica*

Figure 2 shows the temporal change in the average ammonia-N production for the four populations of *Tapes japonica* over five 24 h experiments. The differences in ammonia-N production (Fig. 2) between the four clam populations were greater than the differences in the amount of phytoplankton consumed (Fig. 1). The highest concentration of ammonia-N was produced by the 140 (× 2) gm population of small clams. Despite the differences in the quantity of ammonia-N produced, the periodicity in the observed ammonia-N concentrations was similar for all four populations (Fig. 2). The ammonia-N concentration in the shellfish tank effluent gradually decreased throughout the day with the lowest levels observed between 14:00 and 18:00 h; this was followed by an increase in the average ammonia-N concentration during the night.

For comparison the average turbidity of the phytoplankton culture being metered into the shellfish tanks is also shown in Figure 2. The turbidity increased and reached its peak during the day while the ammonia-N concentration in the shellfish tank effluent was dropping to its lowest level. This suggests that the ammonia-N production by *T. japonica* in our experimental mariculture system is inversely related to the phytoplankton concentration flowing into the shellfish tanks.

In Figure 3 the ammonia-N produced by *T. japonica* per g dry meat per day is plotted against protein-N consumed per gram dry meat per day for the two different size classes of clams. The quantity of phytoplankton consumed was measured as tur-

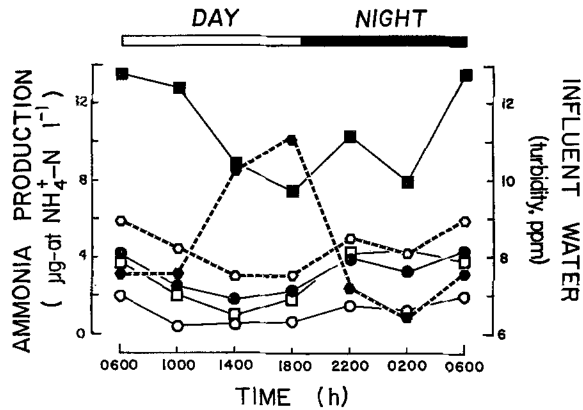

Fig. 2: Temporal changes in the average ammonia-N production by 4 populations of *Tapes japonica* over five 24 h experiments. Symbols as in Figure 1, except open hexagons show average ammonia-N production for the 4 clam populations

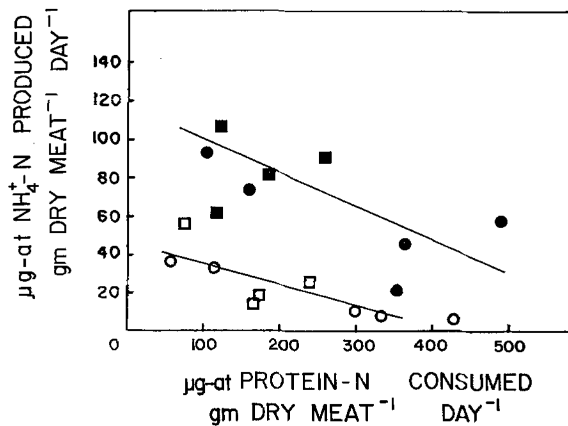

Fig. 3: Ammonia-N production versus protein-N consumed for four populations of *Tapes japonica* comprised of two different size classes of clams. The upper regression line is for the small animals where $Y = -0.18 \times + 118.25$ ($r = 0.78$; d.f. $= 8$) and the lower regression line is for the larger clams where $Y = -0.11 \times + 45.64$ ($r = 0.82$; d.f. $= 8$). Symbols are as in Figure 1

bidity and converted to protein-N using the regression equation protein-N = 31.86 (turbidity) (r = 0.97; d.f. = 29) in order to present the system as protein-nitrogen consumed versus ammonia-N produced. At virtually every level of food consumed the smaller clams, as represented by the upper line, produced ammonia at a greater rate than the larger clams. It is also clear that ammonia-N production and the quantity of phytoplankton, or protein-N, consumed are inversely related for both size classes of clams.

Fragmentation of *Hypnea musciformis*

Hypnea musciformis in a number of tanks fragmented and many of the pieces washed out of the system. This resulted in a decrease in biomass and a decrease in the percent uptake of ammonia-N in these tanks. The process of fragmentation did not appear to be reversible once fragmentation commenced. This suggested that fragmentation might be related to the nutrient concentration, especially since the second *Hypnea* tank in a series had a lower inflowing ammonia-N concentration due to the uptake of the nutrients by the first *Hypnea* tank, and fragmented less often. Figure 4 shows the relationship between the average ammonia-N concentration of the seawater flowing into the *Hypnea* tanks and resistance of the seaweed to fragmentation. At the low ammonia-N concentrations *H. musciformis* could be maintained for longer periods than at the higher ammonia-N concentrations before fragmentation occurred.

