Aquaculture 279 (2008) 160–165

Contents lists available at ScienceDirect

Aquaculture

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

Effect of light on oxygen consumption and ammonia excretion in *Haliotis discus discus*, *H. gigantea*, *H. madaka* and their hybrids

Faruq Ahmed, Susumu Segawa, Masashi Yokota, Seiichi Watanabe *

Department of Marine Bioscience, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477

ARTICLE INFO

Article history:
Received 9 June 2007
Received in revised form 12 March 2008
Accepted 21 March 2008

Keywords:
Abalone
Oxygen consumption
Ammonia excretion
Hybridization

ABSTRACT

Oxygen consumption and ammonia excretion rates of three abalone species, *Haliotis discus discus*, *H. gigantea*, *H. madaka* and their hybrids were measured at 20 °C by incubating for 3 h under dark and light (N=9–13 for each species and hybrid). Animals were fasted before and during the experiment and measurements were made first under dark followed by light on the next day. The rates increased with the increase in body weight and were higher under light than dark. On average, *H. discus discus* had higher oxygen consumption (DD; dark=0.039, light=0.04 ml/g/h) than *H. gigantea* (G; D=0.033, L=0.036) and *H. madaka* (M; D=0.034, L=0.035); the hybrids had varied patterns with respect to their parental species [DD×M (D=0.032, L=0.038); M×DD (D=0.03, L=0.038); G×DD (D=0.035, L=0.04) and DD×G (D=0.03, L=0.034), mother first]. M (0.261, 0.298 μmol/g/h) had the highest ammonia excretion rate while G (0.162; 0.264) and DD (0.229; 0.232) had the lowest under dark and light, respectively. The hybrids had varied patterns in comparison with their parents (DD×M=0.247, 0.32;M×DD=0.177, 0.28; DD×G=0.249, 0.364 and G×DD=0.116, 0.155). The O/N ratios under both conditions in all species and hybrids indicated that they had carbohydrate dominated metabolism. Results demonstrated physiological variability among the species and hybrids indicating necessity of different strategies for their management and aquaculture.

1. Introduction

The abalones *Haliotis discus discus*, *H. gigantea*, *H. madaka* are three economically important species found on the Japanese coasts along the Pacific Ocean and Japan Sea south of Hokkaido (Ino, 1980). Aquaculture and restocking of these species are being conducted as a countermeasure to declining natural stocks. Hybridization of abalones has been suggested as a possible genetic method to increase growth rates and expand aquaculture production (Leighton and Lewis, 1982; Koike et al., 1988; Hoshikawa et al., 1998). Therefore, proper knowledge on the physiology of the species and hybrids is important for the success of aquaculture and restocking program. Oxygen uptake is a direct indicator of metabolic rate and an indirect indicator of capacity for growth (Jobling, 1981; Costa, 1988; Storey and Storey, 1990) including gastropods (Houlihan and Allan, 1982).

Oxygen consumption rate in abalones was investigated by Tamura (1939; *H. discus hannai*: temperature, circadian rhythm), Sano and Maniwa (1962; *H. discus hannai*: light condition), Sagara and Araki (1971; *H. discus discus*, *H. gigantea* now *H. madaka*: light condition), Uki and Kikuchi (1975; *H. discus hannai*: body weight, temperature), Jan et al. (1981; *H. diversicolor supertexta*: light), Jan and Chang (1983; *H. diversicolor supertexta*: decline of ambient oxygen), Barkai and Griffiths (1987; *H. midae*), Segawa (1991; *Sulculus diversicolor aquatilis*: starvation, 1995; *Nordotis discus discus*: temperature), and Harris et al. (1998, 1999; *H. laevigata*, ammonia, low dissolved oxygen). Oxygen consumption rate in *H. gigantea* has not been studied so far.

