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On the role of urea in pond fertilization

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ABSTRACT

Two experiments were conducted to better understand the role of urea in pond fertilization. In Experiment 1, urea dissolved in pond water and incubated in aquaria at approximately 30°C disappeared at a rate of 303 μg urea-N l^{-1} day^{-1} under natural sunlight conditions, and 102 μg urea-N l^{-1} day^{-1} under dark conditions. Total alkalinity decreased in dark aquaria and increased in sun-exposed aquaria, consistent with stoichiometric expectations. Urea hydrolysis was negligible in distilled water exposed to light.

Experiment 2 was a toxicity test. Median lethal concentrations (LC_{50}'s) of urea at 24 and 96 h exposure were 19 700 mg l^{-1} and 16 800 mg l^{-1} for sex-reversed male Nile tilapia (*Oreochromis niloticus*) fingerlings, and 17 000 mg l^{-1} and 16 000 mg l^{-1} for silver barb (*Puntius gonionotus*) fingerlings, respectively. All test fish survived 96-h exposure to 14 000 mg urea l^{-1}, whereas no fish survived 48-h exposure to 22 000 mg urea l^{-1}.

INTRODUCTION

Urea, a major nitrogen (N) fertilizer for land-crop production, is also a popular N source for stimulating algal productivity in pond aquaculture. Purchased in crystalline form urea dissolves quickly in water, but hydrolyzes very slowly to yield carbon dioxide and ammonia according to the following reaction (Chin and Kroontje, 1963):

$$CO(NH_2)_2 + H_2O \rightarrow CO_2 + 2NH_3 \quad (1)$$

Presence of the enzyme urease, which is common among bacteria, fungi, yeasts, algae and higher plants, greatly accelerates urea decomposition (Leftley and Syrett, 1973; Morris, 1974). Although algae play the dominate role in urea decomposition in some marine environments (Mitamura and Saijo,

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1975, 1986), metabolic pathways for algal uptake of urea are still unclear. Release of ammonia in urea-grown algal cultures suggested that urea may be broken down by algae and urea-N utilized as ammonia (Little and Mah, 1970; Uchida, 1976). On the other hand, Healey (1977) reported that freshwater algae may take up urea intact, breaking it down enzymatically once inside the cell. It has been shown that algae of the families Prasinophyceae, Xanthophyceae, Bacillariophyceae, Chrysophyceae, Myxophyceae and Euglenophyceae contain urease, whereas algae of the family Chlorophyceae, including several *Chlorella* spp., contain ATP:urea amidolyase (Leftley and Syrett, 1973).

Urea occurs naturally in aquatic environments as a degradation product of dissolved and particulate organic matter (Mitamura and Matsumoto, 1981), zooplankton excretion, and bacterial degradation of purines and pyrimidines (Mitamura and Saijo, 1986). Studies using urea labelled with radioactive ^{14}C and ^{15}N have clearly demonstrated that urea is rapidly recycled and may represent a significant source of both C and N to marine phytoplankton (Mitamura and Saijo, 1975; Mitamura and Matsumoto, 1981; Kristiansen, 1983; Ignatiades, 1986). At Kaneohe Bay in Hawaii, Harvey and Caperon (1976) reported that daytime urea uptake accounted for approximately 53% of the algal N requirement.

Urea input rates used in aquaculture are orders of magnitude greater than what occurs naturally in aquatic systems. Research presented here describes two experiments designed to improve understanding of urea dynamics and utilization in pond fertilization.

MATERIALS AND METHODS

Two experiments were conducted at the Asian Institute of Technology located approximately 42 km northwest of Bangkok, Thailand (14.2°N, 100.5°E). Experiment 1 examined the decomposition of urea, while Experiment 2 evaluated urea toxicity to Nile tilapia (*Oreochromis niloticus*) and silver barb (*Puntius gonionotus*) fingerlings.

