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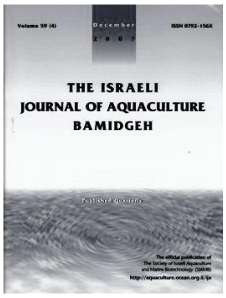
ISSN 0792 - 156X

PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

The Israeli Journal of Aquaculture - Bamidgeh, IJA_66.2014.1027, 6 pages



Use of Natural Zeolite for Ammonia Removal during Simulated Transport of Live Juvenile Sea Bass (*Dicentrarchus labrax*)

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(Received 16.9.13, Accepted 29.11.13)

Key words: *Dicentrarchus labrax*, zeolite, transportation, total ammonia nitrogen

Abstract

This study was conducted to determine the effects of zeolites on total ammonia nitrogen (TAN) removal during a 24-hour-sea bass transport. Five experimental treatments (0 (control), 10, 20, 30 and 40 g/l zeolite) with 3 replications were applied in 14 l plastic tanks. 90 sea bass (total weight 175.07 ± 0.49 g) were stocked in each tank. The zeolites were placed in mesh bags and renewed 8 and 16 hours (h) after the commencement of transfer. TAN contents of tank waters were measured after 8, 16 and 24 h. The results revealed that at the 8th h, 20, 30 and 40 g/l zeolite doses were significantly effective compared with the control. At the 16th and 24th h the lowest zeolite level (10 g/l) was also effective in TAN removal. In general highest TAN removal was achieved with 40 g/l treatment with efficiencies of 18.33%, 34.08% and 20.96% at the 8th, 16th and 24th h respectively. Overall, the results suggest that the use of zeolite in sea bass transportation could bring remarkable benefits and thereby increase fish transportation density and welfare.

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Introduction

European sea bass (*Dicentrarchus labrax*), a member of the recently revised Moronidae family and major species in Mediterranean aquaculture, is distributed from the Black Sea and the Mediterranean Sea to the eastern Atlantic Ocean, Ireland, the Baltic and North Seas as well as across Morocco and Senegal (Karahan, 2009; Stickney, 2000). They are generally first reared in inland facilities to the juvenile stage and then transported live to marine growout cages (Mozes et al., 2011). Because of high transportation fees, farmers tend to maximize stocking density of fish during transportation (Piper et al., 1982; Tang et al., 2009). However elevating stocking density necessitates, stricter attention to water quality, namely dissolved oxygen and ammonia which is toxic to fish (Berka, 1986; Lim et al., 2003; Zhang et al., 2004). Despite the difficulty of ascertaining a precise value for the toxic level of ammonia due to differences in temperature and pH (Berka, 1986), 0.26 mg/l at about pH 8 is considered the maximum safe limit of unionized ammonia for sea bass (Lemarié at al, 2004). Ammonia excretion during transportation can be decreased to a certain degree by reducing the metabolic rate of fish with pre-starvation or anesthetics (Berka, 1986; Lim et al., 2003; Piper et al., 1982; Stickney, 1994). Even when these methods are used, water ammonia can reach toxic levels and cause high mortality especially during long distance transport (Harmon, 2009; Treasurer, 2010) where fish are inevitably held at either low density or need frequent water renewal to avoid ammonia toxicity (Berka, 1986; Santacruz-Reyes and Chien, 2010).

Zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations, which have infinite three-dimensional structures (Mumpton and Fishman, 1977). Although there are some differences in Si/Al ratios, and cation types and quantities, they are all expressed with a general formula, $O.Al_2O_3.9SiO_2.nH_2O$ (Mumpton, 1999). Due to their ion exchange capacity, zeolites have been successfully used to minimize ammonia accumulation in transportation of various freshwater fish (Bower and Turner, 1982; Kaiser et al., 2006; Öz et al., 2010; Singh et al., 2004). However, due to the competition of total ammonia nitrogen (TAN) with other cations (Chiayvareesajja and Boyd, 1993; Emadi et al., 2001; Miladinovic et al., 2004), zeolites have a poor ability to absorb ammonia in salt water as compared to fresh water. Zeolites lose their ammonia absorption abilities in salt water after about 8 h (Emadi et al. 2001). Accordingly, a recent study in gilt head sea bream (*Sparus autara*) (our unpublished data) suggests that the ammonia removal characteristics of zeolites could be advantageous in the transport of marine fish species if renewed every 8 h. Investigation into whether this positive result could be applied to the transport of sea bass would be highly relevant. The present study was conducted to determine the effects of zeolites on TAN levels during a 24-hour live juvenile sea bass transport.

