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Effect of temperature, stocking density and fish size on the ammonia excretion in palmetto bass (*Morone saxatilis* × *M. chrysops*)

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Abstract

Three temperature levels (18, 23 and 28 °C), three stocking density levels (0.72, 1.42 and 2.84 kg m^{-3}) and two sizes of groups (averaging 104 and 173 g respectively) were used to conduct experiments on the effect of temperature, stocking density and fish size on the ammonia excretion (AE) of palmetto bass (*Morone saxatilis* × *M. chrysops*). The AE increased with the increase in temperatures by a significant degree ($P < 0.05$) among different temperature groups. There appeared to be a tendency towards increase of the AE with increased stocking densities, and significant differences ($P < 0.05$) were found among stocking groups. The AE of smaller size-group was significantly higher than that of the larger size-group. Diurnal variation of AE in palmetto bass showed that the AE rose greatly to reach a peak at about 4 h after feeding, and to the lowest values at about 24 h post feeding in all of the experimental groups.

Keywords: temperature, stocking density, fish size, ammonia excretion, palmetto bass

Introduction

In general, oxygen consumption rates are the most commonly used index to measure the metabolic activity of fish. Brett and Zala (1975) described a general parallelism between oxygen consumption rates and ammonia excretion (AE) rates of sockeye salmon. Besides, ammonia is the main nitrogenous excretory product of fish and is derived from dietary protein catabolism. The AE rate is, therefore, considered a metabolic rate of fish (Dabrowski 1986). In addition, the toxicity of nitrogenous excretory product is the limiting factor in intensive fish culture (Brafield 1985). The sublethal environmental ammonia concentrations resulted in the stress of fish and caused retarded growth (Meade 1985; Frances, Nowak & Allan 2000). In such a case, ammonia is considered toxic and must be excreted or converted into a less toxic substance such as nitrites and nitrates (Økelsrud & Pearson 2007).

Changes in quality and quantity of dietary proteins could affect protein metabolism (Lied & Braaten 1984). Furthermore, the AE rate increases positively in response to protein intake (Brett & Zala 1975; Yang, Liou & Liu 2002). As a result, AE could be potentially used as an index of dietary protein utilization for selecting candidate culture fish (Brett & Zala 1975; Jobling 1981; Yigit, Koshio, Aral, Karaali & Karayucel 2003).

On the other hand, high ammonia concentration may limit fish production. Information on AE would be useful in determining the water flow rates and biological filter size required to avoid excess of ammonia concentration (Forsberg & Summerfelt 1992a). As a whole, measurement of AE may be an important indicator of the effect of various environmental and nutritional factors on protein metabolism (Jobling 1981; Forsberg & Summerfelt 1992a; Kelly & Kohler 2003).

It has been stated that the metabolic rate of fish, measured by oxygen consumption or AE, varied directly by temperature (Fry 1971; Brett & Groves 1979; Forsberg & Summerfelt 1992a) and inversely with individual fish mass (Fry 1971; Piper, McElwain, Orme, McCraren, Fowler & Leonard 1982; Cai & Summerfelt 1992). Aside from this, the AE rate is found to be also related to the fish size (Porter, Krom, Robbins, Brickell & Davidson 1987; Cai & Summerfelt 1992; Kikuchi 1995). Palmetto bass (*Morone saxatilis* × *M. chrysops*) have received considerable attention in Taiwan culture, as a food fish and for use in fee-fishing ponds. This study was conducted, therefore, to elucidate the effect of temperature, stocking density and fish size on the AE in palmetto bass, given that minimal information is available in this respect. The data established in this study may be useful for reference on the aquaculture management of this species.