Experiment 1. Urea hydrolysis and decomposition was examined in 50 liters of water contained in 75 liter glass aquaria. Three treatments consisted of pond water exposed to light, pond water without exposure to light, and distilled water. Water temperatures ranged from 28.0°C to 33.6°C during the experiment. Aquaria were suspended in outdoor cement tanks (6 m^3). Water in aquaria and cement tanks were kept at the same level to prevent overheating of aquaria exposed to sunlight. In the dark treatment, aquaria were completely covered with black plastic cellophane to prevent algal photosynthetic activity. There were three replicate aquaria per treatment. Initial urea concentration in pond water treatments was 4 mg urea-N l^{-1}, and 5 mg urea-N l^{-1} in the distilled water treatment. Urea was first dissolved in water before

being added to aquaria. Pond water had the following initial characteristics: pH = 9.0; alkalinity = 510 mg l^{-1} $CaCO_3$; and chlorophyll a = 21 mg m^{-3}. The following water quality measurements were made daily: urea-N (urease method, Parsons et al., 1984), ammonia-N (phenolhypochlorite method; Solórzano, 1969), pH, alkalinity (potentiometric method using 0.02 N HCl to titrate the sample to pH 5.1; APHA, 1985), and minimum-maximum temperatures. Measurements continued until Day 20, when severe rains disrupted the experiment.

Experiment 2. A static bioassay was conducted in 75 liter glass aquaria filled with 60 liters of tap water kept at 25–30°C to evaluate urea toxicity to two species of fish. Sex-reversed male Nile tilapia fingerlings (mean weight of 7.1 g) were exposed to urea concentrations of 1000, 10 000, 14 000, 16 000, 18 000, 20 000, 22 000 and 25 000 mg l^{-1}. Silver barb fingerlings (mean weight of 11.1 g) were exposed to urea concentrations of 1000, 10 000, 14 000, 18 000, 22 000 and 25 000 mg l^{-1}. Fish, acclimatized in aquaria for 1 week, were fed floating pelleted feeds at 5% of body weight. Feces, uneaten feed and other solids were siphoned from the aquaria daily. One third of water in each aquarium was changed daily with the same concentration of urea. An air pump provided constant aeration to aquaria. Accumulative mortality of 20 test fish/treatment fish was determined after 12, 24, 48, 72 and 96 h. A fish was counted dead if it did not respond to touch by glass rod. Water temperature and dissolved oxygen (DO; Yellow Springs meter Model 54A) and pH were measured daily. Total ammonia was analyzed from aquaria where mortality was observed. Probit analysis (Finney, 1971) determined the urea lethal concentration at which 50% of test fish died (LC_{50}) for 24, 48 and 98 h.

Regression analyses were done according to Steel and Torrie (1980). Means are presented ($\pm$ s.e. in parentheses); statistical significance is assumed at $P < 0.05$.

RESULTS

Urea decomposition and uptake. Fig. 1 shows that urea in pond water exposed to sunlight disappeared in 15 days, whereas urea-N concentrations were still approximately 50% of initial values after 19 days in pond water kept in the dark. Linear regression analyses indicate loss rates of 303 $\pm$ 14 μg urea-N l^{-1} day^{-1} (r^2 = 0.94) and 102 $\pm$ 7 μg urea-N l^{-1} day^{-1} (r^2 = 0.79) in pond water under light and dark conditions, respectively. Urea measurements from Days 1–3 after application were contaminated and excluded from analysis. Urea concentrations in distilled water remained almost constant throughout the experiment.

Total ammonia-N concentrations steadily increased under dark conditions, peaking at about 1.8 mg N l^{-1} on Day 16 (Fig. 2). Pond water exposed to