Ammonia is the principal nitrogenous compound excreted by aquatic animals (Colt and Armstrong, 1981) and being toxic to fish, crustaceans and molluscs can limit production in aquaculture (Epifanio and Srna, 1975; Wickins, 1976; Russo, 1985; Allan et al., 1990; Russo and Thurston, 1991). In solution, ammonia exists in a pH- and temperature-mediated equilibrium between the unionized and ionized forms with unionized ammonia considered more toxic (Russo and Thurston, 1991). Ammonia induces detrimental changes in tissue structure, cell function, blood chemistry, osmoregulation, disease resistance, growth and reproductive capacity (Colt and Armstrong, 1981; Russo, 1985; Jeney et al., 1992). Chronic exposure can result in the deterioration of several physiological functions any one of which may be the ultimate cause of death (Russo, 1985). Ammonia may affect gill structure (Smart, 1976), respiratory function (Chen and Lai, 1992; Chen and Lin, 1992; Knoph, 1996) and oxygen consumption (Smart, 1978) in aquatic animals.

Keeping animals healthy in intensive aquaculture depends on preventing accumulation of toxic waste products such as ammonia. Ammonia concentration of 1–2 mM is common in the blood of marine invertebrates (Haberfield et al., 1975). Higher concentrations are presumably toxic because they perturb acid–base balance with too much alkalinity (Hammen, 1980).

Reports on ammonia excretion rate in abalone are scarcer than oxygen consumption. The available reports on ammonia excretion rate

* Corresponding author. Tel.: +81 3 5463 0535; fax: +81 3 5463 0684.
E-mail address: watanabe@kaiyodai.ac.jp (S. Watanabe).

0044-8486/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.aquaculture.2008.03.040

are from Barkai and Griffiths (1987; *H. midae*), Segawa (1991; *Sulculus diversicolor aquatilis*: starvation, 1995; *Nordotis discus discus*: temperature). Information on oxygen consumption and ammonia excretion in abalones *H. gigantea*, *H. madaka*, *H. discus discus* and their hybrids is important due to the impact it has on aquaculture.

Abalones are generally known as light sensitive residing in crevices, caves and cavities in the natural environment (Shepherd, 1973) and in the most shaded areas of the tanks during aquaculture (Jan et al., 1981). Light exposure at a certain level can affect survival and growth of postlarvae (Bernal et al., 2003), juveniles and adults (Ebert and Houk, 1984). The physiological response to light exposure has been reported to be variable at different ontogenetic stages (Sano and Maniwa, 1962; Sagara and Araki, 1971; Jan et al., 1981). As adaptation to different environmental conditions is one of the key factors for commercial suitability of abalone hybrids, information on the physiological response of hybrids of *H. discus discus*, *H. gigantea* and *H. madaka* to light exposure can be useful for understanding their potential and management strategy for aquaculture.

The present study was conducted in the juveniles of *H. discus discus*, *H. gigantea*, *H. madaka* and their hybrids with the objectives (1) to compare respiration and excretion and (2) to observe if light had any effect on respiration and excretion.

2. Materials and methods

2.1. The animals

Juvenile abalones (N=9–13 for each species and hybrid) used in the experiments were produced by artificial insemination, hatched and reared at the Banda Station (Chiba Prefecture, Japan), Field Science Center, Tokyo University of Marine Science and Technology. *Haliotis discus discus* (DD), *H. gigantea* (G), and *H. madaka* (M) were hatched in November, December 2000 and January 2001, respectively. The hybrids, DD×M, M×DD, DD×G, G×DD (mother first) were hatched in December 2000, January 2001 and November 2001, respectively. Genetic status of the hybrids was confirmed by allozyme and mtDNA analyses (Ahmed et al., 2008). The animals were reared under artificial light (light intensity in the tanks ranged from 0.98 to 1.98 μmol/m^2/s) following natural photoperiod cycles throughout the year. Light intensity was estimated by light intensity meter (LI-COR Quantum/Radiometer/Photometer Model LI-189). Body weights were 2.49–10.9 g (2.94–3.85 cm shell length) for *H. discus discus*, 2.92–10.1 g (3.03–3.98 cm) for *H. gigantea* and 2.25–9.9 g (2.86–3.84 cm) for *H. madaka*, 5.1–7.9 g (3.49–3.72 cm) for DD×M, 5.5–9.8 g (3.45–3.98 cm) for M×DD, 4.4–8.6 g (3.21–3.96 cm) for DD×G, and 3.3–6.3 g (3.13–3.67 cm) for G×DD. Only healthy abalones having unbroken shells and uncut feet (Moore et al., 2000) were used for the experiments.

