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Effect of Short-term Elevation Temperature and Salinity Stress on Caspian Roach, *Rutilus caspicus*

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Abstract

Caspian roach, *Rutilus caspicus* must have adaptive mechanisms to control internal homeostasis over a broad range of ambient various such as the heat shock (HS) and salinity changes. This experiment was carried out in two stages. In first stage, thirty juveniles fish (3.2 ± 0.34 g) transferred to 20 L circular tanks, containing three different salinity (5, 10, 15 ppt). Initially, half of the treatments exposed to a HS (26°C for 2 h) while the range of normal temperature was 16.5-17.5°C. At 96 h after transferring, survival rate, hematocrit, plasma Na^+, K^+ and Cl^- and osmolality and gill NKA activity were determined. In the second stage (second 96 h), all of the first treatments were transferred to 15 ppt and similar sampling was done. In first stage, no mortality in 5 and 10 ppt of both non heat shock (NHS) and HS treatments and higher plasma osmolality, ions (except K^+), and hematocrit were observed. Mortality was observed in 15 ppt of NHS and in second stage, both treatments of 15 ppt showed mortality (15-20%). In NHS, significant increase of gill NKA found at in 10 ppt of second stage while in HS treatment was in 10 ppt of first stage. Changes in plasma osmolality and electrolytes in HS treatment were more less similar to NHS treatment. Together, it seems HS and salinity changes resulted to disturbances from an internal fluid shift thus a stress situation and Caspian roach juveniles need to complete ion-osmo regulation systems for adaptation with brackish water.

Keywords: *Heat shock, Salinity, Osmoregulation, Gills, Na+/K+-ATPase activity*

Introduction

In teleost fishes, highly efficient ion/osmoregulatory mechanisms lead to maintenance of body fluid homeostasis, which is necessary for the normal operation of cellular biochemical/physiological processes [1]. Approximately 5% of teleost fish are euryhaline while the most teleost fish are stenohaline and cannot tolerate large changes in salinity [2,3]. Euryhaline teleosts have the ability to adapt to different environmental salinities while maintaining essentially constant their internal milieu by the activation of several osmoregulatory mechanisms, namely in the branchial and renal epithelia [4,5]. Gills, kidney and digestive tract are the main osmoregulatory organs in teleost fishes [4,6]. The rapid response and/or acute transition to changing environmental salinity become a crucial challenge for avoiding significant internal osmotic disturbances. There are two periods of acclimation for euryhaline teleosts to hyperosmotic environments: a) a crisis period (minutes to hours) involving a rapid increase in gill-ion fluxes, activating exist proteins, water transport and/or other mechanisms [7], and elevated plasma ions and osmolality followed by b) a regulatory period (hours to days onward) including increases of gill Na^+/K^+-ATPase (NKA) activity accompanied by a proliferation and development of mitochondrion-rich cells (MRCs) presumably hormonally regulated allowing for increased transport capacity [8], increasing net Na^+ and Cl^- efflux and restoring plasma ions balance [9,10]. Na^+/K^+-ATPase, primary driving force for flux of intra and extra cellular NaCl which is specifically present in high concentrations on the basolateral side of MRCs, plays important roles in maintaining the cell membrane potential by pumping Na^+ out and K^+ in through active transport [9]. Changes in gill NKA activity are observed 2-3 days after transfer from a hypoosmotic to hyperosmotic environment in euryhaline teleosts [11]. In anadromous species [12,13], activation of gill NKA takes place 3-7 days after transfer to SW and also in mullet and killifish, gill NKA activity elevated rapidly within 3 h after transfer from FW to BW or SW [14]. In both of FW and seawater (SW), the regulation of the ion levels and osmolality of body fluids of fishes are doing actively [2]. The plasma osmolalities of euryhaline teleost species of FW and SW origin vary [15] and in a number of euryhaline teleosts showed the effects of changing salinity on plasma osmolality and circulating electrolytes [16-21].

