EXCRETION OF TOTAL AMMONIUM BY SOME MARINE CRUSTACEANS

D. Tyrin, N. Kovatcheva

Russian Federal Research Institute of Fisheries and Oceanography, Russia, Moscow
e-mail: guerrerolatino@mail.ru

At the present time in the world, including in Russia, developing trade in live aquatic animals. The main problem faced by employers is the creation and maintenance necessary base for aquatic animals pre-sale kept in large cities, which are the main market. Red King crab (*Paralithodes camtschaticus, Tilesius*, 1815) and the American lobster (*Homarus americanus*) is one of the main objects of commercial interest.

The most important condition necessary for the maintenance of aquatic habitat parameters is the correct design and further use of closed recycling water supply systems (CRWS). It should be borne in mind that the water temperature in the habitats of the red king crab is in the range from 0 to 14°C, American lobster – from 0 to 20°C, which is a deterrent to the process of biological treatment of recycled water by nitrifying bacteria inhabiting the biofilter. Low water temperature explain the relatively low level of metabolism.

The level of excretion ammonia is one of the major factors considered when designing CRWS. Therefore, the study of this process at different animal species and depending on those or other conditions is the key. Red king crab and american lobster are ammoniotelium species, i.e. excreting ammonia as the main product of nitrogen metabolism. Most part of the ammonia they have released through the gill epithelium. Ammonia (NH_3) is acutely toxic substance and its permissible concentration in circulating water in CRWS for aquatic animals is only 0.05 mg/L. Free ammonia reacts with water to form a less toxic compound – ammonium (NH_4OH or in the ionized form – NH_4^+). Its permissible concentration for marine crustaceans during prolonged keeping and rearing in CRWS is already 0.25–0.5 mg/L (Kovatcheva N. P. et al, 2006). Value of ammonium and free ammonia in total ammonium depends on pH and water temperature (at 5–10°C and pH 8–8.3 ammonium is 90% or more).

In scientific papers this question raised in terms of biochemical mechanism for the excretion of ammonia (Kormanik, Cameron, 1981; Weihrauch and oth., 2002), but not the quantitative aspect, which is more important for the production. In earlier studies in the laboratory of crustacean reproduction and cultivation (VNIRO, Moscow) have been investigated the excretion of total ammonium by red king crab larvae and postlarvae (Shakula L. A. et al, 2008).

The purpose of this study was determination of daily total ammonium excretion amount by market sized red king crab and american lobster in a CRWS per 1 kg of body weight (BW) at different water temperatures – 6 and 12°C.

Experiment was carried out in 2010 in the storage complex with CRWS «LaMareè» Ltd. (Moscow, Russia) under a contract with the laboratory. For the experiment, were selected 20 males red king crabs and 20 males american lobsters. Before landing in the experimental aquatron (fig. 1) animals were kept in the common industrial CRWS with planting density 50–60 kg/m^3 for red king crab, and 60–75 kg/m^3 – for American lobster. The concentration of ammonium in the water in this CRWS was an average of 0.155 mg/L, nitrites – 0.092 mg/L, nitrates – 25.744 mg/L and pH was an average 7.22. The total duration of animals' exposure in the CRWS was 2 – 5 days after catching from the sea. In the aquatron was installed a circulating pump and chiller, but there was no water purification unit. Aquatron were filled with 150 liters of artificial seawater with salinity 32–35‰, prepared from tap water and dry salt produced by "Marine Aquarium" (Russia).

Each crab or lobster was kept in aquatron 1 day without feeding. Before landing, the crabs were measured (total body weight and carapace width). The water sample was taken for hydrochemical analysis. Before boarding the animal aquatron was washed with fresh water. Concentration of ammonium was carried out on a photometer by Sedji-Solorzano method using phenol-hypochlorite reaction. Also measured water temperature and the content of dissolved oxygen (multiparameter instrument «YSI-85», USA), salinity (refractometer), pH (pH-meter «Hanna Instruments pH211», USA), consumption of circulating water (instrumental method). After 1 day crab or lobster was returned to a common CRWS, were taken samples from water, then filled again by new prepared artificial sea water and planted the following animal.