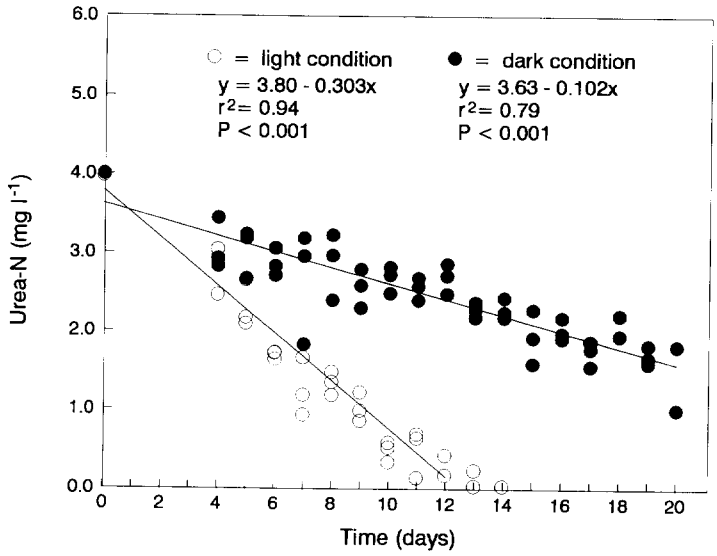

Fig. 1. Disappearance of urea-N in pond water in aquaria exposed to sunlight and kept in the dark (Experiment 1).

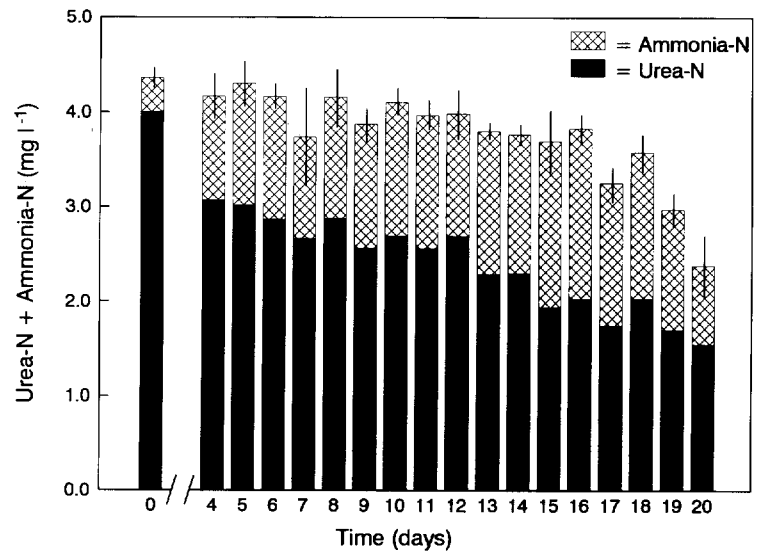

Fig. 2. Accumulative urea-N and ammonia-N concentrations (± s.e.) in aquaria kept under dark conditions (Experiment 1).

light exhibited peak ammonia-N concentrations of about 0.8 mg NH_4-N l^{-1} on Days 4 and 5, after which concentrations gradually decreased until no trace was detected by Day 15 (Fig. 3). Ammonia-N concentrations in distilled water

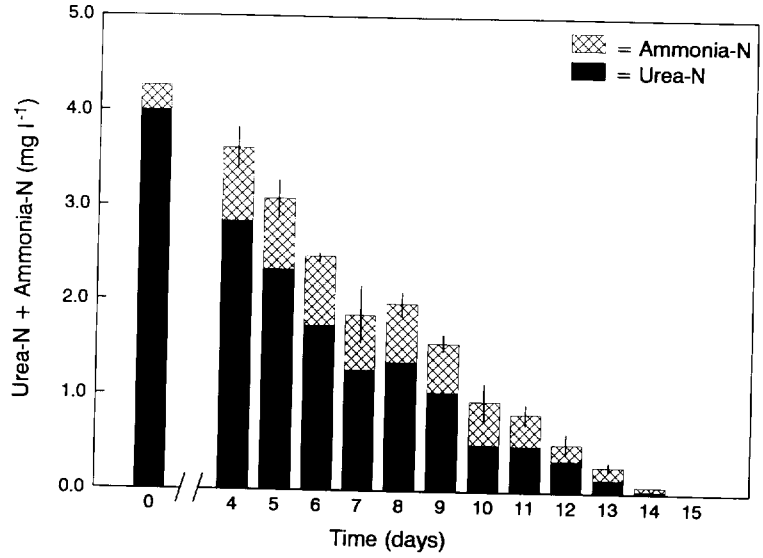

Fig. 3. Accumulative urea-N and ammonia-N concentrations (± s.e.) in aquaria exposed to ambient sunlight (Experiment 1).