Since the most organisms on Earth are ectotherms, such as fish, surviving and adaptation to temperature fluctuations are crucial for

them (Somero, 2010; Tang *et al*., 2014). Rapid water temperature changes (exceeds the optimal temperature range) or exposures to sustained temperatures outside the optimal range (thus, sub-optimal) often result in thermal stresses or lethal conditions (Portz *et al*., 2006). The fishes can be classified into two groups including eurythermal and stenothermal species which tolerance wide and narrow ranges of temperature, respectively [22,23]. Internal electrolyte and osmotic homeostasis in aquatic ectotherms can be influenced by environmental temperature [17,24,25] which is contributed to the regulation of ion-transporting mechanisms by many proteins while stenothermal species have marginally stable of cellular proteins in a limited range of temperature [17,24,26].

The Caspian roach, *Rutilus caspicus* (Yakovlev 1870) belongs to the Cyprinidae, the largest family among FW teleosts, is moderately euryhaline, omnivorous feeding on small crustacea and insect larvae and often lives in areas close to the estuary where water is brackish. Spring and fall migrations, from sea to the river and sea inner migration for spawning and wintering, occur in its life, respectively [27] and also considered as a significant food source for beluga sturgeon, *Huso huso* (L. 1758) in the Caspian Sea [10,28]. This species has been considered for inclusion in the list of threatened species for the region due to over fishing and deterioration of its spawning ground [29]. Since reproduction and sustainability of teleosts species stocks such as Caspian roach negatively are affected due to the human activities in the southern of Caspian Sea, moreover the critical importance of reconstruction such resources, fisheries organization in Iran, using artificial propagation program for releasing millions of different kinds of teleosts larva and juveniles, derived through artificial propagation, to connected rivers to the Caspian sea [10]. Being a eurythermal fish, Caspian roach like *cyprinus carpio* L. must have adaptive mechanisms to control internal homeostasis over a broad range of ambient temperatures particularly in releasing time (May-July) while the fish exposing to the heat shock and salinity changes in transferring processes.

The main goals of the present study were to determine the effects of short-term elevation **t**emperature and direct salinity transferring stresses on the osmoregulatory capability of Caspian roach. Considering the value of gill NKA response it is worth to study of the impact of to environmental stresses such as temperature and salinity on osmoregulatory responses in Caspian roach. The selected salinity treatments were base of the salinity range that Caspian roach are likely to encounter in Bandare Torkaman coastal area, Gorgan bay, southeastern of the Caspian Sea, Iran.

Material and Methods

Animals

Approximately 600 juvenile (aged between 3 and 4 months) Caspian Roach, were obtained from the Sijual Teleost Fish Propagation and Rearing Center, close to the Bandare Torkaman, southeast of the Caspian Sea, Iran. The fish were transferred to Aquaculture research center of the Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. All fish were acclimatized to laboratory conditions for at least two weeks prior to experiments in six 400 l fiberglass tanks, with approximately 150 juveniles in each tank, to avoid the potentially confounding effects of handling stress (e.g. high blood cortisol) on osmoregulation [30]. Fish were fed twice daily with a commercial diet, biomar (0.8; Nersac, France) during holding. Fish were not fed during the experiment. Fish were exposed to ambient photoperiod of approximately 14 h light:10 h dark.

Dechlorinated tap water was used during the experiment and Caspian seawater (SW) with maximum salinity of 15 ppt (obtained from Bandare Torkaman sea shore, Gorgan Bay, Iran) were used for preparing waters of different salinities, ppt. Salinity, temperature (range 16.5-17.5°C), pH (range 8.2-8.6) and dissolved O_2 (7.6-13.3 mg l^{-1}) were measured daily to the nearest 0.1‰, 0.1°C, 0.1 pH unit, 0.1 mg l^{-1}, respectively using a water quality meter (U-10, Horiba Ltd, Japan).

Salinity Acclimation Experiment

The experiment was carried out in two stages including three salinity treatments with three replicates. Feeding was stopped 24 hour before starvation. After adaptation with experimental conditions, initially, half of the treatments exposed to the heat shock (HS), 26°C for 2 hours (h), while the range of normal temperature was 16.5-17.5°C. In first stage, thirty juveniles fish (3.20 ± 0.34 g) directly transferred to the 20 L volume plastic circular tanks, containing three different salinity (5, 10 and 15 ppt). At 96 h after transferring, survival rate, haematocrit, Na^+, K^+, $Cl^-$and osmolality concentrations and gill NKA activity were determined.