The fragmentation did not appear to be specifically related to either light or temperature alone. The seaweed tanks in series had a nearly identical temperature regime (Table 1) but the *Hypnea* in the first tank in each pair usually fragmented more often than, and earlier than, the *Hypnea* in the second tank. The seaweed in

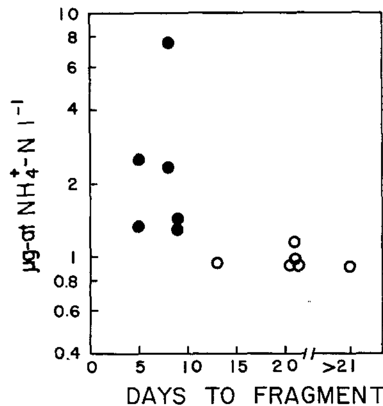

Fig. 4: Relationship between the average ammonia-N concentration of seawater flowing into the *Hypnea musciformis* tank and the resistance of seaweed to fragmentation. Data points are included here if fragmentation of *H. musciformis* was readily visible during experiment or if fragmentation did not occur during the 21 day experimental period. Ammonia-N concentrations are the means calculated from values determined at 4 h intervals over a 24 h period every 4 days. Open circles = 0.80 — 1.16 µg-at ammonia-N/l; closed circles = 1.32 — 7.51 µg-at ammonia-N/l

the control tanks experienced the same lighting regime as the experimental tanks but the control did not fragment during the 21 days of the experiment.

Growth of *Hypnea musciformis*

Excluding all of the *Hypnea* tanks where fragmentation obviously occurred, growth (increase in wet weight) during the 4 day periods between harvest was greater in the tanks receiving the shellfish tank effluent directly than it was in those tanks receiving effluent from seaweed tanks. The percent growth of the weed grown directly in the shellfish tank effluent was 37.4 % (n = 10) and in the second seaweed tank it was 22.0 % (n = 15). In some of these tanks there was actually a slight loss of weight which was possibly due to the onset of fragmentation.

Ammonia-N uptake by *Hypnea musciformis*

The uptake of ammonia-N per gram wet weight of *Hypnea musciformis* was found to be highly correlated with the average ammonia-N concentration of the seawater flowing into the tanks (Fig. 5). The values plotted in Figure 5 are for both day and night samples. The diel variation in ammonia-N uptake followed the diel variation in the ammonia-N concentration flowing into the *Hypnea* tanks. Consequently,

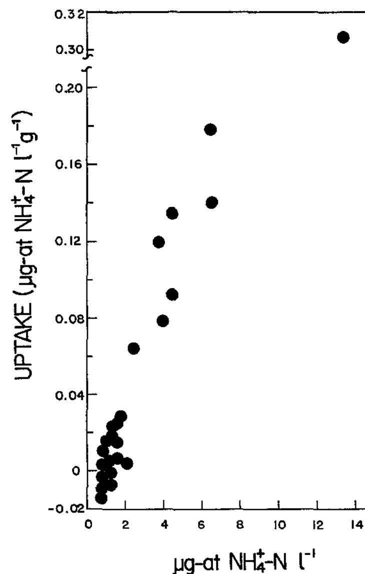

Fig. 5: Uptake of ammonia-N per gram wet weight of *Hypnea musciformis* as a function of the ammonia-N concentration of the seawater flowing into the seaweed tank. A straight line fit to the data follows the form where: Uptake = —0.77 (inflowing ammonia-N concentration) + 0.74; (r = 0.95; d.f. = 25). Data were excluded for tanks in which fragmentation of the seaweed was obvious. Control *Hypnea musciformis* tanks receiving phytoplankton culture only are also included in the graph