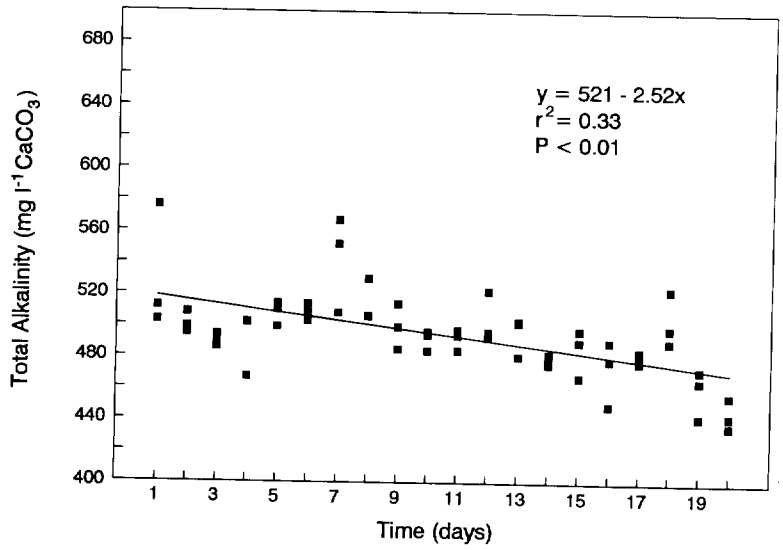

Fig. 4. Decrease of total alkalinity (mg l^{-1} $CaCO_3$) over time in pond water incubated under dark conditions (Experiment 1). Initial urea-N concentration was 4 mg l^{-1}.

remained low throughout the experiment ranging from 0.03 to 0.37 mg NH_4-N l^{-1}. The combined total of urea-N and NH_4-N essentially remained at initial concentrations for both pond water in the dark and distilled water. Under

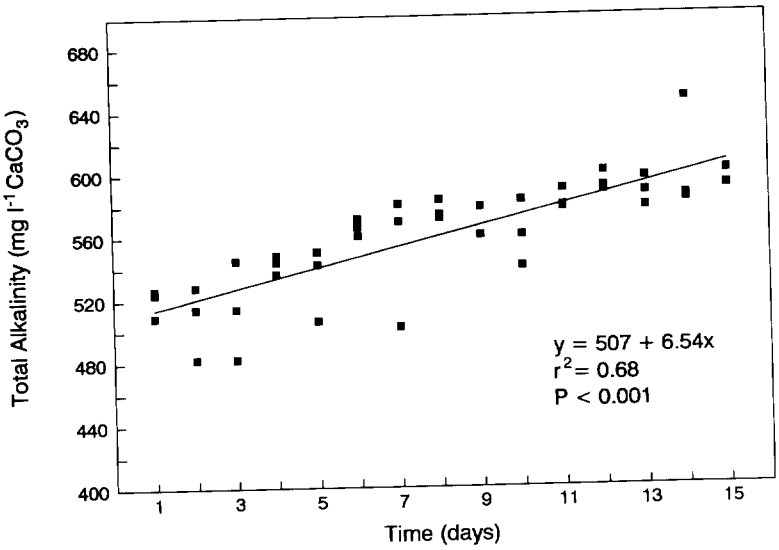

Fig. 5. Increase of total alkalinity (mg l^{-1} $CaCO_3$) over time in pond water incubated under ambient sunlight conditions (Experiment 1). Initial urea-N concentration was 4 mg l^{-1}.

lighted conditions, both forms of N had disappeared by Day 15.

Although alkalinity often showed considerable within treatment variability, a significant linear decrease was observed in the dark treatment (Fig. 4), while a significant linear increase was noted in pond water under lighted conditions (Fig. 5). No significant variations in pH were observed over time for any treatment.

Urea toxicity. Mortalities of tilapia and silver barb exposed to different urea concentrations are presented in Table 1. All test fish survived 96-h exposure to 14 000 mg urea l^{-1}, whereas no fish survived 48-h exposure to 22 000 mg urea l^{-1}. Median lethal concentrations (LC_{50}'s) of urea at 24 and 96 h exposure were 19 700 mg l^{-1} and 16 800 mg l^{-1} for tilapia, and 17 000 mg l^{-1} and 16 000 mg l^{-1} for silver barb.