In the second phase, all of the first phase individuals were transferred to 15 ppt. At second 96 h after transferring, similar sampling mentioned above were performed. Twelve individuals were sampled two times, just before transferring to other salinities (second phase). About 24 individuals, per treatment groups, of this specie were used for the experiment.

Sampling

The six fish from each treatment were anesthetized with clove powder (100 mg/l) and samples of the blood were taken immediately into a 75 mm heparinised capillary tubes by caudal transaction. Capillary tubes with blood samples were centrifuged at 5000g (Hettich: D_78532 Tuttlingen, Germany) for 15 min at 4°C, for the measurement of haematocrit (Hct) and aliquots of plasma were stored at -80°C [10].

Analytical Techniques

Plasma Ion and Osmolality Measurements

Plasma Na^+, K^+ and Cl^- concentrations were measured using an ion-selective electrodes (Electrolit analyzer mod EI-99IE, Germany) and results reported in mEq·l^{-1}. Plasma osmolality was determined in fresh samples using a freezing point depression (OSMOMETER AUTOMATIC model. Roebling, Germany) and reported as mOsmo1. l^{-1} [10].

Gill Na^+/K^+-ATPase Activity

Gill NKA activity was measured according to the McCormick (1993) microassay protocol with some modifications [10,31]. Gill filament samples from the leftside second arch were served by fine point scissors, from the anesthetized fish and immersed in 100 µl of ice-cold SEI buffer (150 mmol.l^{-1} sucrose, 10 mmol.l^{-1} EDTA, 50 mmol.l^{-1} imidazole, pH 7.3) and frozen at -80°C.

The thawed filaments were homogenized with pestle in SEI buffer containing 0.1% deoxycholic acid and centrifuged at 8000g for 60 s to remove large debris. For the assay 25 µl of the supernatant were added to 500 µl of assay mixture (Imidazole buffer (50 mM) Phosphoenolpyruvate (2.8 mM), NADH (0.22 mM), ATP (0.7 mM), Lactic Dehydrogenase (4.0 U), Pyruvate Kinase (5.0 U). Assays were run in two sets of duplicates, one set containing the assay mixture and the other assay mixture plus ouabain (1.0 mM; Sigma-Aldrich Chemical Co., St. Louis, MO, USA) to specifically inhibit NKA activity. ATPase activity was detected by enzymatic coupling of ATP dephosphorylation to NADH oxidation measured at 340 nm with a spectrophotometer (Photometer clinic Π, Iran) for 10 min at 30°C. Total protein concentrations were determined by modification of the Bradford (1976) dye binding assay with a bovine serum albumin (BSA) standard at 630 nm and the results expressed as µmoles ADP/mg protein/hour.

Statistical Analysis

All the data are expressed as means with standard deviation (SD). Analysis the data of plasma ions, osmolality and gill NKA activity between groups was carried out using one-way analysis of variance (ANOVA) by SPSS (17) in individuals. Statistically significant differences were expressed as $p < 0.05$.

Ethical Statement

The collection and use of experimental animals in this study complied with Iranian animal welfare laws, guidelines and policies, and was approved by the Gorgan University of Agricultural Sciences and Natural resources, College of Fisheries and Environment, Gorgan, Iran and the Portuguese Animal Welfare Law (Decreto-Lei no.197/96) and animal protocols were approved by CIIMAR/UP.

Results

Survival

No mortality occurred in 5 and 10 ppt of non-heat shock (NHS) treatments in first stage (Figure 1). However, after 96 h exposure mortality was significantly higher in 15 ppt of NHS treatments in first stage (Figure 1). There were a non-significant mortality in 5 and 10 ppt of heat shock (HS) treatments in first stage (Figure 1). In the second stage, a significant mortality was observed in 10 ppt of HS treatment and 15 ppt of both NHS and HS treatments (Figure 1).