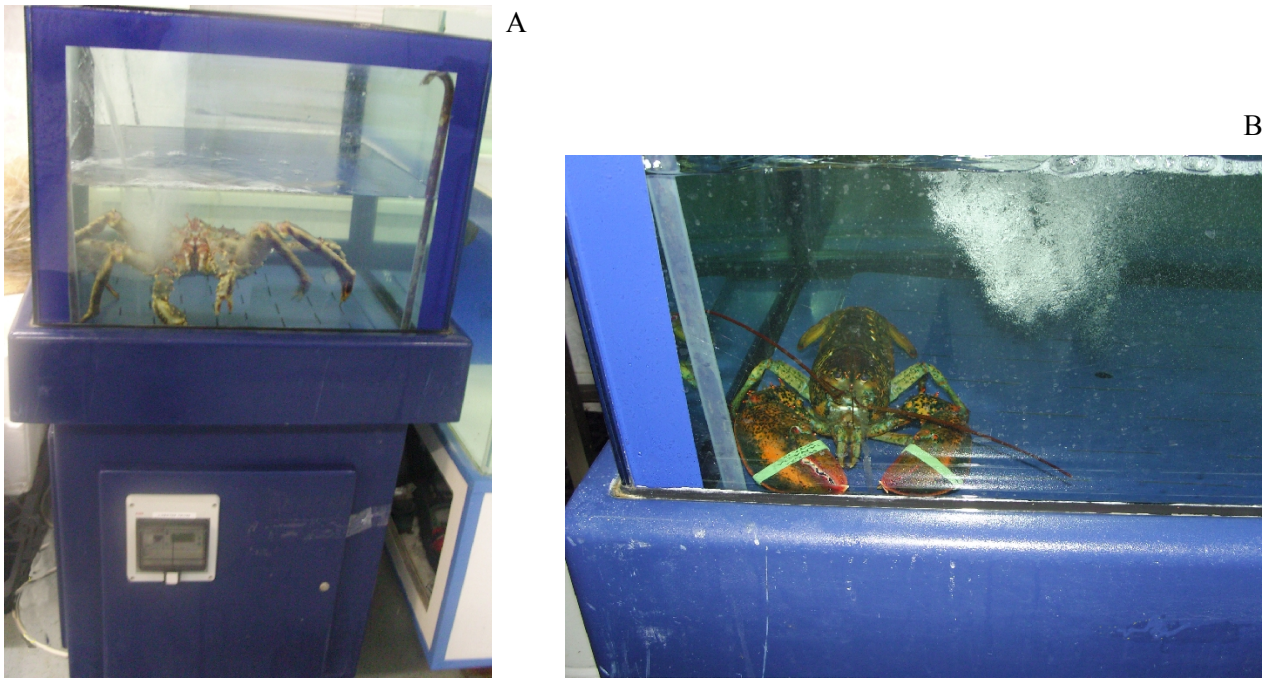

Fig. 1. Aquatron with red king crab (A – left) and american lobster (B – right)

Ranges, average values of the measured indicators and the brief bioassay are summarized in the Table 1. The results of hydrochemical analysis of water are presented in the Tables 2 and 3.

Table 1. Hydrochemical parameters in the experimental aquatron and bioassay indicators of animals

Indicators (an average)	Temperature, °C	
	5.9–6.9 (6.5)	12–12.5 (12.3)
Body weight, kg (BW): red king crabs american lobsters	 1.66–2,68 (2.108) 0.58–0.79 (0.703)	 1.95–2.74 (2.436) 0.66–0.78 (0.739)
Carapace width of crabs, mm	149–171 (160)	155–174 (167)
Planting density, specimens per m^3	6.7	
Biomass, kg/m^3: red king crabs american lobsters	 11,1–17,9 (14.1) 3,9–5,3 (4.6)	 13,0–18,3 (16.2) 4,4–5.2 (4,8)
The average specific water consumption, l/h per 1 kg BW: red king crabs American lobsters	 207.47 556.86	 176.03 518.31
Dissolved oxygen in water, mg / l: red king crabs American lobsters	 9.7–12.2 (10.6) 10.3–12.4 (11.4)	 8.8–9.6 (9.2) 8.4–9.6 (9)
pH of water: red king crabs American lobsters	 7.63–8.02 (7.83) 7.42–7.73 (7.56)	 7.72–8.29 (7.96) 7.64–7.86 (7.8)

