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INFLUENCE OF SALINITY ON PHYSIOLOGICAL RESPONSE OF THE BEARDED HORSE MUSSEL *Modiolus barbatus* and NOAH'S ARK SHELL *Arca noae*

MATEA VLAŠIĆ[1], NIKŠA GLAVIĆ[2*], JAKŠA BOLOTIN[2], ENIS HRUSTIĆ[3], IRIS DUPČIĆ RADIĆ[2]

[1]Ministry of Agriculture of Republic of Croatia, Fisheries Directorate, Vukovarska 2, 20000 Dubrovnik, Croatia; e-mail: matea.vlasicmprrr@gmail.com

[2]University of Dubrovnik, Institute of Marine and Coastal Research, Damjana Jude 12, 20000 Dubrovnik, Croatia; e-mail: niksa.glavic@unidu.hr; bolotinjaksa@yahoo.com; iris@unidu.hr

[3]Ruđer Bošković Institute, Center for Marine Research, Giordano Paliaga 5, 52210, Rovinj, Croatia; e-mail: enis.hrustic@irb.hr

* Author for correspondence

Abstract

Vlašić M., Glavić N., Bolotin J., Hrustić E., Dupčić Radić I.: Influence of salinity on physiological response of the bearded horse mussel *Modiolus barbatus* and Noah's ark shell *Arca noae*. Ekológia (Bratislava), Vol. 37, No. 4, p. 345–357, 2018.

Bearded horse mussel *Modiolus barbatus* and Noah's ark shell *Arca noae* are a species of interest for the diversifying shellfish aquaculture on the south-eastern coast of the Adriatic. In this study, oxygen consumption (OC), total ammonia excretion (TAM) and clearance rate (CR) responses to the changes in seawater salinity (37, 30, 25 and 20) were investigated in the laboratory. There is a statistically significant influence of salinity on oxygen consumption and TAM excretion of Noah's ark shell, while the time of exposure to different salinities is significantly correlated to TAM excretion by the bearded horse mussel. Mean OC of Noah's ark shell ranged from 0.14 ± 0.06 to 0.54 ± 0.27 mg $O_2 g^{-1} h^{-1}$ and that of bearded horse mussel from 0.18 ± 0.17 to 0.26 ± 0.14 mg $O_2 g^{-1} h^{-1}$. Mean values of TAM excretion of Noah's ark shell ranged from 2.14 ± 1.52 to 7.22 ± 6.04 μmol $g^{-1} h^{-1}$ and for bearded horse mussel from 0.98 ± 0.53 to 2.78 ± 2.96 μmol $g^{-1} h^{-1}$. Salinity and exposure time have a significant influence on the CR of Noah's ark shell, whilst salinity has been found to be the determining factor for the bearded horse mussels' CR. Mean values of Noah's ark shell CR ranged from 0.96 ± 0.54 to 4.18 ± 1.15 l $h^{-1} g^{-1}$ and for bearded horse mussel from 2.43 ± 0.99 to 4.23 ± 0.84 l $h^{-1} g^{-1}$. Higher oxygen consumption to total ammonia excretion (O:N) ratios at lower salinities indicated the use of proteins as a metabolic substrate for both species. Noah's ark shell has greater energy expenditure related to respiration and TAM excretion than the bearded horse mussel.

Key words: shellfish, salinity, clearance rate, oxygen consumption rate, ammonium excretion rate, O:N ratio.

Introduction

Bivalves *Arca noae* Linnaeus, 1758 and *Modiolus barbatus* (Linnaeus, 1758) have become species of interest in recent years and significant research has been carried out on them.

Various biological data was documented for bearded horse mussel, such as age, growth rate, reproduction and aquaculture potential (Mladineo et al., 2007; Peharda et al., 2007, 2013). Energy budget and other physiological responses were also investigated for bearded horse mussel (Ezgeta-Balić et al., 2011; Pörtner, 2012). Age, growth rate, reproductive biology and aquaculture potential studies were performed on Noah's ark shell (Peharda et al., 2002, 2006, 2013). Literature shows very little data on the physiological response of Noah's Ark shell (Glavić et al., 2018).

Estuaries and coastal areas are under a strong influence of tides and freshwater from rivers (Levinton et al., 2011). Therefore, salinity is one of the most important abiotic ecological factors in the estuaries and coastal seas (Berger, Kharazova, 1997). Bivalves respond to changes in the ambient salinity by closing shells and decreasing the rate of feeding and breathing; but gradually, they recover their osmotic balance and return to normal physiological conditions (Almada-Villela, 1984). Several studies have been carried out on the effect of changes in salinity on oxygen consumption (OC), total ammonia (TAM) excretion e and clearance rate (CR) (Navarro, 1988; Navarro, Gonzalez, 1998; Tang et al., 2005), but there is no available information, to our knowledge, on the influence of salinity on the metabolism of *Arca noae* and *Modiolus barbatus*. Metabolic responses to changes in the environment are important when selecting the farming area and our research contributes to the estimates of farming opportunities of the investigated species. The aim of this study was to determine OC, TAM excretion, O:N ratio and CR of bearded horse mussel and Noah's ark shell related to abrupt changes in water salinity (37, 30, 25 and 20 psu).

