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Influence of salinity on the physiological conditions in mussels, *Perna perna* and *Perna viridis* (Bivalvia: Mytilidae)

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Abstract: *Perna* genus was introduced to Venezuela, but nowadays, *Perna perna* and *Perna viridis* coexist and are commercially exploited from their natural beds. The aim of this work was to determine the effect of salinity on the physiological conditions of these species by studying RNA/DNA and Protein/DNA ratios. The organisms were collected from natural beds at La Esmeralda, Sucre State, Venezuela, and acclimatized for 15 days under laboratory conditions at 25ºC, 36‰ salinity, pH between 7 and 8 and more than 90% of oxygen saturation. Later, they were divided in two groups: for one group, the salinity concentration was increased (36 to 45‰), and for the other, the salinity was decreased (36 to 15‰). The rate of change was 1‰ every day. Ten organisms per group of both species were taken at each of 15, 20, 25, 30, 36, 40 y 45‰ salinity concentrations. Protein (colorimetric method) and nucleic acids (RNA and DNA by fluorometric method) concentrations were measured in the digestive gland, gills and adductor muscle tissues. Results indicate that *P. perna* can physiologically compensate the increase in salinity, but not when the salinity decreased, when proteins are the most needed macromolecules. The Protein/DNA index is directly related to salinity changes in both cases. *P. viridis* shows physiological compensation to salinity increases and decreases. The RNA/DNA index value in both cases supports this. Digestive gland and muscle tissues are the regulating tissues in both species. These results show that *P. viridis* has a higher degree of adaptability to salinity changes and, therefore, a greater potential for aquaculture than *P. perna.*.

Key words: *Perna perna*, *Perna viridis*, RNA/DNA, salinity.

Perna perna started colonizing Venezuelan coasts more than a hundred years ago (Soot-Ryen 1955) and its biology has been widely studied. *Perna viridis* was accidentally introduced eight years ago and the results of this process have not been quantified yet. The invading mussel, *P. viridis*, colonized the east coasts of Venezuela, in the Gulf of Paria, on the northern coast of Sucre State and Margarita Island. Both species are sessile bivalves, from the intertidal zone and sublitoral water of the tropical zone. They have commercial value in the seafood market as live shellfish and now they are a source of work and income for Venezuelan fishermen.

Only a few papers about the invading mussels have been published: Rylander *et al*. (1996) studied the status of green mussel *P. viridis*, Segnini *et al*. (1998) studied the salinity and temperature tolerances of *P. perna* and *P. viridis.* Viñoles *et al*. (2000) measured the effect of acclimatization temperature on growth of *P. viridis* and Bracho *et al.* (2000) determined the effect of feeding in *P. viridis.* Tejera *et al*. (2000) evaluated the growth of both species under experimental conditions. The aim of this work was to determine the effect of salinity on the physiological conditions of these species by studying the RNA /DNA and Protein/DNA ratios.

MATERIALS AND METHODS

Mussels (*Perna viridis* and *Perna perna*) were collected from natural beds at La Esmeralda, Estado Sucre, Venezuela (10°40'35"N, 63°30'50"W). They were brought to the laboratory, cleaned of epibiotic organisms and acclimatized for 15 days under laboratory conditions at 26°C, the pH was between 7 and 8 and oxygen 90% saturation, Mussels of each species, total lengths of 10-20 mm, measured in antero-posterior direction, were used. They were fed daily with microalgal (*Chaetoceros gracilis*, *Isochryis aff* (T-Iso) and *Tetraselmis chuii*) in rations of 30,000 cel/ml. Once the laboratory acclimatization time was complete, they were divided in two groups: for one group, the salinity concentration was increased (36 to 45‰), and for the other, the salinity was decreased (36 to 15‰). The rate of change was 1‰ every day. When the prescribed salinity concentration was reached (15, 20, 25, 30, 36, 40 y 45‰), ten organisms from each species were extracted and approximately 10 mg of digestive gland, gills and adductor muscle tissues were dissected. The samples were frozen immediately with dry ice and stored to a temperature of -17°C until nucleic acid and protein concentrations were measured. For DNA and RNA the fluorometric method proposed by Karsten & Wollenberger (1972, 1977) was used, with a modification in a ribonuclease concentration, which was increased from 25 µg/ml to 100 µg/ml, and incubation time to 30 minutes instead of 20 minutes in order to be sure that all the RNA was hydrolyzed by the enzyme. For protein determinations, the Bradford method was adopted (1976), using blue comassie reactive, and its concentration was calculated with the aid of a calibration curve prepared in a relative proportion and sensitive to an optical spetrophotomether in µg/ml of serum of bovine albumine.