Table 2. Changes in the ammonium concentrations in water (Red King crab)

Number of crab	Initial concentration of NH_4^+, mg/L	Final concentration of NH_4^+, mg/L	Δ, mg/L
	6°C		
1	0.293	0.535	0.242
2	0.237	0.319	0.082
3	0.310	0.473	0.163
4	0.230	0.371	0.141
5	0.250	0.338	0.088
6	0.365	0.636	0.271
7	0.280	0.335	0.055
8	0.277	0.397	0.120
9	0.258	0.327	0.069
10	0.443	0.580	0.137
Average	0.294	0.431	0.137

12**°C**			
1	0.481	0.821	0.340
2	0.531	0.833	0.302
3	0.506	0.865	0.359
4	0.529	1.028	0.499
5	0,547	1.014	0.467
6	0.496	1.016	0.520
7	0.407	0.787	0.380
8	0.373	0.870	0.497
9	0.307	1.084	0.777
10	0.503	1.098	0.595
Average	0.468	0.942	0.474

In the experiment with red king crab at the water temperature 12°C mean concentrations of ammonium was 3.46 times higher than at 6°C.

Table 3. Changes in the ammonium concentrations in water (american lobster)

Number of lobsters	Initial concentration of NH_4^+, mg/L	Final concentration of NH_4^+, mg/L	Δ, mg/L
6°C			
1	0.389	0.42	0.031
2	0.419	0.435	0.016
3	0.447	0.454	0.007
4	0.251	0.256	0.005
5	0.223	0.231	0.008
6	0.202	0.213	0.011
7	0.187	0.192	0.005
8	0.129	0.131	0,002
9	0.164	0.21	0.046
10	0.13	0.157	0.027
Average	0.25	0.27	0.02
12°C			
1	0.16	0.234	0.074
2	0.141	0.161	0.02
3	0.115	0.134	0.019
4	0.133	0.143	0.01
5	0.072	0.128	0.056
6	0.097	0.118	0.021
7	0.116	0.12	0.04
8	0.08	0.089	0.09
9	0.068	0.077	0.09
10	0.048	0.058	0.1
Average	0.1	0.13	0.02

In the experiment with american lobster at both water temperatures average difference of ammonium concentrations was approximately the same level.

However, the difference between the ammonium concentrations is only an intermediate result. Daily excretion of the total ammonium was calculated by dividing the difference between final and initial concentration of total ammonium in water, expressed in milligrams per kilogram of BW (Fig. 3, 4).

For a day, on average crabs were excreted 9.73 mg total ammonium per kilogram of BW at 6°C and 29.16 mg total ammonium per kilogram of BW at 12°C. That way, at 12°C red king crab was excreted total ammonium at 3.04 times more than 6°C. This is due to accelerated metabolism at the water temperature 12°C and increasing in general activity of crabs, because 4°C and 12°C – average water temperature in the Norwegian Sea in winter and summer respectively.