Materials and methods

Fish and rearing conditions

Palmetto bass juveniles, obtained from a private fish farm in the southern Taiwan, were maintained in an outdoor concrete pond (8.0 × 7.9 × 0.8 m) until required for the trial. Before each experiment, fish were transported from the holding pond to six indoor recirculating freshwater tanks (2.0 × 1.7 × 0.7 m), each holding 1700 L of water at a water depth of 0.5 m. The tanks were maintained under a 12 L:12 D photoperiod and supplied with filtered, aerated and thermostatically controlled (26 ± 0.5 °C) well water at a flow rate of 96 L min^{-1}. Fish were acclimated for 2 weeks before commencing the experiments. During the period of acclimation and experiments, fish were fed to satiation twice daily with commercial floating pelleted feed (0.45 cm diameter). The proximate composition of the feed on wet basis was 8.9% moisture, 51.1% protein, 8.0% lipid and 11.5% ash.

Experiment 1 – effect of temperature on AE

The experiment involved three temperature treatments of 18, 23 and 28 °C with two replicates per treatment. This temperature range was selected to reflect nearly natural culture condition in Taiwan. Temperature in each tank was adjusted at a rate of approximately 1 °C day^{-1} to attain the experimental temperature. On the final day of acclimation, 60 fish with even size were selected (31.41 ± 6.91 g) and randomly restocked in each tank. Total ammonia nitrogen (TAN) was determined 21 days after the commencement of the experiment. On the day of measuring, fish were fed twice, once at 09:00 hours and then at 15:00 hours.

Experiment 2 – effect of stocking density on AE

The experimental water temperature was maintained at 26 ± 0.5 °C in both experiment 2 and experiment 3 and was reached within 3 days. This temperature was selected to optimize growth and feed efficiency for palmetto bass (Woiwode & Adelman 1991). At the end of acclimation, the fish were weighed and measured before the start of the experiment. The fish were randomly distributed into each tank at an evenly sized mean weight of 30.34 g (SD = 5.88). They were divided into two replicate groups of 40, 80 and 160 fish, representing low, medium and high density groups of 0.7, 1.4 and 2.8 kg m^{-3}. On day 21, AE was analysed and fish were fed once at 09:00 hours.

Experiment 3 – effect of fish size on AE

After acclimatization, a random sample of those larger fish were sieved and graded into two weight groups (mean): 101–109 (104) and 169–180 (173) g. The number of fish held in each of the four tanks (two replicates per treatment) varied with fish size: 61 fish in 104 g groups and 36 fish in 173 g groups. AE were measured on day 21 and day 42. Fish were fed once at 09:00 hours on the day of measuring.

AE analysis

Two 125 mL samples of tank water were collected and pooled together and then three sub-samples were analysed for TAN concentration using the automated phenate method (American Public Health Association, American Water Works Association & Water Environment Federation 1995). Ammonia excretion was calculated by stopping tank inflow for 1 h and measuring the resulting decrease in tank AE (Forsberg & Summerfelt 1992b). On the day AE was analysed, samples of tank water at the start (and the end) of stopping inflow were collected at 09:30 (10:30) hours, 13:30 (14:30) hours, 17:30 (18:30) hours, 21:30 (22:30) hours and next day 09:30 (10:30) hours. Ammonia excretion was calculated for each tank and each sampling time using the formula:

$$\text{mg TAN} \times \text{kg}^{-1} \times \text{h}^{-1} = ([\text{TAN}]_{60} - [\text{TAN}]_0) \times \text{tank water volume (L)} \times \text{biomass}^{-1}(\text{kg})$$

where $[\text{TAN}]_0$ and $[\text{TAN}]_{60}$ = concentration at time 0 and 60 min after the inflow water was shut off.

A measurement of total fish weight was taken on the next day when AE was measured.

Statistical analysis

Data was analysed using one-way analysis of variance (ANOVA), comparisons between treatment means were made with Duncan's multiple range test, statistical results were considered significant if $P < 0.05$ in experiments 1 and 2. Student's *t*-test was used to assess whether the means of two size-groups in experiment 3 were statistically different from each other at the 5% significance level.

Results

The AE in palmetto bass of the 18, 23 and 28 °C groups is presented in Fig. 1. The AE increased with the increase in temperatures and were significantly different ($P < 0.05$) among the groups.

On day 21, the daily AE in palmetto bass of the three temperature groups is given in Fig. 2. The AE reached sharp peaks about 4 h after feeding was initiated. It then declined, and elevated again to moderate peaks at about 12 h, before dropping to the lowest values at 24 h for all the temperature groups.