Water quality measurements during the 96-h experiment gave the following ranges: temperatures from 25.4°C to 30.5°C, DO concentrations from 3.1 to 8.2 mg O_2 l^{-1}, and pH from 8.2 to 8.9. Total ammonia-N concentrations, measured in aquaria where fish mortality was observed, varied between fish species. In aquaria with tilapia, mean total ammonia concentration was 5.9 ± 0.3 mg NH_4-N l^{-1}, of which approximately 1.2 mg N l^{-1} was present as unionized ammonia (NH_3-N). For silver barb, mean total ammonia concentration was 2.0 ± 0.1 mg NH_4-N l^{-1}, or approximately 0.4 mg NH_3-N l^{-1}.

TABLE 1

Toxicity of urea to *Oreochromis niloticus* and *Puntius gonionotus* fingerlings (Experiment 2). Number represents accumulative total of dead fish

Number of fish	Exposure time (h)	Concentration of urea (mg l^{-1})							
		1000	10 000	14 000	16 000	18 000	20 000	22 000	25 000
Oreochromis niloticus									
20	12	0	0	0	0	0	1	4	10
20	24	0	0	0	1	2	3	17	20
20	48	0	0	0	2	5	11	20	20
20	72	0	0	0	4	8	16	20	20
20	96	0	0	0	5	15	19	20	20
Puntius gonionotus									
20	12	0	0	0		0		11	20
20	24	0	0	0		2		17	20
20	48	0	0	0		5		20	20
20	72	0	0	0		10		20	20
20	96	0	0	0		20		20	20

DISCUSSION

Negligible urea decomposition rates in distilled water supported observations of Chin and Kroontje (1963) that chemical disassociation of urea is slow and requires enzymatic activity. In pond water, linear regressions from Experiment 1 gave urea decomposition rates of 102 μg urea-N l^{-1} day^{-1} under dark conditions, and 303 μg urea-N l^{-1} day^{-1} when exposed to daylight (Fig. 1). Assuming 12 h of daylight per 24-h day and similar rates between treatments under nighttime/dark conditions, urea decomposition in pond water exposed to daylight was about 17 μg urea-N l^{-1} h^{-1} as compared to about 4 μg urea-N l^{-1} h^{-1} in water kept completely dark. These results support investigations demonstrating a strong relationship between urea decomposition and algal photosynthetic activity (Ignatiades, 1986; Mitamura, 1986a,b). In eutrophic Kaneohe Bay (Hawaii), Harvey and Caperon (1976) obtained dark decomposition rates of about 30% of daytime measurements, a proportion similar to that found in this study. It is not clear whether dark uptake rates represent effects of residual algal urease, bacterial activity, and/or heterotrophic uptake by algae (Ignatiades, 1986). It has been reported, however, that urea assimilation by algae is suppressed by ammonium concentrations of 1–2 μg-at N l^{-1} (Kristiansen, 1983; Mitamura, 1986b). Results here do not support those conclusions, as Fig. 3 indicates high urea uptake rates in the presence of up to 1 mg ammonia-N l^{-1} (71 μg-at N l^{-1}).

Mitamura and Saijo (1975) and Mitamura and Matsumoto (1981) showed that urea can be a source of both N and C for phytoplankton in coastal waters.

In the former study 38–84% of decomposed urea-C was assimilated, yet urea-C represented only about 0.5% of total inorganic C incorporated. This supports the possibility that algae cleave urea into ammonia and CO_2 internally (Healey, 1977). A pond fertilized with urea at a rate of 0.4 g urea-N m^{-2} day^{-1} would receive approximately 0.34 g urea-C m^{-2} day^{-1}. Although this would represent less than 10% of the algal-C requirement in a productive pond, urea could still make a small but significant C contribution in a C-limited system.