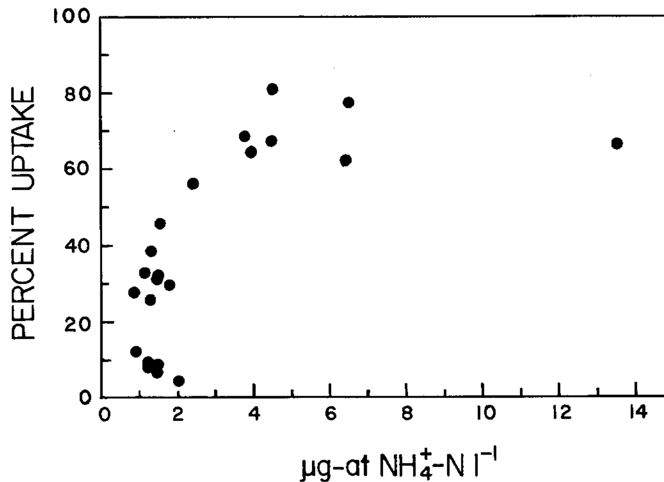

Fig. 6: Percent uptake of ammonia-N by *Hypnea musciformis* as a function of the ammonia-N concentration of the seawater flowing into the seaweed tank. The values are derived from the same data presented in Figure 5 except that the negative uptake (excretion) data were omitted

any direct influence of light on ammonia-N uptake was probably masked by the diel fluctuations in ammonia-N production by *Tapes japonica* (Fig. 2). Support for this is provided by uptake studies with an ammonia-N starved laboratory culture of *H. musciformis* where dark uptake of ammonia-N was about 90 % of the uptake rate in the light, over the range of 12–18 µg-at/l (Haines, unpublished data).

At inflow ammonia-N concentration of 1.1 µg-at/l, or less, excretion (shown as negative uptake in Fig. 5) was observed in six out of eight tanks. Perhaps *H. musciformis* in these tanks was fragmenting but not to the extent that it was readily visible, or the seaweed may have been excreting intracellular nitrogen prior to the onset of fragmentation.

On a percentage basis, uptake of ammonia-N increased with increasing concentration of the nutrient in the inflowing water, reaching a plateau of about 70 % uptake of the available ammonia-N above an inflowing concentration of 4 µg-at/l (Fig. 6).

The pigmentation of *Hypnea* changed when it was maintained in our system. The natural light brown color changed to a yellow-brown color in tanks receiving low levels of ammonia-N, and to a dark red-brown in the tanks receiving the highest ammonia-N concentrations. Lapointe et al. (1976) observed similar changes in pigmentation in *Hypnea* and *Gracilaria* species in their mariculture system utilizing sewage-enriched seawater.

DISCUSSION

Feeding activity of *Tapes japonica*

The quantity of phytoplankton consumed by the four populations of clams in our flow through mariculture system fluctuated over a 24 h period (Fig. 1). The pattern

of change, which reflected a change in the algal cell density of our phytoplankton culture was similar for all four populations except that a biomass of small clams consumed slightly more of the available phytoplankton than an equal biomass of large clams. This might be expected since it is well known that filtration rate is dependant upon body size, being relatively higher for smaller animals (Winter, 1973). The fact that there was not a greater difference in the quantity of phytoplankton consumed by an equal biomass of different size clams may indicate that our system was food limited. The phytoplankton culture was metered into the shellfish tanks at a constant rate and this rate probably resulted in an algal cell density immediately surrounding the animals which was below the level where filtration rate is reduced, and yet high enough to stimulate a near-maximum feeding efficiency for both size classes and weight groups of *Tapes japonica* (Winter, 1969; Winter & Langton, 1976; see also Hildreth & Crisp, 1976). Therefore the differences in the quantity of phytoplankton consumed by equal biomasses of clams may reflect the absolute difference in filtration rate for the animals of different sizes.

In a mariculture system such as ours the percentage of the available phytoplankton consumed by the shellfish might be expected to remain constant. This was not the case; a greater percentage of the available phytoplankton was consumed at night (Fig. 1). The reasons behind this are not yet fully understood but it may be that the increasing phytoplankton concentration flowing into the tank during the day stimulated an increased feeding efficiency which was maintained for a number of hours after the algal cell concentration had decreased during the night (Winter & Langton, 1976).

Ammonia production by *Tapes japonica*

The ammonia-N production by *Tapes japonica* in our experimental system showed a similar daily pattern of change for all four populations (Fig. 2). The decrease in ammonia-N concentration from the shellfish tank effluent during the day contrasts with the algal cell density of the inflowing phytoplankton culture, which increased during the day. It therefore appeared as if the ammonia-N concentration in the shellfish tank effluent was inversely related to the influent algal cell concentration. Since the quantity of phytoplankton consumed by the clams reflected the phytoplankton concentration coming into the tanks (Fig. 1) and, the ammonia-N concentration was inversely related to the inflowing phytoplankton concentration (Fig. 2) it follows that the ammonia-N production was inversely related to the quantity of food consumed, as shown in Figure 3. Little data exists on the influence of nutritional levels on the rate of ammonia excretion for bivalve molluscs, except for starved animals (Bayne, 1973, 1976; Bayne et al., 1976). However, the effects of nutrition have been more thoroughly investigated for zooplankton where it has usually been found that nitrogen excretion increases with an increased ration (Corner et al., 1965; Butler et al., 1970; Takahashi & Ikeda, 1975). In contrast to this more general pattern, Martin (1968) found an inverse relationship between ammonia excretion and the quantity of phytoplankton ingested by natural zooplankton populations. However, in a more