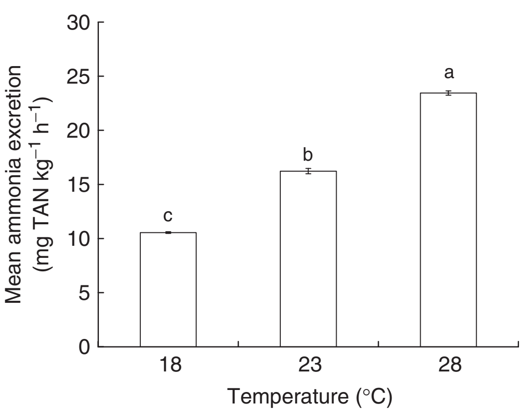

Figure 1 Rates of ammonia excretion (mean ± SD) in palmetto bass at 18, 23, and 28 °C groups. Vertical bars not sharing the same letter are significantly different, $P < 0.05$.

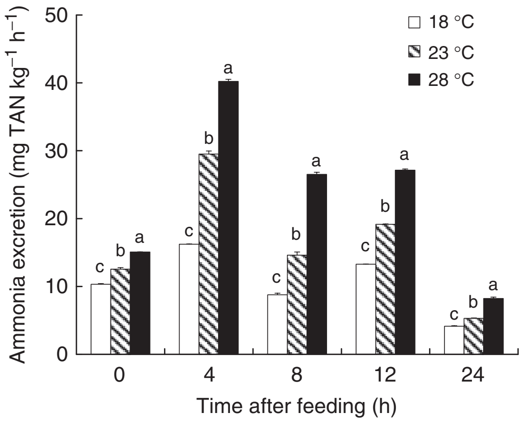

Figure 2 Daily ammonia excretion rate of palmetto bass at 18, 23 and 28 °C groups. Vertical bars not sharing the same letter are significantly different, $P < 0.05$.

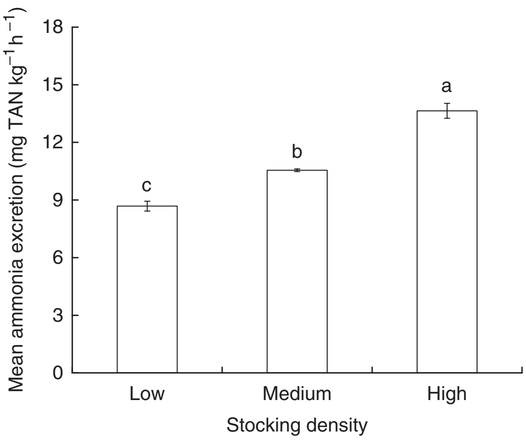

Figure 3 Rates of ammonia excretion (mean ± SD) of palmetto bass at different stocking densities over a period of 24 h. Vertical bars not sharing the same letter are significantly different, $P < 0.05$.

The AE of palmetto bass for different stocking densities is detailed in Fig. 3. There appeared to be a tendency toward increasing AE with increased stocking densities, and significant differences were found among stocking groups. In regard to the daily AE, the highest daily AE values occurred 4 h after feeding and the lowest values were found 24 h after feeding in all the three stocking densities (Fig. 4). Difference in the AE among all the stocking densities was significant in spite of measuring time.

The AE was inversely related to fish size as shown in Table 1. The AE of smaller size group was significantly higher than that of the larger size group during both analysing periods. The AE was higher on the first measuring day than the second, regardless of the

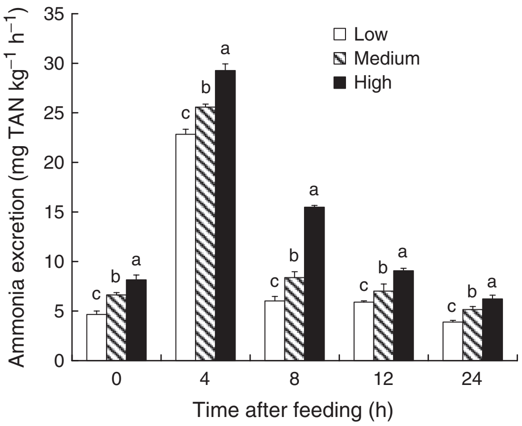

Figure 4 Daily ammonia excretion rate of palmetto bass at high, medium and low stocking densities after feeding. Vertical bars not sharing the same letter are significantly different, $P < 0.05$.