Urea decomposition and uptake rates in natural waters often follow first-order kinetics, or a hyperbolic function of ambient concentrations (Harvey and Caperon, 1976; Horrigan and McCarthy, 1981; Kristiansen, 1983; Mitamura, 1986a). Those studies are in contrast to the linear decomposition rates illustrated in Fig. 1. Mitamura and Saijo (1975), however, also reported linear urea decomposition rates in coastal waters of Mikawa Bay of 0.24 μg urea-N l^{-1} h^{-1} and 0.02 μg urea-N l^{-1} h^{-1} under light and dark conditions, respectively. These rates are about two orders of magnitude less than our results in pond water, differences which probably reflect both lower ambient urea concentrations and algal productivity in Mikawa Bay. Warmer temperatures in our study also may have facilitated urea metabolism; maximum decomposition rates occur at about 30°C with a Q_{10} value of 1.8 (Mitamura, 1986b).

Urea loss rates reported for fertilized systems more closely compare to our results. Pautong (1991) measured a decomposition rate of 350 μg urea-N l^{-1} day^{-1} in culture tanks receiving 21 kg urea-N ha^{-1} $week^{-1}$. In fertilized flooded rice fields Cao et al. (1983) reported a disappearance of 15 kg urea-N ha^{-1} 5 $days^{-1}$ ($\approx$ 300 mg urea-N m^{-2} day^{-1}), while Mosier et al. (1989) reported a disappearance of 80 kg urea-N ha^{-1} 4 $days^{-1}$ ($\approx$ 2000 mg urea-N/m^2/day). Assuming a pond of 1 m depth, equivalent decomposition rate from Experiment 1 would be approximately 300 mg urea-N m^{-2} day^{-1}.

Because of apparent linearity (Fig. 1), urea decomposition rates can be used to estimate efficient fertilization frequency schedules based on desired rates of urea-N input and maintenance of a N-limited system. Actual urea loss rates in earthen ponds may be greater than reported here, however, as contact with bacteria-rich sediments may accelerate urea decomposition (C.E. Boyd, personal communication, 1992).

Alkalinity changes due to urea decomposition depend on ammonia production and oxidation processes. Urea hydrolyzed in water yields NH_3 and CO_2 [1]. Ammonia is basic and combines with water to produce NH_4^+ and OH^- [2].

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^- \qquad (2)$$

If NH_4^+ is oxidized to NO_3^-, the net result is two equivalents of H^+ per oxidized mole of urea and a reduction of total alkalinity (Hunt and Boyd,

1981). Experiments by Hunt and Boyd (1981) demonstrating alkalinity losses through urea oxidation were conducted in the dark, preventing any algal uptake of ammonia. Similar reductions in alkalinity were noted in dark aquaria from Experiment 1 (Fig. 4). But if phytoplankton utilize NH_4^+ before it can be oxidized to nitrate, according to equation [2] alkalinities should increase with the hydrolysis of urea. Increased alkalinities observed in aquaria exposed to sunlight in Experiment 1 (Fig. 5) support this conclusion. Vlek and Craswell (1979) also reported an increase in pH and alkalinity in flooded rice fields after urea application. Magnitudes of alkalinity losses and gains in Experiment 1 were greater than can be accounted for by urea decomposition alone, and probably reflect additional ammonia production and recycling from metabolized particulate and dissolved organic N in pond water.

Urea toxicity to fish appears to be of little concern. Tests showed that urea concentrations of up to 14 000 mg l^{-1} resulted in no silver barb or tilapia mortalities after 96 h (Table 1). This concentration approximates weekly applications of > 900 kg urea-N ha^{-1} day^{-1} in a 1 m deep pond, easily 200 times reasonable loading rates. Results for tilapia and silver barb compare with 96-h mortality thresholds of 1000 mg l^{-1} for *Labeo rohita* fry (Triphati et al., 1974) and 20 000 mg l^{-1} for *Oreochromis mossambicus* (Planichamy et al., 1985). Sarkar and Konar (1985), however, reported that feeding activity in *O. mossambicus* can be affected by urea concentrations of about 400 mg l^{-1} or less.

Urea appears to be an excellent fertilizer for stimulating natural food production in pond aquaculture. Urea is readily utilized by phytoplankton as a source of N and possibly C, increases alkalinity under N limiting conditions, and has no apparent toxicity to fish at reasonable loading rates. Urea is also relatively inexpensive. On a gross weight basis urea is often ten times more expensive than commercially available manures; but as per kg of available (i.e., soluble) N, urea may be up to 7 times less expensive than chicken manure (Knud-Hansen et al., in press).

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