Material and methods

Sampling site and procedures

For the measurements of OC and TAM excretion, individuals of *Arca noae* and *Modiolus barbatus* were collected by autonomous diving in November 2015 and April 2014, respectively in Bistrina Bay (42° 52'11.41"N, 17° 42 '06.73"E) located in Mali Ston Bay (south-eastern Adriatic) . The animals for CR measurements were collected at the same place, *Arca noae* in May 2016 and *Modiolus barbatus* in June 2016. Mali Ston Bay is one of the most important areas for the production of shellfish in Croatia, with annual salinity fluctuations from 17.48 to 36.93 psu (Jasprica et al., 1997).

Bivalves were transferred to the Institute for marine and coastal research, where they spent seven days acclimating to the ambient aquarium conditions (salinity 37±1.0 psu, temperature 20±1 °C). Experimental shellfish were fed daily, in the morning with the green algae *Tetraselmis suecica* (Kylin) Butcher, 1959, at a density of 3000 cells ml^{-1} (0.43 mg l^{-1}dry algal mass, according to Widdows, Staff, 2006). Abiotic water parameters (temperature, salinity) were measured daily by using the WTW Profiline Cond 3110 conductivity and temperature probe, whilst dissolved oxygen (DO) concentration was measured by using the Oxyscan graphic oxygen probe (UMS Gmbh, Germany).

Experimental design

Measurements of OC and TAM excretion were carried out on the organisms under different salinities: 37, 30, 25 and 20 psu. Sample groups of individuals were transferred from the sea water of ambient salinity into the waters of prepared different salinities. Sea water of different salinity was prepared by diluting the filtered (10/5/1 μm) and sterilized (UV-B lamp) aquarium sea water with the calculated portions of distilled water. Physiological response for each salinity was measured after 24 and 120 hours (1 and 5 days) to determine the acclimation to changed salinity. Measurements were performed in triplicate groups with five individuals per group (a total of 15 individuals per species). OC measurement was performed at the end of the period between two feedings to avoid the influence of

feeding (Widdows, Staff, 2006). Size of animals selected for the experiment was 40–50 mm for both species (usual market size).

OC, TAM excretion and CR measurements

OC and TAM excretion measurements were performed for each shellfish in a 429.39 ml volume closed respiratory chamber. Prior to introducing individuals in the chamber, they were cleaned from epibionts. After placing the shellfish in the chamber, it was closed and filled with pure sea water. Prior to physiological measurements, the oxygen enriched seawater was circulated through the chamber for an hour to allow the bivalves opening of the shells. Then the chamber inlet and outlet valves were closed, and the oxygen concentration decrease was measured for 40 minutes with the Oxyscan graphic probe (UMS Gmbh, Germany). No significant drop in oxygen was observed in a closed control chamber with pure sea water without shellfish during the measurement period.

After the experiment, the volume of individual shellfish was measured, and the soft tissue was dried in a drying chamber at 60 °C for 24 h to a constant mass and subsequently weighted. The dry weight (DW) of shellfish was taken as the basis for calculating the specific (per gram) physiological rate. Dissolved oxygen spent during one hour, that is, VO2 (mg O_2 h^{-1}) by individual shellfish was calculated according to Widdows, Johnson (1988) using equation:

$$VO_2 = 60 \times \left[C\ (t_0)\ - C\ (t_1) \right] \times\ (Vr) / (t_1 - t_0)$$

where: t_0 and t_1 - initial and final points (min) of the measurement period, C (t) - oxygen concentration in water at time t, Vr - volume of respirometeric chamber reduced by the volume of shellfish.

TAM excretion was measured simultaneously with the consumption of oxygen. After opening the chamber where OC was measured, 50 ml of seawater sample was taken, preserved with 2 ml of 1 M phenol in 95% ethyl alcohol vol/vol solution and stored in the refrigerator until spectrophotometric measurement conducted within 72 hours. Reference sample for ammonia concentration in the seawater at the beginning of each experiment was sampled the same way. These TAM concentrations were subtracted from the TAM concentrations obtained at the end of each experiment. The concentration of TAM was determined spectrophotometrically at 634 nm from the non-filtered samples using indophenol blue method (Solorzano, 1969) modified by Ivančić, Degobbis (1984). The TAM excretion rate was calculated according to Sobral, Widdows (1997) using equation:

$$U\ =\ (T - C) \times (V/1000) / t$$

where: U - TAM excretion rate (μmol NH_4-N h^{-1}), T - concentration of TAM (μM) in the sample, C - concentration of TAM (μM) in the control sample, V - volume (ml) of chamber, t - time (h).