Materials and Methods

This study was conducted at the Beymelek Unit of Mediterranean Fisheries Research Production and Training Institute, Antalya, Turkey. Naturel zeolite (clinoptilolite) material (0.8-1.2 cm diameter) was provided from a private company (Gordes Zeolite, İzmir, Turkey). Its chemical composition is given in Table 1. The zeolite was washed with distilled water and dried overnight at 65°C and put into experimental bags to provide various treatment levels; 0 g/l (control; Z0), 10 g/l (Z10), 20 g/l (Z20), 30 g/l (Z30), 40 g/l (Z40). Overall, five experimental groups were tested in triplicate 14 l plastic tanks filled with full strength sea water (38.13 ± 0.11 ppt) passed through a 25 µm automatic filter (Model:6″ M100-4500, Timex, Ankara, Turkey). No water exchange was made throughout the study. 90 sea bass (total weight 175.07 ± 0.49 g) produced at the institute's hatchery were stocked in each tank. A custom made platform kept in darkness and gently shaken with an electric motor was used to simulate real transport conditions. The shaker was stopped for 15 minutes every two hours. The zeolite bags were replaced with new ones 8 and 16 h after commencement of the experiment in accordance with the findings of Emadi et al. (2001). During the trial pure oxygen was supplied to all the tanks.

Table 1. Chemical composition of zeolite used in the present study*.

Component	*% (Weight)*
SiO_2	67.11
Al_2O_3	11.84
Fe_2O_3	1.47
MgO	1.15
CaO	2.18
Na_2O	0.38
K_2O	3.44
Moisture	12.43

*Taken from the supplier (Gordes Zeolite, İzmir, Turkey)

Water samples were taken at the 0th, 8th, 16th and 24th h, and filtered through a fiberglass microfilter (Watman GF/A). Dissolved oxygen, temperature and pH of the tanks were measured at each sampling and recorded as 9.52 ± 0.82 mg/l, 27.1 ± 0.1°C, 7.5 ± 0.03 respectively. Ammonia was determined according to the phenate method using a spectrophotometer (Helios-α, Thermo Scientific, Cambridge, UK) (APHA, 1995). Data were tested with one-way ANOVA using statistical software (JMP 8.0) after checking for normality and homogeneity with the Shapiro-Wilk, and Bartlett, tests respectively. Tukey HSD *post hoc* test was used to differentiate significant treatments. Repeated ANOVA was also used to analyze the effects of zeolite treatments on the changes in TAN levels in relation to time.

Results

TAN levels detected over the study period were within the range of safe levels for sea bass (Lemarié et al., 2004; Ruyet et al., 1995) and no mortality occurred. TAN level was 0.13 mg/l at the beginning of the experiment after which there was an increasing trend in relation to time, regardless of the treatments. However, this increase was less in the zeolite treated tanks, being significantly different ($p<0.05$) from the control particularly after 8 h (Table 2).

Table 2. TAN concentrations of experimental groups at varying times (mg/l)

Treatments	*Time (hours)*		
	8	*16*	*24*
Z0	2.27 ± 0.06^{a}	4.18 ± 0.23^{a}	4.88 ± 0.16^{a}
Z10	2.08 ± 0.04^{ab}	3.37 ± 0.06^{b}	4.21 ± 0.05^{b}
Z20	1.99 ± 0.06^{bc}	3.06 ± 0.14^{bc}	4.17 ± 0.08^{b}
Z30	1.93 ± 0.02^{bc}	2.87 ± 0.06^{bc}	3.90 ± 0.11^{b}
Z40	1.86 ± 0.03^{c}	2.75 ± 0.02^{c}	3.86 ± 0.09^{b}

Values with different superscripts in the same column are significantly significant ($P<0.05$).

There appeared to be a dose dependent effect of zeolite on TAN removal at 8, 16 and 24 h in the present experiment. Repeated ANOVA analyses revealed highly significant treatment, time and treatment × time interaction effects (Table 3), suggesting that a differential treatment effect with time was operative, particularly between 16 and 24 h.

Table 3. Results of repeated measures of ANOVA test for zeolite and time on TAN levels.

Variation sources	*Degree of freedom*	*F*	*P*
Zeolite	4	74,503	0.0001
Time	2	1198,921	<0.0001
Zeolite × Time	8	3,6392	0,00431

There was a common trend in efficiency at the 8th and 16th h whereas at the 24th h, a decrease in TAN removal efficiency compared with 16th h was observed. Highest TAN removal occurred in Z40 with 18.33 % at the 8th, 34.08% at 16th and 20.96% at 24th h (Fig. 1).