Osmoregulatory Indicators: Plasma Ions and Osmolality

Blood parameters from stages 1 and 2 are presented in Figures 2 and 3, respectively. Plasma Na^+ and Cl^- levels increased significantly in most of treatments compared with control in first and second stages (Figures 2a, 2b, 3a and 3b) except Cl^- levels in 5 and 10 ppt of fist stages (Figure 2b). Plasma K^+ levels were not altered by 96 h acclimation (first stage) to 5, 10 or 15 ppt (Figure 2c) however were significantly lower following second stage except in 5 ppt of HS treatment (Figure 3c). Plasma osmolality showed an significant increase only in 15 ppt of NHS at first stage (Figure 2d) however all of treatment in the second stage showed significant increase (Figure 3d).

Blood haematocrit showed significant increase in in 5 and 15 ppt of NHS while there was not significant change at HS treatment at first stage (Figure 2e). The all treatments in second stage showed significant increase compare to control group (Figure 3e).

Gill NKA Activity

Na^+/K^+-ATPase activity in gill was rather similar to the initial levels in the FW control treatment after 96 exposures in 5, 10 or 15 ppt and only HS treatment at 10 ppt showed significant increase in first stage (Figure 2f). At the second stage (192 h), only 10 ppt of NHS showed a significant increase in gill NKA activity compared with the control group (Figure 3f). In NHS treatment, 5 and 10 ppt showed higher NKA activity rather than 15 ppt while in HS treatment NKA activity of individuals at 15 ppt was higher than 5 ppt (Figure 3f). In each salinity, NKA activity of 5 and 10 ppt at NHS were higher than HS treatments while it was inverse at 15 ppt (Figure 3f).

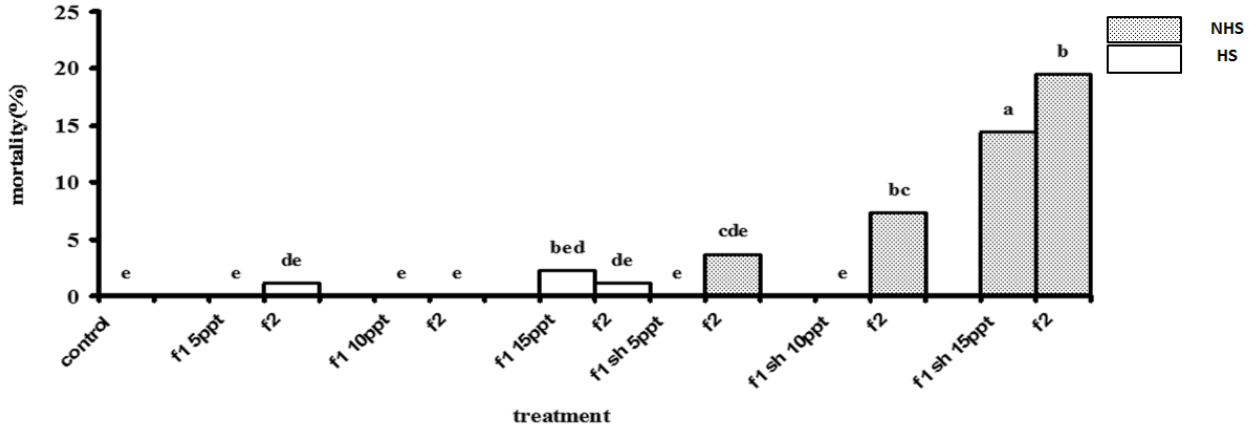

Figure 1: Mortality percentage of *R. caspicus* in the first (96 h) and second stages (192 h), dotted bars, of salinity acclimation in 5, 10 and 15 ppt with (HS) and non-heat shock (NHS). Values are means + s.d. (n = 6). Bars with the same lower case letters are not significantly different from each other ($P < 0{\cdot}05$).