Using the values of specific OC and TAM excretion, O:N atomic ratio was calculated and expressed according to Hawkins et al. (2002) using the equation:

$$O/N = (mg\ O_2/16) / (mg\ NH_4/14)\ .$$

CR can be determined by measuring the decrease in concentration of suspended algal cells added to the seawater. A closed metering system was used in which CR was measured in 5 l water tank over a period of 2 hours (Widdows, Staff, 2006). Bivalves were cleaned from epibionts, left for 20 minutes to open the shells and then *Tetraselmis suecica* algal cells were introduced at a concentration of 20 000 cell ml^{-1}. Mixing of water was achieved by aeration. Every 30 minutes for a period of 2 hours, a sample of 20 ml was taken to determine the algal concentration, which was performed using the Hach DR2500 spectrophotometer by reading the absorbance of visible light at 750 nm. Separate string of known algal concentrations was analysed for absorbance to produce the regression equation of the dependence of absorbance on algal concentration. Subsequently, polynomial equation of that regression curve was used to calculate the algal concentrations in 20 ml samples from the absorbance data, similar to Rodrigues et al. (2011). No significant drop of algal cell concentration was observed during the experimental period in the control tanks without shellfish. The CR of each bivalve was calculated using the following equation (Coughlan, 1969):

$$CR\ (l\ h^{-1}) = Vol\ (l)\ \ x\ (lnC1 - lnC2)/time\ interval\ (h)$$

where: Vol - volume of water, C1 and C2 - cell concentrations at the beginning and end of time interval (h).

In order to standardize the values of physiological rates and eliminate the influence of different animal weight on OC, CR and TAM excretion, all the physiological rates were converted to a specific physiological rate per gram of dry mass of the animal. Standardized rates were calculated according to Bayne, Newell (1983) using the following equation:

$$Ys = (Ws/We)^b \times Ye$$

where: Ys - the physiological rate for the animal of standard mass (1 g), Ws - standard mass (1 g), We - the observed mass of the individual in grams, Ye - the uncorrected (measured) physiological rate, b - the mass exponent for the physiological rate. The mean b exponent mass was 0.67 for the CR and 0.75 for the OC of bivalve (Savina, Pouvreau, 2004), and a value of 0.78 was used for the TAM excretion (Hawkins et al., 2000).

Energetic expenditures were respiratory energy expenditure (R) and energy lost as excreta (U). Calculation of R and U (all in $Jg^{-1}h^{-1}$) was as follows (Widdows, Johnson, 1988):

$$R = VO_2 \left(ml\ O_2 g^{-1} h^{-1}\right) \times 20.33\ J\ ml^{-1} O_2;$$

$$U = mg\ NH_4 g^{-1} h^{-1} \times 19.4\ J\ mg^{-1} NH_4.$$

Statistical analysis

The data collected were tested for variance homogeneity using Levene's test using the Statistica package 12.0. Normality of data was estimated by Kolmogorov-Smirnov and Liliefors test. For the analysis of OC, TAM excretion, O:N ratio and CR with respect to different salinity and exposure time to specific salinity, 'nested' ANOVA and Tukey post-Hoc analysis was used. To compare the metabolic rates between *Arca noae* and *Modiolus barbatus*, a t-test was used. Significant probability for all analyses was given as $p < 0.05$, if not expressed by an exact number.

Results

Respiration rate, that is, OC rate

Significant difference in OC was observed for Noah's ark shell ($F = 9.964$; $p = 0.000$) exposed to different salinities. Difference was significant between groups kept at 25 and 30 psu (Tukey, $p = 0.001$). Mean OC values ± SD are shown in Table 1.

Bearded horse mussel did not show statistically significant difference in OC at different salinities and at different exposure times. Mean OC values ± SD are shown in Table 2.

T a b l e 1. Mean values (± SD) of *Arca noae* oxygen consumption, respiration energy loss, TAM excretion, excretion energy loss, O:N ratio and clearance rate for different salinities after one and five days of exposure.