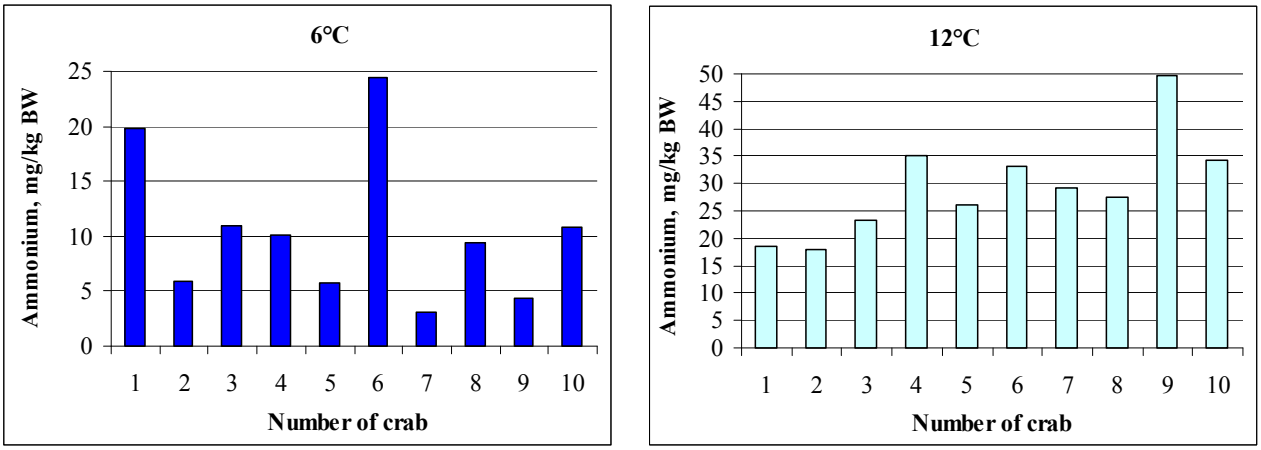

Fig. 3. Daily excretion of total ammonium by red king crab

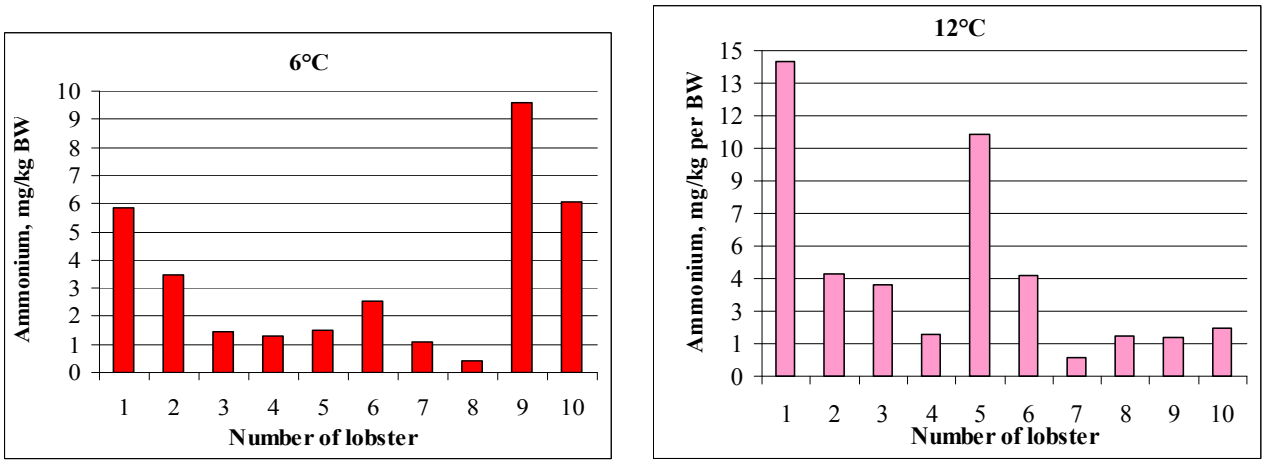

Fig. 4. Daily excretion of total ammonium by american lobster

At the water temperature 6°C american lobsters excreted on average 3.32 mg ammonium per 1 kg BW, and 4.63 mg/kg BW at 12°C respectively. A slight difference may indicate better adaptive ability of american lobster (it is known that lobster is much more stable to changes in water temperature than red king crab). Peaks of concentrations which are demonstrate on diagrams probably due to their different exposure after catching, since the storage complex was carried out continuously acceptance of new parties.

Conclusions

1. At the water temperature 6°C red king crab excreted on average 9.73 mg of total ammonium per kilogram of BW, and 29.16 mg at the water temperature 12°C respectively.

2. At the water temperature 6°C american lobster excreted on average 3.32 mg of total ammonium per kilogram of BW, and 4,63 mg at the water temperature 12°C respectively.

References

Kovatcheva N., Kalinin A., Epelbaum A., Borisov R., Lebedev R. 2006. Early life history stages of the Red King crab *Paralithodes camtschaticus* (Tilesius, 1815). Part 1. Biology and culture. M., VNIRO Publishing, 115 p.

Kormanik G., Cameron J. 1981. Ammonia excretion in the seawater Blue Crab (*Callinectes sapidus*) occurs by diffusion, and not Na^+/NH_4^+ exchange. // Journal of Comparative Physiology, v. 141, P. 457–462.