First stage

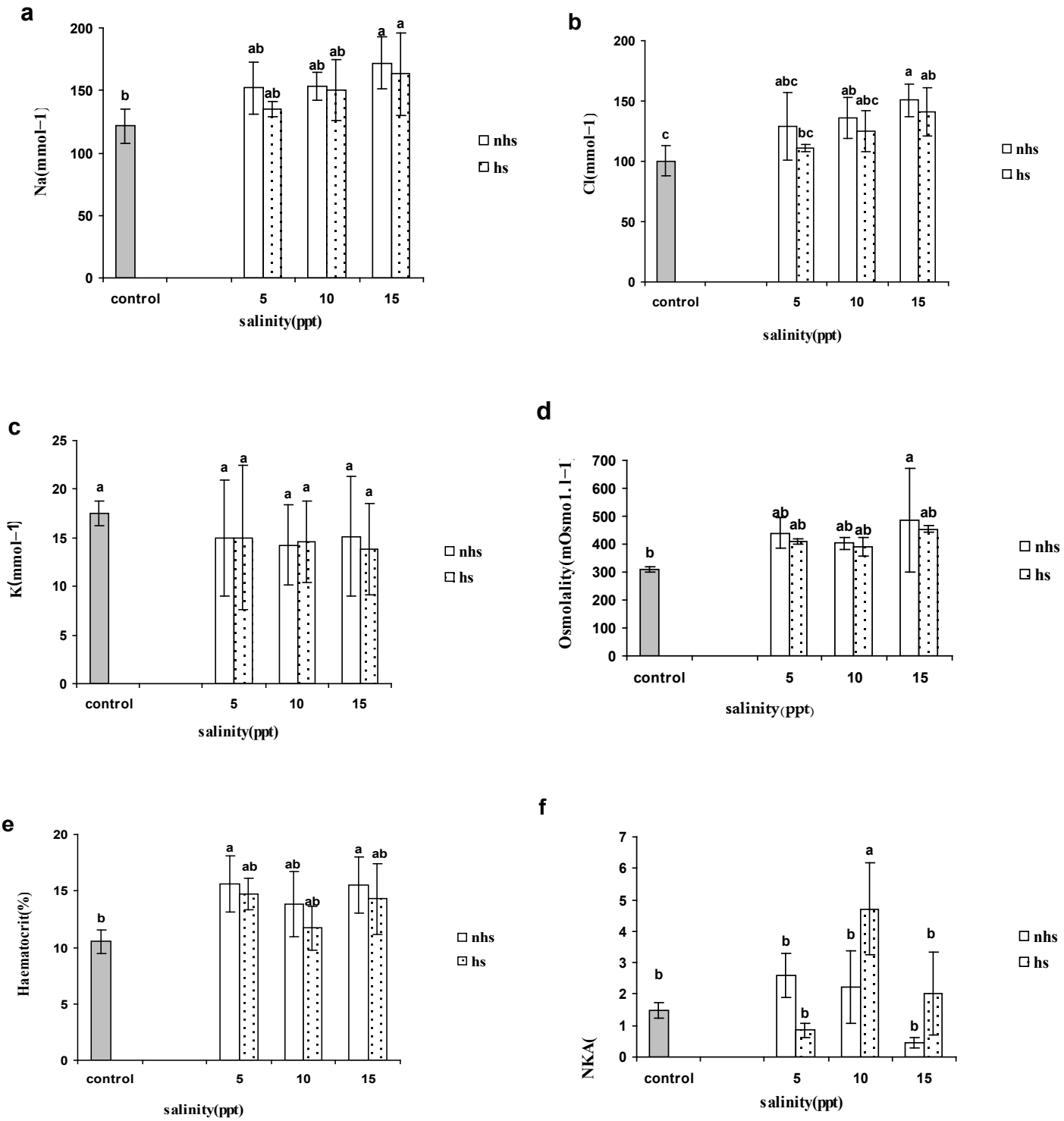

Figure 2: Plasma (a) sodium, (b) chloride and (c) potassium concentrations (mEq·l^{-1}), (d) osmolality (mosmo.kg^{-1}) and (e) haematocrit (%) as well as (f) gill Na^+/K^+-ATPase activity (µmoles ADP/mg protein/hour) of *R. caspicus* transferred from 0 to 5, 10 and 15ppt and acclimated for 96h at each salinity, first stage. Dotted bars represent heat shock (HS) treatment. Values are means ± S.E.M. (n=6 whole times). Bars with the same lower case letters are not significantly different from each other (P<0.05).