RESULTS

The RNA/DNA ratio, Protein concentration and Protein/DNA ratio and the standard deviation (SD) for the digestive gland are shown in Table 1 for both species. For the RNA/DNA ratio in *P. viridis*, the values for salinity change are practically constant. In *P. perna,* when the salinity was increased, the initial value (2.58 ± 0.06 at 36‰) remained almost constant up to 40‰ and was reduced to 2.40 ±

TABLE 1

Mean values and standard deviations of RNA/DNA ratio, Protein concentration (µg/mg) and Protein/DNA ratio in the digestive gland of Perna viridis *and* Perna perna *at different (increase and decrease) salinity concentrations (n = 10 organisms/ species/ salinity concentration)*

Salinity concentration (‰)	RNA/DNA	Protein(µg/mg)	Protein/DNA
Perna viridis			
45	3.96 ± 0.05	387.81 ± 9.91	101.95 ± 2.79
40	3.89 ± 0.01	406.88 ± 6.03	96.74 ± 1.31
36	3.91 ± 0.15	396.02 ± 8.85	95.33 ± 4.19
30	4.05 ± 0.07	414.56 ± 8.75	101.97 ± 4.04
25	3.99 ± 0.05	424.60 ± 7.62	102.76 ± 1.84
20	3.99 ± 0.14	410.59 ± 6.90	102.30 ± 2.12
15	3.98 ± 0.17	383.85 ± 9.12	109.03 ± 3.97
Perna perna			
45	2.40 ± 0.01	110.54 ± 9.55	42.67 ± 3.51
40	2.53 ± 0.07	110.42 ± 9.32	43.06 ± 3.98
36	2.58 ± 0.06	116.39 ± 9.59	47.10 ± 4.09
30	2.55 ± 0.02	121.68 ± 7.38	48.43 ± 2.79
25	1.92 ± 0.21	95.12 ± 9.95	31.66 ± 3.48
20	1.94 ± 0.22	89.02 ± 9.81	29.87 ± 2.06
15	1.52 ± 0.15	77.46 ± 5.98	20.63 ± 3.43

TABLE 2

Mean values and standard deviations of RNA/DNA ratio, Protein concentration (μg/mg) and Protein/DNA ratio in the gills of *Perna viridis* and *Perna perna* at different (increase and decrease) salinity concentrations (n = 10 organisms/ species/ salinity concentration)

Salinity concentration (‰)	RNA/DNA	Protein(μg/mg)	Protein/DNA
Perna viridis			
45	1.47 ± 0.05	304.54 ± 5.41	90.43 ± 3.36
40	2.03 ± 0.04	323.95 ± 4.16	122.53 ± 1.86
36	2.02 ± 0.03	324.68 ± 9.34	122.98 ± 2.94
30	2.05 ± 0.03	310.33 ± 9.50	118.83 ± 3.70
25	2.04 ± 0.02	296.09 ± 5.12	113.62 ± 2.36
20	1.58 ± 0.01	290.37 ± 9.99	113.60 ± 3.92
15	1.68 ± 0.02	301.14 ± 7.47	117.82 ± 2.91
Perna perna			
45	1.55 ± 0.01	208.08 ± 7.71	36.96 ± 1.37
40	2.22 ± 0.00	198.09 ± 7.28	40.35 ± 1.58
36	2.50 ± 0.07	185.05 ± 4.92	37.74 ± 1.82
30	2.37 ± 0.02	179.26 ± 6.91	36.02 ± 1.38
25	1.96 ± 0.01	165.76 ± 5.55	31.10 ± 1.11
20	1.49 ± 0.01	133.96 ± 8.55	23.97 ± 1.38
15	0.63 ± 0.01	100.21 ± 5.57	15.81 ± 2.34

0.01 for 45‰. When it was decreased, the index fell from 2.58 ± 0.06 (36‰) to 1.52 ± 0.15 at 15‰. The protein concentration followed the same pattern as the RNA/DNA ratio for *P. viridis*. *F*or *P. perna,* there were not large differences when salinity was increased, but the values decreased from 116.39 ± 9.59 at 36‰ to 77.46 ± 5.98 at 15‰. The Protein/ DNA ratio had the same response as Protein concentration for both species.