recent publication Takahashi & Ikeda (1975) have demonstrated that the excretion rates, measured using a method similar to Martin's, have to be corrected for the uptake of ammonia-N by the phytoplankton culture which is serving as a food source for the zooplankton. They divided the zooplankton excretion rate into an apparent excretion rate and the uptake rate by the phytoplankton and have shown that the apparent excretion rate was inversely related to the phytoplankton concentration in the experimental flask. This situation is very similar to what takes place in our mariculture system. It would appear that our ammonia-N production data for *Tapes japonica* only approximates an actual excretion rate and would need to be corrected for ammonia-N uptake by the phytoplankton being continuously metered into the shellfish tanks. Nevertheless, our ammonia-N production data accurately reflects the levels of ammonia which are available for culturing commercially valuable seaweeds such as *Hypnea musciformis*. One other possibility has to be considered before we may conclude that nutritional levels are or are not inversely related to ammonia-N excretion for *T. japonica*. Bivalves will normally satisfy their metabolic energy requirements by using carbohydrates, then lipids and then protein (Gabbott & Bayne, 1973; Bayne, 1976). So, it is possible that at the lower food levels the protein in the phytoplankton would be utilized for the metabolic requirements and that the resulting deamination of the protein would increase the rate of ammonia excretion at the lower food levels.

Growth and ammonia-N uptake by *Hypnea musciformis*

The growth rates of *Hynaea musciformis* and uptake of ammonia-N per gram of seaweed observed in this experiment were lower than in a previous study (Haines, 1976), but the maximum percent uptake was higher. This may be explained by the use here of lower dilution rates, lower ammonia-N concentrations, and a higher biomass of *H. musciformis* per unit volume of the tanks. In this experiment we are trying to maximize nutrient removal, whereas in the previous study the emphasis was on maintaining a maximum growth rate and avoiding nutrient limited growth.

Fragmentation of *Hypnea musciformis*

The fragmentation observed in this experiment appeared to be related to high levels of ammonia-N, but it does not necessarily follow that high concentrations of ammonia-N alone caused the fragmentation. In the previous study fragmentation did not occur in *Hypnea musciformis* cultures receiving either *Tapes japonica* effluent or deepwater when supplemented with 4–12 μg-at ammonia-N/l and a chelated trace metals + vitamins mix (Haines, 1976). The *Tapes japonica* in that study were fed algal cultures grown in deep water supplemented with the chelated trace metals + vitamins mix 85 % of the time, and with cultures grown in unsupplemented deepwater alone the remaining 15 % of the time. In the more recent experiments, no

supplement was used in the algal culture. There were also differences in light intensity and temperature between the two experiments. Previously the temperature was lower in the *Hypnea* tanks receiving supplemented deep water, where the average temperature was 22–23° C (maximum 24.6° C), and in the tanks receiving shellfish effluent where the average temperature was 26.1° C (compare with Table 1). Light intensity was also undoubtedly higher than in the previous study because the tanks used in the present experiment were shallower (0.1 m versus 0.3 m depth) and translucent rather than opaque. Furthermore this study was completed during the summer months, rather than in the winter when light intensity is lower. Our experimental conditions may have induced, in the *Hypnea musciformis* that fragmented, an increased requirement for a trace metal or vitamins caused by using an ammonia rich, high light intensity, and high temperature environment. Lapointe et al. (1976) observed fragmentation of *H. musciformis* in their tanks at the Harbor Branch Foundation (Florida, USA). They suggested that fragmentation was caused by high light intensity, rather than the high temperatures (above 28° C) in their non-nutrient limited cultures. The total dissolved-N concentration in their tanks was at least 10–20 times higher than those in our experiments. It therefore seems unlikely that the trace nutrient deficiency at high light intensities and high temperature that we suggested as a possible cause of fragmentation would occur in their system. From these studies of *H. musciformis* it is difficult to identify a specific factor(s) which causes fragmentation especially since our control *H. musciformis* did not fragment. A comprehensive study on nutrient, light, and temperature interaction is necessary before the mechanism causing fragmentation will be understood.

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