Second stage

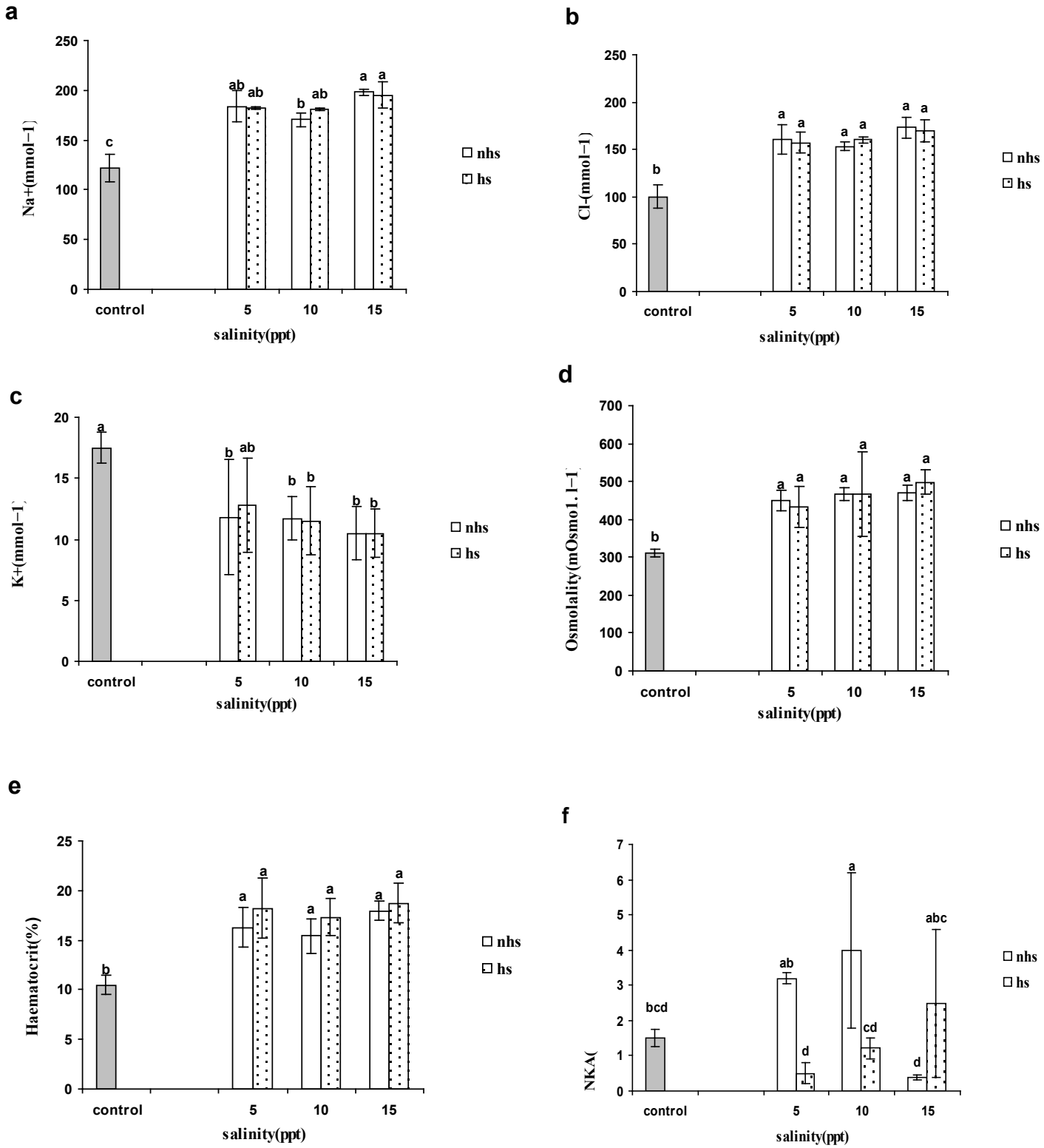

Figure 3: Plasma (a) sodium, (b) chloride and (c) potassium concentrations (mEq·l^{-1}), and (d) osmolality (mosmo.kg^{-1}) and (e) haematocrit (%) as well as (f) gill Na^+/K^+-ATPase activity (µmoles ADP/mg protein/hour) of *R. caspicus* transferred from first stage (0 to 5, 10 and 15ppt) to second stage (15 ppt) and acclimated for second 96h (192h) at given salinity (5, 10 and 15ppt). Dotted bars represent heat shock (HS) treatment. Values are means ± S.E.M. (n=6 whole times). Bars with the same lower case letters are not significantly different from each other (P<0.05).

