Aquaculture 319 (2011) 162–167

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Ammonia excretion of octopus (*Octopus vulgaris*) in relation to body weight and protein intake

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ARTICLE INFO

Article history:
Received 3 February 2010
Received in revised form 17 May 2011
Accepted 11 June 2011
Available online 2 July 2011

Keywords:
Octopus vulgaris
Ammonia excretion
Body weight
Protein intake

ABSTRACT

The rate of total ammonia excretion (U, mg TAN d^{-1}) in relation to body weight (W, 490–3460 g) and absolute protein feeding rate (APFR, 0.00–11.90 g d^{-1}) was studied in octopus (*Octopus vulgaris*). Using multiple regression analysis, the data obtained were fitted to the equation $U = 13.91 + 0.07W + 0.02W \times$ APFR, which explained 95% of the variance observed. According to this equation, there was a significant interaction between W and APFR ($W \times$ APFR), the coefficient of W varying linearly with APFR ($b' = 0.07 + 0.02$APFR). When U was expressed as a function of body weight (Ur, mg TAN kg^{-1} body weight d^{-1}), Ur basically varied as function of APFR, showing a value of 84 mg TAN kg^{-1} body weight d^{-1} when the octopus was not fed and 265 mg TAN kg^{-1} body weight d^{-1} when protein intake was 9 g d^{-1}. U increases linearly with body weight from 10 to 57 g TAN kg^{-1} feed dry matter d^{-1} when APFR is 9 g d^{-1}.

1. Introduction

Octopus vulgaris is characterised by fast growth, which may exceed 5% of body weight per day and high food efficiency, 30–60% of ingested food being incorporated in its own weight (Mangold and Boletzky, 1973; Mangold, 1983; Aguado Giménez and García García, 2002; García García and Cerezo Valverde, 2006; Rodríguez et al., 2006). However, in trials in tanks and in cages in the Mediterranean, these high performance figures have only been obtained between 16 and 21 °C, while mortality increases significantly at temperatures above 22 °C (Aguado Giménez and García García 2002, García García et al., 2009), although satisfactory results have been achieved in small specimens (50–150 g) up to temperatures of 25 °C (Miliou et al., 2005).

In the Mediterranean Sea, the water temperature varies widely from 10 to 14 °C in winter to 25–27 °C in summer (García García et al., 2009), limiting the ongrowing of this species to 7–8 months of the year. This has led to the suggestion that *O. vulgaris* may be reared in a recirculating aquaculture system (RAS) with temperature control (Aguado Giménez and García García, 2002; Miliou et al., 2005). In a RAS, it is especially important to know the rate of nitrogen excretion in relation to dietary protein to help both with the design of biological filters and with the daily management of the facility. The effect of diet on excretion rate has been widely described in fish, and the daily excretion rate of nitrogen directly related to the nitrogen consumed (Rychly, 1980; Beamish and Thomas, 1984; Chakraborty et al., 1992; Ballestrazzi et al., 1994; Dosdat et al., 1995; Engin and Carter, 2001; Yang et al., 2002; Sun et al., 2007).

However, in cephalopods information on this subject is scarce, although, as in most aquatic organisms, cephalopods are ammonotelic organisms, nitrogen end-products being eliminated mainly as soluble ammonia (Potts, 1965; Boucher-Rodoni and Mangold, 1985, 1988, 1989; Katsanevakis et al., 2005), although urea has been reported to represent another significant source of waste nitrogen in some species (Hoeger et al., 1987). In *O. vulgaris*, Boucher-Rodoni and Mangold (1985) studied ammonia excretion during feeding and starvation in specimens varying in weight from 492 to 660 g. Subsequently, Katsanevakis et al. (2005) studied the effect of weight and temperature on fasting specimens of 19 to 1210 g. However, no information exists concerning how excretion rates may vary as a result of food intake and, more specifically, protein intake.

The purpose of the present study was to evaluate the effect of body weight and level of protein intake on the rate of ammonium excretion in *O vulgaris*, and to develop an equation for estimating nitrogen waste due to these two factors, since such information would be useful in aquaculture, particularly in recirculating aquaculture systems (RAS).

2. Materials and methods

The octopus specimens (body weight, 490–3,460 g) were caught by trawling in the Mediterranean Sea. After transport to the aquaculture laboratory, the animals were placed in a 4 m^3 tank containing PVC tubes as shelters and with an open flow-through seawater system. The octopuses were fed once per day to satiation with fillet of the frozen bogue (*Boops boops*). Octopuses were considered to have become

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doi:10.1016/j.aquaculture.2011.06.017

acclimatised to the captive conditions when they consumed the amount of food approximately corresponding to their body weight and water temperature according to Aguado Giménez and García García (2002), which occurred in our case before two weeks. Then the octopuses were brought to the incubation chamber where the ammonium excretion rate was to be determined.

Experiments to determine the excretion rate were performed in six incubation chambers, three of them with a water storage volume of 168 l and three of 49 l. The smaller volume was used for small specimens weighing less than 700 g. All the incubation chambers were covered by a methacrylate sheet, with a 6 mm diameter hole for taking water samples (Fig. 1). All aquaria contained a PVC tube as refuge for the octopuses, two flexible tubes for bubbling air and a small underwater pump to ensure that the water was mixed well (Fig. 1). The incubation chambers formed part of a recirculating experimental system, and were equipped with mechanical and biological filters, an ultraviolet lamp and a heat pump to regulate the water temperature (Fig. 1). Each incubation chambers housed a single octopus and experiments always took place after a two weeks acclimation period. During this period, the octopus was fed fillet of the frozen bogue, except the day before the experiment in which food was not supplied. Temperature was 19 ± 0.8 °C, dissolved oxygen values of above 80% saturation (Cerezo Valverde and García García, 2005), salinity of 38 ppm and a 12L/12D photoperiod.