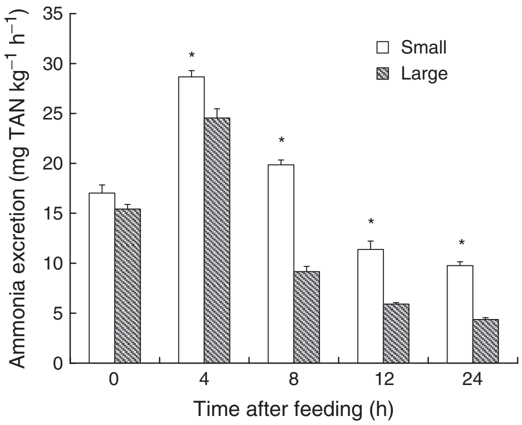

Figure 5 Daily ammonia excretion rate of two size-groups of palmetto bass on day 21. Each data represent an average value from replicates ± SD. The columns marked with asterisk are significantly different between the size-groups at the same sampling time.

Table 1 Rates of ammonia excretion (mean ± SD) of two size-groups of palmetto bass[1]

	Mean ammonia excretion* ($mgTAN\,kg^{-1}\,h^{-1}$)	
	104 g fish	173 g fish
Day 21	17.34 ± 0.62	11.88 ± 0.45
Day 42	14.30 ± 0.40	8.57 ± 0.54

[1]Values of size-groups in day 21 and day 42 are significantly different ($P < 0.05$) respectively.
*Mean excretion rate over a 24-h period.

size of the group. Figure 5 illustrates the daily changes in the AE between two size groups of palmetto bass on day 21. The AE rose sharply to reach a peak 4 h after feeding, and then declined to the lowest value at 24 h post feeding. Moreover, the AE of the smaller size group was higher than that of the large size group, irrespective of measuring time. A similar trend of hourly change was found in the AE measuring on day 42, with the exception of a slight elevation in AE of the larger size group 12 h after feeding (Fig. 6).

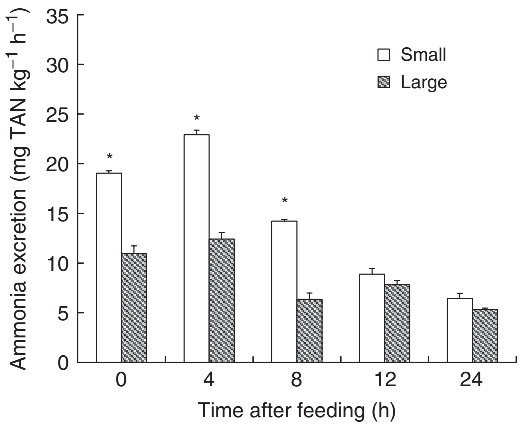

Figure 6 Daily ammonia excretion rate of two size-groups of palmetto bass on day 42. Each data represent an average value from replicates ± SD. The columns marked with asterisk are significantly different between the size-groups at the same sampling time.

Discussion

Given that ammonia represents the end nitrogenous metabolite excreted by fish, the measurement of AE is then recognized as an indicator to evaluate the metabolic activity of fish (Brafield 1985; Dabrowski 1986). Generally, temperature has great effect on metabolic rate of fish, and has been consequently shown to be related to the AE of fish. The increase in AE with increasing water temperatures found in present study agrees with reports for other fish species (Dabrowski 1986; Cai & Summerfelt 1992; Kikuchi 1995; Person-Le Ruyet, Mahe, Le Bayon & Le Delliou 2004). In higher water temperatures, higher energy demand is met, partially, through the transamination and deamination of dietary amino acids with the resultant excretion of ammonia, and the release of carbon skeletons utilized as an energy source (Lied & Braaten 1984; Forsberg & Summerfelt 1992a).