Salinity (psu)	Day	Respiration rate mg $O_2 g^{-1} h^{-1}$	Respiration energy loss J $g^{-1} h^{-1}$	Excretion rate µM$NH_4 g^{-1} h^{-1}$	Excretion energy loss J $g^{-1} h^{-1}$	O:N ratio	Clearance rate l $h^{-1} g^{-1}$
37	1	0.38±0.24	5.36±3.46	5.53±4.58	1.94±1.60	35.02±100.38	4.19±1.15
30	1	0.48±0.18	6.85±2.51	7.22±6.04	2.36±2.14	11.21±21.81	1.29±0.58
30	5	0.54±0.28	7.73±3.95	5.46±5.37	1.91±1.88	13.75±20.21	2.04±0.32
25	1	0.14±0.07	2.00±0.95	2.14±1.52	0.75±0.53	7.71±9.92	3.01±0.66
25	5	0.16±0.13	2.29±1.82	5.00±4.42	1.62±1.56	3.44±4.19	3.05±1.47
20	1	0.42±0.41	5.99±5.83	4.11±4.13	1.34±1.44	13.02±22.88	0.96±0.54
20	5	0.38±0.30	5.44±4.21	2.60±2.80	0.91±0.98	22.36±26.23	2.66±1.06

T a b l e 2. Mean values (± SD) of *Modiolus barbatus* oxygen consumption, respiration energy loss, TAM excretion, excretion energy loss, O:N ratio and clearance rate for different salinities after one and five days of exposure.

Salinity (psu)	Day	Oxygen consumption mg $O_2 g^{-1}h^{-1}$	Respiration energy loss J $g^{-1}h^{-1}$	TAM excretion µMNH4$g^{-1}h^{-1}$	Excretion energy loss J $g^{-1}h^{-1}$	O:N ratio	Clearance rate l $h^{-1}g^{-1}$
37	1	0.21±0.16	2.92 ± 2.30	1.21 ±0.78	0.40±0.28	32.20±61.81	2.43±0.99
30	1	0.23±0.12	3.20 ± 1.67	1.02 ±0.94	0.36±0.33	30.88±38.58	2.86±0.82
30	5	0.25±0.16	3.63 ± 2.22	1.29 ±0.65	0.45±0.23	11.40±10.11	3.22±0.51
25	1	0.26±0.13	3.74 ± 1.85	2.34 ±1.35	0.82±0.47	8.21±8.56	3.72±0.49
25	5	0.26±0.15	3.77 ± 2.09	1.98±1.11	0.60±0.43	15.75±26.32	4.23±0.84
20	1	0.18±0.17	2.63 ± 2.48	0.98 ±0.53	0.34±0.19	11.42±9.21	2.75±0.21
20	5	0.17±008	2.48 ± 1.07	2.78 ±2.96	0.97±1.03	12.51±14.51	2.79±1.00

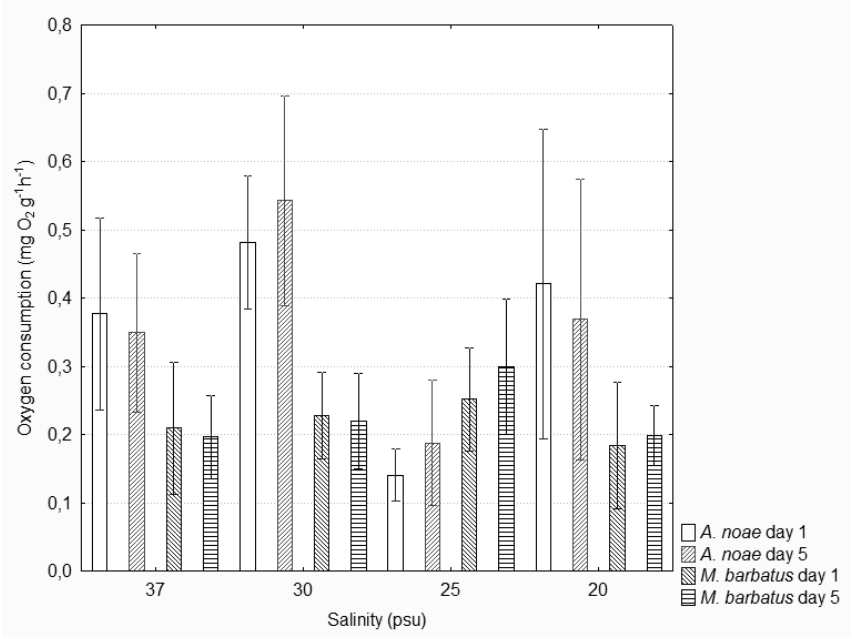

Fig. 1. Comparison of oxygen consumption (mg $O_2 g^{-1}h^{-1}$) between Noah's ark shell and bearded horse mussel. Values are given as mean ± SD, N = 15.

A comparison of OC between the investigated species is shown in Table 3. OC were found to be significantly different between the species at all tested salinities, except for the measurements performed after the exposure for 5 days at 25 psu and after 24 h at 20 psu. *M. barbatus* exhibited lower OC than *Arca noae* at all salinities (Fig. 1).

T a b l e 3. Summary of t-test results for oxygen consumption, TAM excretion, O:N ratio and clearance rate between Noah's ark shell and bearded horse mussel.