Discussion

Acclimated *R. caspicus* to higher salinity had higher plasma osmolality and ions (except K^+), and hematocrit. Salinity of 5 and 10 ppt represents the more natural salinity range of Caspian roach. However, acclimation to 15 ppt has allowed us to test the osmoregulatory abilities of *R. caspius* under more challenging conditions. In the first stage, the direct transfer to 5 and 10 ppt in both NHS and HS treatments resulted to no mortality which might indicate relative ability of Caspian roach to tolerate such environmental condition changes. However, the observed mortality in 15 ppt of NHS and/or HS treatment might express imposed stress due to synergism effect of stressors. In NHS, two times increase in gill NKA found in 10 ppt after transferring from first to second stage which might be contributed to rather intermediate salinity at first stage then increase positively with salinity at the second stage. In HS treatment, detected higher gill NKA at 10 ppt at first stage might be related to the stress of HS and requiring stabilizing the energy consuming then reduced to the half in second stage as regulatory period. Changes in plasma osmolality and electrolytes in HS treatment were more less similar to NHS treatment which might be occurring of initial dehydration. However, it seems that HS and salinity changes have some physiological effects on ion regulation in this fish. Together, these data representing disturbances from an internal fluid shift potentially due to water loss and elevated plasma osmolality which may be problematic resulting in a stress situation and mortality.

Survival

The observation of no mortality duration of direct transfer to 5 and 10 ppt in both NHS and HS treatments at the first stage, indicating relative ability of this fish to tolerate such salinity changes. [32] expressed the metabolic cost of osmoregulation is reduced in brackish water (BW), because the blood-medium osmotic gradient is minimal. In contrast, gradual transfer of Caspian roach to different salinities (5, 10 or 15 ppt) resulted no mortality [10]. The goldfish *Carassius auratus* (L. 1758) showed high survival under chronic exposure to salinities of 5 and 10 ppt while significant mortality was observed at salinities of 15 and 20 ppt [33]. Such observation might be related to different level of endocrine and ionoregulatory pathways developing, as has been suggested in some works [34] on smoltification in salmonids or due to social hierarchies, which can also influence ionoregulatory capacity [35] and also change in chloride cells morphology and restoration of homeostasis [36,37]. The direct transferring of Common carp, *Cyprinus carpio* (L.). to environmental salinity (2.5, 5, and 7.5‰) showed a great adaptation and higher survival rate [38]. [39] reported survival rates of lake trout (*S. namaycush*), brook trout (*S. fontinalis*) and Atlantic salmon (*Salmo salar*) were 80%, 50% and 100%, respectively following direct transferring to 30 ppt. [40] reported direct transfer of FW-adapted white sturgeon juveniles from FW to 16 ppt was associated with 25 to 30% mortality, indicating that these fish have some ability to tolerate large changes in salinity for up to 5 days. [32] reported Nile tilapia, *Oreochromis niloticus*, which were transferred directly to full strength SW (36 ppt) for 14 days whole mortality occurred in this period. However, *O. mossambicus* and its hybrids showed not survive by direct transfer to salinities of 35 psu [25,41]. The observed mortalities in 15 ppt of NHS treatments might be related to osmotic shock of direct transfer to this salinity and also delay in gill NKA activation in response to osmotic challenge that is proposed to reflect changing gene expression but also transcript expression and protein synthesis [42-45]. In second stage, both treatments of 15 ppt showed mortality (15-20%) which might be indicted to imposed stress because of HS and subsequent apoptosis [46] and also osmotic challenge of transferring to this salinity on the other word synergism effect of stressors.