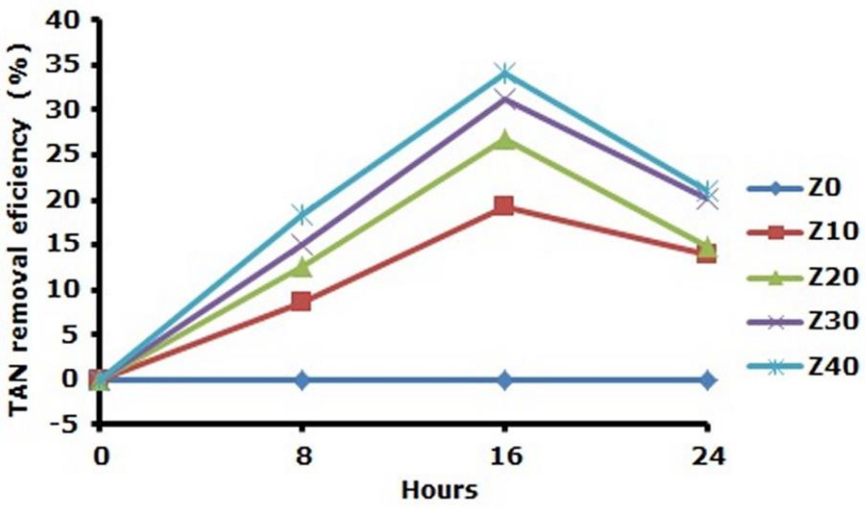

Fig. 1. TAN removal efficiency of various levels of zeolite in juvenile gilthead sea bass transportation at 8, 16 & 24 hrs relative to the control. Note that Z0 was considered 0.

Discussion

The best ammonia removal efficiency of zeolite was obtained at a salinity of below 10 ppt (Emadi et al. 2001). Our results from the present experiment at 38.13 ppt showed that all zeolite treatments up to 40 g/l bound TAN in all sea bass transportation tests except after 8 h when the 10 g/l zeolite was ineffective. TAN removal was most effective with treatment Z40 – 40g/l, (34.08%) at the 16 h. This efficiency level was better than the highest removal (24.65%) obtained with the same zeolite treatment at 24th h of transfer in juvenile gilthead sea bream (our unpublished data). The increase of the zeolite efficiency with tank water TAN concentrations in the present experiment, particularly at 16th h, is consistent with observations of Miladinovic et al. (2004), Emadi et al. (2001) and our previous unpublished study. However, unexpected trends in the values which do not match this pattern occurred at the 24th h, because lower TAN removal was seen at the 24th h rather than after the 16th h. This could be due to a higher nitrification activity or microbial activity at the 24th h than 16th h (Fig. 1), consistent with the existence of nitrification activity in the experimental tanks even if the tank water is renewed every 10-12 hours (Eshchar et al. 2006).

TAN retention values of zeolites in transport of several freshwater fish species including rainbow trout (*Onchoryncus mykiss*), ornamental fish (*Carassius auratus*, *Haplochromis obliquidens)* and Indian carps (*Catla catla, Labeo rohita, Cirrhinus mrigala*) ranged between 18-93% (Bower and Turner, 1982; Kaiser et al., 2006; Öz et al., 2010; Singh et al., 2004). This large variation in TAN removal could be due to differences in zeolite type and mining area (Miladinovic et al., 2004; Mumpton and Fishman, 1977) rather than fish species. In general, however, the literature suggests that TAN removal efficiency was closer to maximum level when zeolite was added at a level higher than 20 g/l. Moreover, there is general consensus that zeolite is effective in removing ammonia in transporting many fresh water fish species.

The present findings clearly indicate that natural zeolite utilization is effective in reducing TAN level during transport of marine fish. This could create new use of zeolite. Future studies should focus on the use of zeolite types in various marine fish species at differing doses and stocking densities. Considering the numerically best overall efficiency, 40 g/l zeolite application can be recommended for live transport of juvenile sea bass. However the cost of this treatment under practical conditions needs further elaboration. Based on the results, with 40 g/l zeolite roughly 12 kg of zeolite (three renewals) for a 100 l transportation water tank would be required, and 24 h of transportation would cost ~$1.2 per 100 l water ($0.1 per kg of zeolite in Turkey). This cost would be much lower for short distance transport. In conclusion, zeolite application could reduce transportation costs considerably by increasing fish density per volume of water.

Acknowledgements

The General Directorate of Agricultural Research and Policy, Ministry of Food, Agricultural & Livestock supported this research under grant no. (TAGEM/HAYSUD/2011/09/01/01). The authors wish to express their gratitude to Talip Devrim Özgen, for their support during the study, and Gordes Zeolite for generously providing the zeolite material.

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