Salinity (psu)	Day	Oxygen consumption		TAM excretion		O:N ratio		Clearance rate	
		t	*P*	*t*	*P*	*t*	*P*	*t*	*P*
37	1	-2.247*	0.032	3.481*	0.001	-0.091	0,927	2.591*	0.032
30	1	-4.668*	0.000	3.925*	00.00	1.673	0,105	-3.482*	0.008
30	5	-3.591*	0.001	3.087*	0.004	-0.412	0,682	-4.343*	0.002
25	1	3.104*	0.004	-0.352	0.727	0.140	0,889	-1.866	0.104
25	5	1.980	0.059	2.391*	0.025	1.599	0,123	-1.556	0.158
20	1	-1.991	0.056	2.011	0.054	-0.262	0,795	-6.823*	0.000
20	5	-2.296*	0.031	-0.163	0.871	-1.235	0,226	-0.212	0.836

Note: * significant difference.

TAM excretion rate

There was a statistically significant difference in Noah's ark shell TAM excretion (F = 2.896; $p = 0.039$) due to different salinity and there was the largest difference between the group of bivalves exposed one day (24 hrs of influence) to salinity of 25 psu and a group exposed one day to salinity of 30 psu (Tukey, $p = 0.044$). The mean values of TAM excretion ± SD are shown in Table 1.

There was a statistically significant difference in the influence of the exposure time to TAM excretion of bearded horse mussels (F = 4.304; $p = 0.006$). The mean values of bearded horse mussel TAM excretion are shown in Table 2. The lowest values of TAM excretion rate were recorded for the bivalves exposed to 20 psu for one day (24 hrs of influence) and the highest after five days (120 hrs of influence) of exposure to 20 psu (Fig. 2). The post hoc Tukey test results showed the difference between shellfish exposed one day and five days to salinity of 20 psu (Tukey, $p = 0.018$).

A comparison of TAM excretion between the investigated species is presented in Table 3. There was a statistically significant difference between the two species, except for those exposed to 25 psu for one day and to 20 psu for one and five days.

O: N ratio

The mean values of O:N ratio of Noah's ark shell ± SD are shown in Table 1. There was no statistically significant difference in its O: N ratios due to different salinities.

The mean values of the O:N ratio of bearded horse mussel ± SD are shown in Table 2. The influence of salinity and exposure time on O:N ratio was not statistically significant.

A comparison of the O:N ratios of *A. noae* and *Modiolus barbatus* due to different salinities and exposure time showed no statistically significant differences between the species. The lowest values of O:N ratio for both species were determined at 25 psu (Fig. 3).

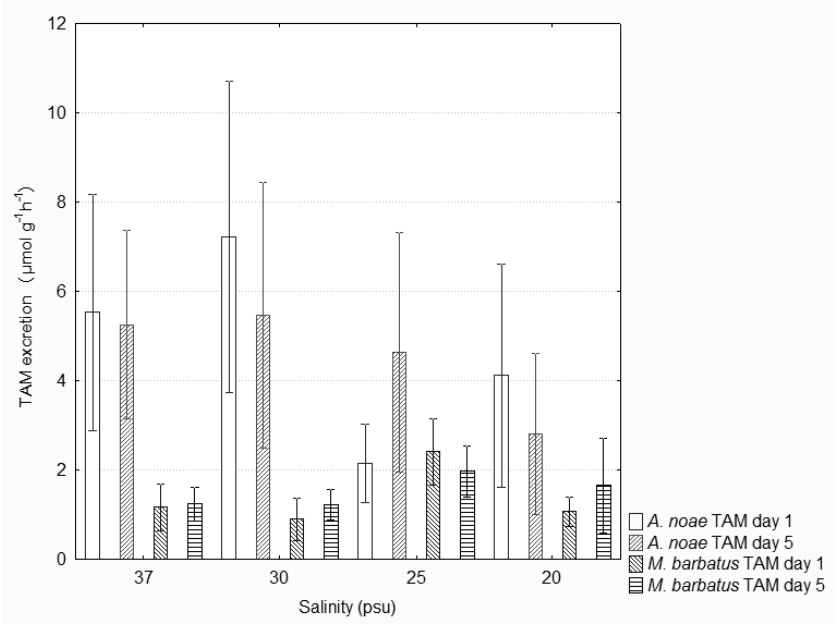

Fig. 2. Comparison of TAM excretion (µmol NH_4 $g^{-1}h^{-1}$) between Noah's ark shell and bearded horse mussel. Values are given as mean ± SD, N = 15.