Shakula L. A., Kovatcheva N. P., Nazartceva M. U., Timofeeva J. V. 2008. Excretion ammonium by Red King crab *Paralithodes camtschaticus* (Tilesius, 1815) at different stages of the life cycle under cultivation. // Tez. reports Third int. scientific-practical conference "Marine coastal ecosystems. Algae, invertebrates and their products", Vladivostok, TINRO-Center, P. 271–272. [in Russian]

Weihrauch D., Ziegler A., Siebers D., Towle D. 2002. Active ammonia excretion across the gills of the green shore crab (*Carcinus maenas*): participation of Na^+/K^+-ATPase, V-type H^+-ATPase and functional microtubules. // The Journal of Experimental Biology, V. 205, P. 2765–2775.

SALTWATER MUSSELS (FAMILY MYTILIDAE) – PROSPECTIVE SOURCE OF HIGH-ACTIVE HYDROLYTIC ENZYMES

R.U. Vysotskaya, V.S. Skidchenko

Institute of Biology Karelian Research Centre RAS, Russia, Petrozavodsk
e-mail: rimma@bio.krc.karelia.ru

An economically and environmentally pressing challenge of today is waste-free sustainable utilization of natural biological resources, a substantial part of which is extracted from the World Ocean. Researchers' attention to marine macro- and microorganisms remains high, since they are a source of physiologically active substances used in human and veterinary medicine, food industry, agriculture and other sectors. Marine flora and fauna supply substances employed in genetic engineering, as fungicides, immunity stimulators, anti-radiation products, diagnostic and health food preparations. Widely used alongside with antibiotics, toxins, pigments, polysaccharides, and proteins are enzymatic preparations, including hydrolases, which decompose natural biopolymers to easily digestible and metabolisable components. Particular focus is on proteolytic enzymes (Mukhin and Novikov, 2001). Little studied in terms of applied enzymology are glycosidases from marine organisms. Since hereditary deficit of acid glycosidases causes lysosomal storage disorders, such studies would promote development of means and methods to fight this disease.

The present study aimed to investigate the activity of lysosomal glycosidases in different organs of the bivalve *Mytilus edulis* L. Mussels are typical inhabitants of the littoral and sublittoral zones of the White Sea. Besides, they are widely used in mariculture, both in Russia and in many European countries.

The material for the study was collected at the Kartesh facility of the RAS Zoological Institute's White Sea Biological Station. Mussels *M. edulis* were captured from the sublittoral zone in the Chupa Bay, Gulf of Kandalaksha. Mussels of one size were placed in 16 litre aquaria with salt water, natural temperature and light regimes, and forced aeration. After the mussels had grown acclimated to laboratory conditions they were subjected to biochemical study. Mussel organs were frozen, taken to the laboratory and stored at – 80°C until analysis. The material was then homogenized in 0.25 M sucrose solution with 0.001 M EDTA and 0.1% triton X-100 detergent, pH 7.4. The tissue/sucrose ratio was 1:9. The homogenates were clarified by centrifugation in K-24 centrifuge at 12 000 rpm. Lysosomal enzyme activity and protein content were determined in the supernatant fluid using techniques adjusted to the study object (Vysotskaya and Nemova, 2008). β-glycosidase (EC 3.2.1.21) activity was determined using the sodium para-nitrophenyl-β, D-glucopyranoside substratum with 0.15 M citrate-phosphate buffer (pH 5.0). β-galactosydase (EC 3.2.1.23) activity was determined by its reaction with para-nitrophenyl-β, D-glucopyranoside with citrate buffer (pH 4.0).

Enzyme activity was calculated as μM of para-nitrophenol formed through the reaction per 1 g of wet weight of tissue per minute and per 1 mg of protein.

The study showed the activity of lysosomal glycosidases in organs of mussels from the White Sea to be quite high (Table). Notably high is the β-glycosidase activity in the mussels' digestive gland. We have never observed such high glycosidase activity in our studies of lysosomal enzyme activity in other aquatic organisms. Absolute values of acid glycosidase and galactosydase in the digestive gland of the mussels are an order of magnitude higher than in fish, even in lysosome-rich organs such as kidneys and liver (Vysotskaya and Nemova, 2008).

Table. Glycosidase activity in organs of the mussel *Mytilus edulis* (μM para-nitrophenol / g wet weight / min), n = 7

Organ	β-glycosidase	β-galactosidase
Digestive gland	1.48–1.57	0.474–0.640
Gills	0.022–0.041	0.123–0189
Foot	0.012–0.013	0.040–0.048