The water flow was discontinuous, taking advantage of the time that circulation was suspended to determine ammonia production. When the flow was restarted, the TAN concentration in each incubation chamber was restored. The experiments began at 08:00 hours – when water was flowing through the aquarium – and a flexible plastic tube was used to take a 100 ml sample to measure the initial total ammonia nitrogen in mg $N\,l^{-1}$ (TAN, mg $N-NH_3+N-NH_4^+\,l^{-1}$). All the samples were fixed with 0.1 ml HCL (2N) and were kept in a refrigerator until measurement (always within 24 hours). An ion selective electrode with an ammonia-permeable membrane (ORION Model 95-12) coupled to a display (Orion Model 720 A) was used to measure TAN. Measurements were made in 50 ml samples, adding 1 ml of a commercial solution (Ionic Strength Adjustor (ISA), Orion), which contains a strong base to displace the equilibrium towards the formation of ammonia gas, a pH indicator to ensure the correct value, and a chelating agent that prevents the formation of salts with the ammonia and interference from other ions. The electrode membrane was replaced before each series of readings and calibration was carried out with five standards of known concentration (0.014, 0.028, 0.139, 0.560 and 1.399 mg/l $N-NH_3+N-NH^{+4}$) made from synthetic sea water and a standard solution of 0.1 M of NH_4Cl.

Also measured were water temperature, dissolved oxygen and pH using a multiparametric probe (model YSI 556). After taking the water samples, incubation chamber was sealed to make the accumulation of ammonia in the incubation chamber. After 1 hour, the temperature, dissolved oxygen saturation and pH were measured again and a sample was taken to measure the TAN. The water flow was then re-established (the concentration of ammonia in the water started to decrease). At the end of this period the oxygen saturation values were always above 70%, and therefore within the range considered optimal for this species (Cerezo Valverde and García García, 2005).

After 1 hour, the above procedure was repeated, and so on for 24 hours. In this way, a measure of ammonia production was obtained for each two hour cycle. When the experiment finished, the specimen was weighed (W, in g) and its sex was determined by observation of hectocotylus of the males, which is found at the extremity of the third right arm.

The absolute rate of excretion in each one hour period was calculated as the difference in TAN concentration between the measurement made when the water flow was interrupted (TAN_i, in mg l^{-1}) and that obtained just before re-connecting the flow water (TAN_f, in mg l^{-1}), as follows $(TAN_f\text{-}TAN_i)V$, where V is the volume of aquarium water in litres.

Bogue fillets were supplied at 11:00 hours, while uneaten food was withdrawn after 4 hours, dried with absorbent paper and weighed. Previously, three groups of octopuses had been established according to body weight: 400–1000 g, 1000–2000 g and above 2000 g. In the first group seven octopuses were used while in the other two groups five octopuses were used, since the greatest changes in U due to body weight could occur for smaller specimens. No food was provided to one specimen of each of these groups, while the rest were given different amount of food (Table 1). Proximate analyses of the diet were based on AOAC procedures (1997): 74.6% moisture, 17.0% protein, 3.9% lipids, and 4.5% ash. The digestibility of bogue protein is 98% (Mazón et al., 2007). The amount of bogue intake by each octopus is shown in Table 1, where it is expressed as daily specific feeding rate (SFR, % body weight d^{-1}) and as daily absolute protein feeding rate (APFR, g d^{-1}).

All measurements were made between November and April to avoid mature females, since in our geographical conditions the gonads mature between May and June. Mature males, on the other hand, are found throughout the year and from the time they reach 500–600 g.

The excretion rate (U, expressed in mg TAN day^{-1}) was measured in 17 octopuses. The independence of the variables body weight (W, in g)

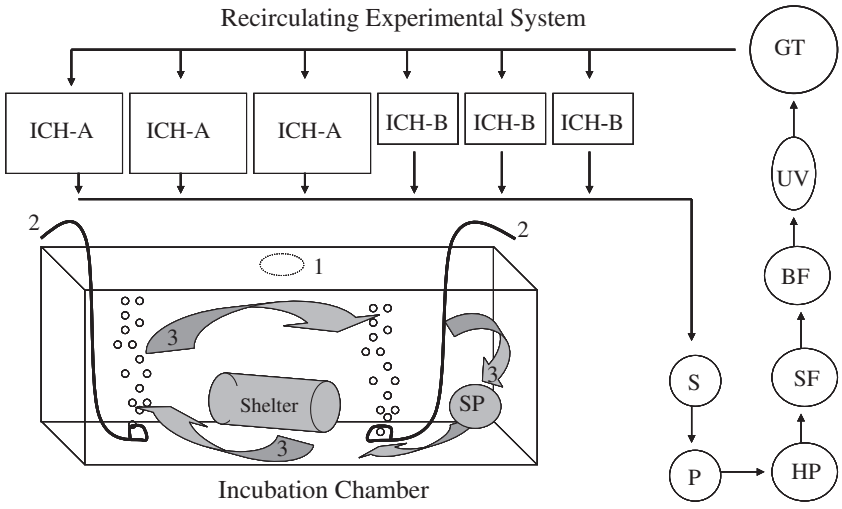

Fig. 1. Schematic diagram of the recirculating experimental system for excretion studies: ICH-A: 168 l incubation chamber, ICH-B: 49 l incubation chamber, S: solids filter, P: pump, HP: heat pump, SF: sand filter, BF: biological filter, UV: ultraviolet lamp, GT: gas transfer. Incubation chamber. 1: 6 cm circular aperture to take water samples, 2: outlets for bubbling air, 3: seawater circulation, SP: submergible pump to mix water, Shelter: PVC pipe to octopus.