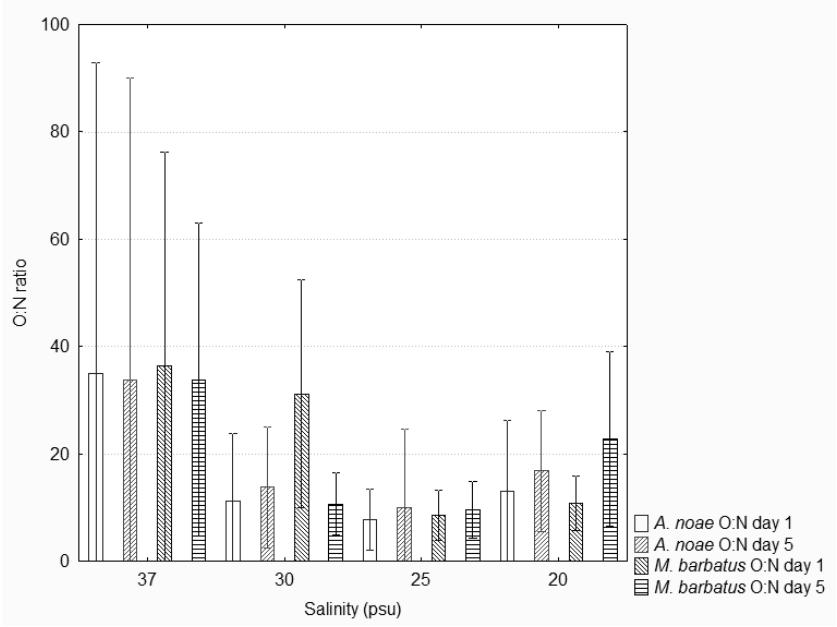

Fig. 3. Comparison of O:N ratio between Noah's ark shell and bearded horse mussel. Values are given as mean ± SD, N = 15.

Clearance rate

There was a statistically significant difference in the CR of Noah's ark shell (F = 15.814; p = 0.000) due to different salinity. The statistically significant difference (F = 3.255; p = 0.034) also occurred due to time of exposure. The mean values of Noah's ark shell CR ± SD are shown in Table 1. The lowest values were observed for groups exposed to salinity of 20 psu for one day, and the highest values were observed at ambient salinity (37 psu). The post hoc Tukey test showed a significant difference between the groups of bivalves exposed to the following conditions: 1. one day at 20 psu and one day at 25 psu (Tukey, p = 0.039), 2. one day at 20 psu and five days at 25 psu (Tukey, p = 0.019). There was a statistically significant difference in the bearded horse mussel CR (F = 7.456; p = 0.000) due to different salinity. The mean CR ± SD at different salinities are shown in Table 2. The lowest values were observed for bivalves exposed to ambient salinity, and the highest at 25 psu after five days of exposure. Values of CR increased to reach a maximum at 25 psu and then decreased. The influence of exposure time was not statistically significant, although the measured values were somewhat larger after five days. The post hoc Tukey test showed a significant difference in the CR between the bivalves exposed to 37 psu for one day and to 25 psu for five days (Tukey, p = 0.002).

Comparison of the CR between species is shown in Table 3. Significant differences in CR between the species were observed for all sets of conditions, except for the groups exposed to 25 psu for one and five days and to 20 psu for five days (Fig. 4).

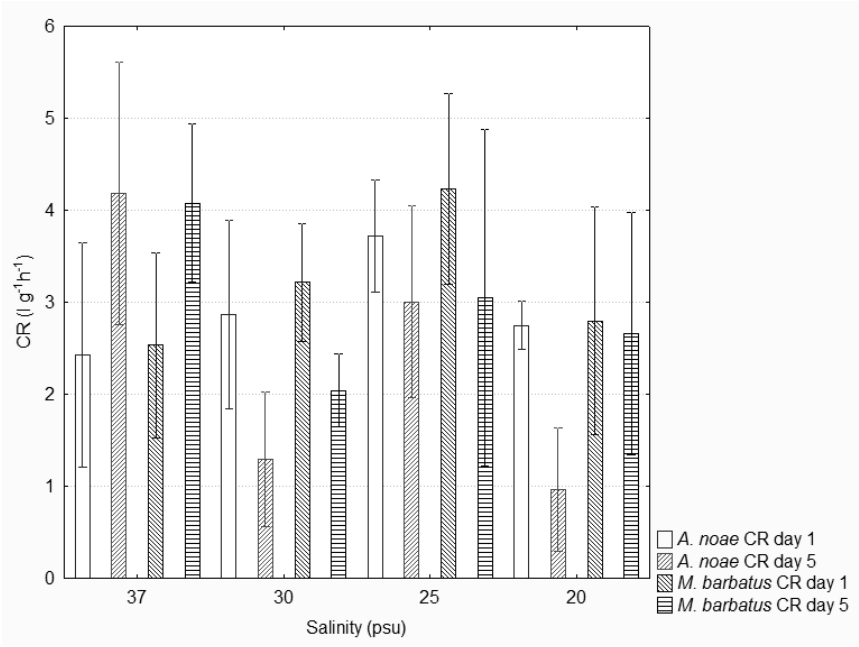

Fig. 4. Comparison of clearance rate (CR) between Noah's ark shell and bearded horse mussel. Values are given as mean ± SD, N = 15.