In an intensive aquaculture system, production of toxic metabolic wastes is one of the major factors limiting stocking density of fish. In our study, the higher value of AE at higher stocking densities has demonstrated that the AE is proportional to the stocking density. The finding is consistent with some related reports (Piper *et al.* 1982; Cai & Summerfelt 1992). Under high stocking density conditions, the AE increased with the increasing fish biomass. On the other hand, higher levels of social hierarchy (aggressive behaviour) at higher stocking density, associated with elevated metabolic rate (Rosa, Beckerb & Oliveira 2006), will result in the increase in AE.

The AE is also influenced by fish size, as indicated by some reports (Jobling 1981; Cai & Summerfelt 1992; Zhang, Goodwin, Pfeiffer & Thomforde 2004). In this study, the AE was inversely related to fish size. This is in accordance with the results from other studies (Ming 1985; Porter *et al.* 1987; Almendras 1994; Kikuchi 1995; Zhang *et al.* 2004). It is found in this study that there's a tendency for the AE to increase with time in all the experimental size-groups, and this is the reason why extra AE was determined on day 42. As indicated by previous studies (Porter *et al.* 1987; Zhang *et al.* 2004), this is a function of amount excreted/unit biomass that the smaller fish excrete metabolites at a markedly higher rate than the larger fish.

In addition to temperature and fish size, AE is also affected by feeding (Ming 1985; Gallagher & Matthews 1987; Seginer 2008). After feeding, fish usually increase their metabolic rate as observed by an increase in AE (Kaushik & Dabrowski 1983; Seginer 2008). Therefore, fish will exhibit a diurnal variation of AE. From the four times of AE measurements, our data showed that AE reached a sharp peak 4 h after feeding, as found by Brett and Zala (1975) for the sockeye salmon and Porter *et al.* (1987) for the gilthead seabream. However, disparate results were reported as 3–6 h for Japanese flounder (Kikuchi 1995), 3 h for sea bass (Almendras 1994), 5–6 h for cod (Lied & Braaten 1984), 10 h for common carp (Kaushik 1980) and 12 h for rainbow trout (Gunther, Brune & Gall 1981). These discrepancies are probably due to the difference in fish species, feeding time and feeding frequency (Fry 1971; Forsberg & Summerfelt 1992a; Seginer 2008).

Probably due to the two feeding times, there appeared to be two peaks in our temperature experiment, similar observation was described by Rychly and Marina (1977) and Porter *et al.* (1987). In contrast, different results with one single peak were obtained by many other authors, despite of feeding frequency. It may be assumed that the fluctuation of nitrogen excretion is related to fish activity and oxygen consumption, of which their daily rhythm had been examined. Further, it was reported that the cortisol has an influence on the AE in some fish species and would exhibit a diurnal rhythm, reflecting the circadian biorhythms in the AE (Webb & Wood 1998).

According to Brett and Zala (1975), the AE of fish starved over 24 h represented a near approximation of the endogenous nitrogen fraction, whereas the exogenous nitrogen was derived from the food intake or more precisely, nitrogen intake. Therefore, post-feeding increasing AE is considered as the oxidation of exogenous rather than endogenous amino acids. When the fasting time was not long enough, the measured AE might partially be derived from dietary protein, in other words, the AE was not fully endogenous nitrogen fraction. In this regard, the varied food deprivation time of 10 h for sea bass, 25 h for cod or even 1 week for common carp and rainbow trout were demonstrated by some researchers (Kaushik 1980; Lied & Braaten 1984; Almendras 1994). The baseline level of AE was observed 24 h after feeding in our data, however, the partitioning of endogenous nitrogen in AE obtained in the present study needs further proof.

In conclusion, AE increased with increasing temperatures and stocking densities. On the contrary, AE varied inversely with individual fish size. Diurnal variation of AE in palmetto bass showed that AE increased to a sharp peak at about 4 h after feeding was initiated, and to the lowest value at about 24 h.

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