2.2. Experimental procedure

Abalones were collected from the culture tanks at the Banda Station and transported to the Laboratory of Population Biology, Tokyo University of Marine Science and Technology, Japan. After bringing to the laboratory in Tokyo, the biofouling attached to the animals was cleaned and they were reared at 20±2 °C water temperature and 35±1 ppt salinity. The animals were acclimatized for at least two weeks to the experimental condition (similar to Banda station) before the initiation of oxygen consumption and ammonia excretion measurements. They were fed ad libitum *Eisenia bicyclis* and *Undaria pinnatifida* during the rearing period. At the time of measurement, the abalones were transferred to 400 ml glass bottles placed inside another tank containing seawater that was filtered with GF/C filter (Whatman, 1.2 μm pore size), aerated for 12–24 h and then left alone for several hours to avoid oxygen super-saturation. The salinity and temperature of the water were the same as that of the rearing tanks. All measurements started at 10.15 to 10.30 am. The abalones were incubated inside the glass bottles for 3–3.5 h. Animals were fasted at least one day before and during the experiment and measurements were made first under dark followed by light at the same time on the next day. Immediately after the measurement, shell length, whole wet body weight and body weight in seawater of each individual were quantified. The latter two were used for calculating the volume of the organism (V) following the equation:

$$V = (m_a - m_w)/d_w.$$

Where

m_a the mass of abalone in air,
m_w the mass of abalone in sea water,
d_w the density of sea water (assumed to be 1.025 g cm−3) (Donovan and Carefoot, 1997).

Before weighing whole wet body weight, the abalones were put on filter paper for 1–2 min to remove excess moisture. Oxygen concentration was measured using Winkler Titration method and ammonia concentration was measured using indophenol method (Strickland and Parsons, 1968). Oxygen consumption rate and ammonia excretion rate were calculated following the equations:

$$R = \{(I - F)1000(V - v)\}/t$$

$$E = \{(F - I)1000(V - v)\}/t.$$

Where

R oxygen consumption rate,
E ammonia excretion rate,
I concentration of oxygen/ammonia at the beginning of incubation,
F concentration of oxygen/ammonia at the end of incubation,
V volume of glass bottle,
v volume of abalone,
t incubation period.

Oxygen consumption rate (R) on individual basis is expressed as ml O_2/ind./h and ammonia excretion rate (E) on individual basis is expressed as μmol NH_4–N/ind./h. The relationships between oxygen consumption rate and body weight were expressed as $R=aW^b$, where R, a, W and b were the oxygen consumption rate, the intercept, body weight and the slope, respectively. The relationships between ammonia excretion rate and body weight were expressed as $E=aW^b$, where E, a, W and b were the ammonia excretion rate, the intercept, body weight and the slope, respectively. O/N ratio, the index of metabolism is expressed by the ratio of oxygen consumption and ammonia excretion rates by atoms.

2.3. Statistical analyses

Tests of significance were conducted for the differences between dark and light for both oxygen consumption and ammonia excretion rates under each species and hybrid by Students' t-test. One-way ANOVA was used to find the differences between the species and hybrid types in both dark and light for both oxygen consumption and ammonia excretion rates. The tests were deemed significant at $P<0.05$.

3. Results

3.1. Oxygen consumption rate

The oxygen consumption rates on individual basis (ml O_2/ind/h) under dark ranged from 0.058 to 0.298 in *H. madaka*, 0.097 to 0.349 in