Discussion

Koehn, Bayne (1989) defined stress as a change in the environment that resulted in decrease in net energy balance. This paper analyses the physiological response of two shellfish species to changes in salinity. The first response of bivalves to a stressful change in salinity is the closure of the shells that separates the animal from the outside environment. This response helps in the prevention of osmotic stress but provides only short-term protection from adverse conditions (Berger, Kharazova, 1997). Pierce, Greenberg (1972) noted that salinity decrease caused the increase of free amino acids degradation and TAM excretion, while increase in salinity had the opposite effect.

OC values for bearded horse mussel and Noah's ark shell partly overlap. Our results are consistent with the results obtained for the mussel *Mytilus edulis* by Hawkins et al. (1985) and for the dog cockle *Glycymeris glycymeris* by Savina, Pouvreau (2004). Ezgeta-Balić et al. (2011) documented OC of 0.31 to 0.67 mg $O_2 g^{-1} h^{-1}$ for bearded horse mussels. The results of this study are from 0.14 to 0.54 mg $O_2 g^{-1} h^{-1}$, being somewhat lower than those obtained by Ezgeta-Balić et al. (2011). This could be caused by different seasons in which the experiments were conducted as well as by the different phases of the gametogenetic cycle.

Noah's ark shell showed fluctuating OC values with the highest value at 30 psu and the lowest at 25 psu. In some studies, with salinity drop, OC decreased as recorded for the ark shell *Anadara broughtonii* by Shin et al. (2006) and for the gren-lipped mussel *Perna viridis* by Wang et al. (2011). In other studies, the decreasing salinity increased OC in the clam *Meretrix meretrix* (Tang et al., 2005) and the mussel *Mytilus galloprovincialis* (Hamer et al., 2008). OC of the coot clam *Mulinia lateralis* and brown mussel *Perna perna* oscillated with the highest value at 20 psu (Williams, 1984; Resgalla Jr. et al., 2007) and at 16 psu for the clam *Meretrix meretrix* (Tang et al., 2005).

In this experiment, salinity changes did not significantly affect OC of bearded horse mussel, which was consistent with the study of Paganini et al. (2010) for the clam *Potamocorbula amurensis.* Different salinity regimes did not affect OC of *Mytilus edulis* in field research, although the highest OC was recorded at 25 psu (Landes et al., 2015). Williams (1984) noticed a higher OC at 20 psu that could be a referendum of salinity because it was similar to the average salinity of the habitat. In both investigated species in our experiment, an intensive physiological response was observed at 25 psu, and this should be further explored.

The effect of salinity on the TAM excretion was significant for Noah's ark shell, while the influence of exposure time on the TAM excretion was significant for the bearded horse mussel. Noah's ark shell demonstrated higher values of TAM excretion than bearded horse mussel. The highest TAM excretion values of bearded horse mussel overlapped with the lower values of the Noah's ark shell. Our results of TAM excretion are in accordance with the values given for the scallop *Argopecten purpuratus* by Navarro, Gonzalez (1998), and for the mussel *Mytilus edulis* by Bayne, Thompson (1970), Bayne, Scullard (1977b) and Gilek et al. (1992). The value of the TAM excretion rate that Navarro (1988) noticed for the mussel *Choromytilus chorus* kept at 18 psu corresponded to the lowest values for bearded horse mussel exposed to 20 psu for one day in our study. Presumably, there was an influence of type of food (*Tetraselmis suecica*) on TAM excretion due to the dietary conditions and higher content of

proteins in the mentioned algal species (Brown, 1991). Feeding with algal species *T. suecica* could increase TAM excretion rate (Bayne, Scullard, 1977a). Bivalves from the brackish environment have higher values of TAM excretion (Gilek et al., 1992). That paper also showed the variability of all the measured physiological responses for shellfish collected in the Vråganskär area that was unpolluted and brackish, similar to Mali Ston Bay.

Ratio of consumed oxygen and excreted nitrogen can help to reveal the nature of the substrate used to maintain metabolism (Corner, Cowey, 1968). Low value of O:N ratio (≤ 10) is connected to significant protein decomposition (Bayne, Newell, 1983) indicating the state of stress (Widdows, 1978). Metabolism relying more on carbohydrates and lipids produces values of O:N ratio higher than 30, while protein decomposition results in values below 30 (Bayne, Thompson, 1970). Similar to our study results, the O:N ratio decreased with reducing salinity in the Chilean scallop *Argopecten purpuratus* (Navarro, Gonzalez, 1998) and in the green-lipped mussel *Perna viridis* (Wang et al., 2011).