The RNA/DNA ratio, Protein concentration and Protein/DNA ratio and the standard deviation (SD) for the gills are shown in Table 2 for both species. For *P. viridis* the values of the RNA/DNA ratio are nearly constants, between 25 and 40‰ and decrease at the extremes (1.47 ± 0.05; 1.58 ± 0.01 and 1.68 ± 0.02 at 45, 20 and 15‰ respectively). For *P. perna* starting at 36‰, for salinity increase or decrease, there was a reduction (from 2.50 ± 0.07 at 36‰ to 1.55 ± 0.01 at 45‰ and to 0.63 ± 0.01 at 15‰). For the protein concentration in *P. viridis*, the fluctuation was small in all the range studied. For *P. perna,* there was a reduction of up to nearly half its original value (185.05 ± 4.92 μg/mg at 36‰ to 100.21 ± 5.57 μg/mg at 15‰). The Protein/DNA ratio showed the same pattern as Protein concentration for both species.

The RNA/DNA ratio, Protein concentration and Protein/DNA ratio and the standard deviation (SD) for the abductor muscle are shown in Table 3 for both species. For *Perna viridis*, for salinity increase or decrease, the differences were not appreciable on the values of the three indexes studied. For *Perna perna,* there was a reduction for salinity increase or decrease (from 1.74 ± 0.02 at 36‰ to 0.87 ± 0.01 at 45‰ and to 1.09 ± 0.01 at 15‰), respectively.

DISCUSSION

The results obtained for the RNA/DNA ratio during salinity changes indicate that *Perna viridis* shows physiological compensation to salinity increases and decreases. The almost constant value of RNA/DNA index in both cases supports this. In this species, the digestive gland and muscle tissues are the regulator tissues, but the gill tissue was the most affected by salinity changes. However, the protein of abductor muscle was increased when it

TABLE 3

Mean values and standard deviations of RNA/DNA ratio, Protein concentration (μg/mg) and Protein/DNA ratio in the abductor muscle of Perna viridis *and* Perna perna *at different (increase and decrease) salinity concentrations (n = 10 organisms/ species/ salinity concentration)*

Salinity concentration (‰)	RNA/DNA	Protein(μg/mg)	Protein/DNA
Perna viridis			
45	3.06 ± 0.04	266.71 ± 4.00	217.59 ± 5.34
40	3.06 ± 0.05	256.57 ± 5.56	208.66 ± 5.56
36	3.01 ± 0.12	251.20 ± 7.07	205.62 ± 3.66
30	2.46 ± 0.21	262.48 ± 6.69	173.24 ± 8.43
25	3.03 ± 0.03	250.46 ± 1.81	206.67 ± 2.84
20	2.96 ± 0.08	243.15 ± 7.09	204.52 ± 9.78
15	2.58 ± 0.04	241.70 ± 6.60	203.87 ± 7.34
Perna perna			
45	0.87 ± 0.01	127.71 ± 6.76	73.47 ± 3.60
40	1.72 ± 0.02	171.72 ± 7.02	98.47 ± 4.28
36	1.74 ± 0.02	154.42 ± 6.05	88.14 ± 3.48
30	1.79 ± 0.01	134.69 ± 9.02	76.77 ± 8.22
25	1.58 ± 0.01	113.94 ± 8.12	62.96 ± 4.65
20	1.39 ± 0.01	100.49 ± 6.84	55.50 ± 3.47
15	1.09 ± 0.01	80.98 ± 6.96	45.03 ± 3.72

is needed to compensate the stress at 45‰ in gill tissue. This is evidenced by the protein /DNA ratio in muscle tissue. Furthermore, the abductor muscle is an energetic storage reserve and it is related with the need of the bivalves for an energy source easily available for opening and closing the valves (Nusetti & Morales 1988).