In our experiment, Noah's ark shell had O:N ratio over 30 when kept at 37 psu and below 30 at lower salinities. This indicates a stress when the environmental salinity was changed. Values of O:N ratio were above 30 for the bearded horse mussel at 37 psu and for the ones exposed to 30 psu for one day. For the bearded horse mussel, due to the stage of the gametogenetic cycle and the period of the fastest growing, lower value of the O:N ratio was expected at ambient salinity, but it has shown enough energy for both activities as noted by Ezgeta-Balić et al. (2011). Regardless of disadvantageous results for bearded horse mussel there was no statistically significant difference in the O:N ratio between the species.

CR is the indicator of feeding activity. Changes in salinity limit maximum feeding rate, and salinity fluctuations can cause severe disruption to normal diet physiology (Navarro, González, 1998), as also evidenced by the results of our study. For Noah's ark shell , the CR values were reduced by salinity decrease, which was consistent with the results of Wang et al. (2011) for the green-lipped mussel *P. viridis* and Kang et al. (2014) for the clam *Mactra veneriformis*. The values measured after 5 days of exposure to a particular salinity differed significantly from the values achieved after one day. Pleissner et al. (2013) suggested that filtration was strongly influenced by the rate of salinity change, that is, faster the salinity decreased or increased, a more pronounced change was in CR. In this study, an abrupt change of salinity was provoked. The highest observed values of CR were consistent with the results obtained by Widdows et al. (1990) for the species *Arca zebra* at salinity of 36 ± 0.5 psu and Albentosa et al. (2007) for the clam *Ruditapes decussatus* at ambient salinity. The lower values were consistent with the results documented by Navarro (1988) for the mussel *Choromytilus chorus* at 18 psu and Resgalla Jr. et al. (2007) for the brown mussel *Perna perna* at 20 psu. The CR values were not reduced with salinity decline for the mussel *M. odiolusbarbatus*, unlike the ark shell *Arca noae* in our study. Upward trend to one point and then a decrease in CR was found in the brown mussel *Perna perna*, which showed the lowest values at 15 and 40 psu, and the highest value at 20 psu (Resgalla Jr. et al., 2007). In this paper, the values of OC and TAM excretion for *Modiolus barbatus* were the highest at 25 psu, and the O:N ratio was the lowest, suggesting that salinity of 25 psu increased the energy needs due to the osmoregulatory energy expenditure. Increased energy needs could be the reason for greater filtration at this salinity. Furthermore, increased CR caused greater TAM excretion and OC.

Ezgeta-Balić et al. (2011) observed a much lower CR (0.17 to 0.23 $lh^{-1}g^{-1}$) for the mussel *M. barbatus*. Experimental shellfish were fed with somewhat larger amount of different food, *Isochrysis galbana* (Prymnesiophyceae). The individuals used by Ezgeta-Balić et al. (2011) were collected in November; while for this study, the samples were collected at the end of May when the bivalves were in a different phase of the gametogenetic cycle. Since *Modiolus barbatus* grows faster from May to August (Peharda et al., 2007), it would have higher energy needs that could cause a greater need for nutrition. Due to the whole range of differences during research, it was difficult to compare results, especially due to the lack of information on the cumulative effects of different impacts.

Conclusion

For Noah's ark shell, salinity reduction to 30 and 20 psu, respectively, increased OC and TAM excretion, and reduced CR presumably due to protein mobilization as a metabolic substrate during a reduced food intake period. From the results, it was likely that for the Noah's ark shell, salinity of 25 psu was favourable. Strong reaction of both species to salinity of 25 psu may be the result of natural occurring changes in Mali Ston bay salinity caused by river Neretva and submarine groundwater discharges.

Our research has shown that the investigated species had similar physiological responses at lower salinities of 20 and 25 psu. From the aspect of energy loss, Noah's ark shell lost more energy than the bearded horse mussel. Bearded horse mussel was more tolerant to salinity changes, which was indicated by the O:N ratio greater than 30 at ambient salinity and 30 psu, whereas Noah's ark shell achieved O:N ratio greater than 30 only at ambient salinity. Noah's ark shell achieved the highest CR at ambient salinity, and exhibited lower CR at reduced salinity. In contrast to that, the bearded horse mussel showed a higher CR when exposed to lower salinity than the ambient, achieving higher energy gain. Therefore, in an area such as the Mali Ston Bay, which is subject to constant changes in salinity, the bearded horse mussel seems to be more the economical choice for farming. Physiological condition indexes could be used to determine the degree of stress that bivalves experience during farming. In order to determine the influence of stress, there was need for data collecting on normal seasonal variations in the physiological indexes of *M. barbatus* and *Arca noae*.

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