Perna perna can physiologically compensate the increase in salinity, but it cannot occur when the salinity decreased, when proteins are the most needed macromolecules. This is evidenced by the Protein/DNA index, which is directly related to salinity changes in both cases. The RNA/DNA ratio in gill tissues decreased with salinity concentration decrease until half of its initial value at 45‰. For a decrease in salinity concentration (from 36 to 15‰), its value decreased to a quarter of its initial value, possibly due to the fact that these tissues are more exposed to the natural environment and continuously subjected to fracture and wear. Because of this, they need to regenerate constantly and also maintain their growth (Gómez 1991).

The protein concentration for *P. perna* was constant as salinity concentration increased for all the tissues (digestive gland, gills and muscle). For salinity decrease, the digestive gland showed the highest decrease of protein concentration, the lowest concentration of salinity registered for these macromolecules was of 15‰. These results confirm that the digestive gland is a central organ of the general metabolism, which drives the energy movement and redistribution between the different organs. This tissue has a role in the metabolic activity, having a higher amount of RNA for the formation of new tissues in protein synthesis. Jordan (1990) described that this gland directed its proteins for the maintenance of its basic metabolic functions and the growth in the natural environment. Furthermore, the role of the gland has been shown to be part of energetic storage transfer (Gómez 1991). Nusetti & Morales (1988) demonstrated that the digestive gland in *P. perna* has an increase in cellular size jointly with protein accumulation, implying a high rate of protein biosynthesis and a low cellular proliferation (Protein /DNA). These results agree with those obtained by Segnini de Bravo *et al.* (1998) who reported that green mussel, *P. viridis*, has wider tolerance limits to salinity changes than brown mussel, *P. perna.* Also, these results suggest

that *P. perna* may colonize salinities environments between 25 and 45‰ with adaptable physiological regulations and without endangering its cellular volume growth (Protein/DNA). The salinities of 25 and 15‰ would like to be stressing for this species because the physiological condition was decreased up to 50% of its initial value. These results agree with Hicks *et al*. (2000) who reported that *P. perna* can colonize environments with salinities concentrations ranged between 15 and 50‰. From all the data obtained, we can infer that *P. perna* only have an environmental parameter limit in Venezuelan coasts, the temperature, because it is known that its reproductive cycle occurs between 21 and 25ºC, the upperwell temperature in our coasts (Vélez, 1971).

P. viridis appears to be physiologically tolerant to the environmental (salinities and temperature) extremes that characterize Venezuelan coasts habitats, suggesting that there are no biotic or abiotic barriers to its future rapid expansion throughout our coastal waterways. Because of that, it is necessary further studies and monitoring for this species. From this, we can infer that *Perna viridis* has a higher degree of adaptability to salinity changes and therefore, has a greater potential for aquaculture than *Perna perna*.

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RESUMEN

El género *Perna* es exótico en Venezuela. En la actualidad *Perna perna* y *Perna viridis* coexisten y son explotados comercialmente de sus bancos naturales. El objetivo de este trabajo es determinar el efecto de la salinidad sobre la condición fisiológica de *P. perna* y *P. viridis*, mediante la relación ARN/ADN y Proteínas/ADN. Los organismos se colectaron en la Esmeralda, Edo. Sucre, Venezuela y fueron transportados al laboratorio donde se aclimataron por 15 días a la salinidad de 36‰, pH entre 7-8 y la saturación de oxígeno de más del 90%. Posteriormente, se dividieron en dos grupos, a un grupo se le aumentó la salinidad (de 36 a 45‰) y a otro se le disminuyó (de 36 a 15‰). La tasa de cambio era de 1 ‰ cada día. Se tomaron 10 mejillones, de cada una de las especies a las salinidades de 15, 20, 25, 30, 36, 40 y 45‰. Luego se midió la concentración de proteínas (método colorimétrico) y ácidos uncleicos (método flurométrico) en los tejidos digestivo, branquial y muscular. Los resultados indican que *P. perna* en salinidades crecientes puede compensar fisiológicamente, no así a salinidades bajas, donde las proteínas son las macromoléculas más comprometidas, reflejándose en el índice Proteína/ADN. Mientras, *P. viridis* presenta respuestas compensatorias tanto a incrementos de salinidades como a diluciones de ésta, lo cual se corrobora con los valores casi constantes del índice ARN /ADN en ambos casos. En esta especie el tejido más regulador son el muscular y el de la glándula digestiva. Estos resultados demuestran que *P. viridis* tiene un mayor grado de adaptación a los cambios de salinidad y por consiguiente un mayor potencial para la acuacultura que *P. perna*.

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