Osmoregulatory Indicators: Plasma Ions and Osmolality

Salinity challenges typically alter plasma osmolality and electrolytes levels in euryhaline teleosts with an initial crisis stage followed by a regulatory stage [7,10,11,12,20]. The observed plasma ion concentrations were in the range of other teleost fish species [47]; see reviews by [48]. The elevated plasma osmolality, and electrolytes (except plasma K^+) of Caspian roach in the most treatments of both stages might be contributed to the occurring of initial dehydration due to osmotic efflux of water from the fish by osmosis and diffusional ion influx of electrolytes from the environment [39,45,49]. Blood osmolality in teleost fish is 280-360 mmol kg^{-1}, and is tightly regulated in a species-dependent range of salinities [15]. In present study based on comparison to euryhaline fish, the values of Na^+, Cl^- and osmolality are relatively high suggesting that the fish have relatively poor salinity tolerance, or that they are in a temporary state of ion imbalance. Also increased levels of electrolytes concentrations had not returned to initial concentration (control treatment) as has been reported in [50]. The rather similar results observed in gradual transferring of Caspian roach to different salinity levels (5, 10 or 15 ppt) [10].

In general changes in hematology can be explained by changes in ionoregulatory status [40] and it is one of the secondary stress responses in fish [51,52]. Hematocrit showed a positive correlation with salinity in both stages, as has been reported by (*Platichthy flesus*: [53]; *Gymnocypris przewalskii*: [10,20,54,55] and in most anadromous teleosts studied to date (*Oncorhynchus mykis*: [56]; *O. tshawytscha*: [57]; *S. salar*: [58]) or sturgeon (*Acipenser oxyrinchus, A. brevirostrum*: Baker *et al.*, 2005). By attention to previous finding which indicating to the important role of effective hormones such as cortisol, prolactin, in acclimation phase to osmotic and environmental challenges (several hours to several days after stress) [8], this result might be interpreted by measuring hormonal changes but not done in the present study thus measuring hormones which are involving in response to environmental challenges could be helpful in future study.

Gill Na^+/K^+-ATPase Activity

The assessment of osmoregulatory status/ability of teleosts has been achieved by using the branchial NKA activity responses (mRNA and protein expression) [1,59]. The alterations in gill NKA activity in relation to environmental salinity are diverse, but two typical situations seem to prevail: (i) a direct relationship, characteristic of anadromous species, in which higher salinities induce higher values of NKA activity [60] and (ii) a U-shaped relationship, described for some euryhaline teleosts, in which lower values of NKA activity occur at intermediate salinities and higher values at low and high salinities [10,11,45,61-63]. There are

various reports which express/state no effect of salinity on NKA activity (*Gillichthys mirabilis*: [64]), conversely, strong effect of medium salinity on gill NKA activity (*Scophthalmus maximus*: [65]), positive correlation between environmental salinity and NKA activity (*Oreochromis mossambicus*: [66]; *Onchorhynchus keta*: [67]) and negative correlation (*F. heteroclitus*: [68]; *O. mossambicus*: [20,69]). Short- and long term acclimation to environmental salinity have examined intermediate metabolism changes in euryhaline fishes [70-74]. Gaumet *et al.* (1995) suggested that NKA activity is generally lowest in fish living in a medium whose salinity is equivalent to that of the blood. An ecologically theory would state that fish would be adapted to spend the least amount of osmoregulatory energy in environmental salinities they evolved to live in. Also, physiologically we would expect the energy consuming NKA activity to be minimal at environmental salinities isosmotic to blood [10,75]. According our results most changes occurred in 10 ppt of NHS treatments (Figure 2f). The observed two times increase in gill NKA of 10 ppt after transferring from the first to second stage (15 ppt) might be contributed to rather intermediate salinity at first stage then increase positively with salinity at second stage. Adaptation of Caspian roach individuals to different salinity presumably can be results increasing the number of entering ions to the body thus occurring water loss by osmosis. In continue, increasing/or tendency to increase in gill NKA activity occurring to absorb water from the external environment [21] however, decreased significantly at the salinity of 15 ppt. The latter might be due to the increase of plasma Na^+ and Cl^- as the major electrolytes in the body fluid and their critical role in osmoregulation [59] which result to increase plasma osmolality under higher salinity condition thus decrease of NKA activity. The similar results have been reported in the juvenile largemouth bass adapted to saline waters [21] and Mozambique tilapia [76]. In juvenile turbot (*Scophthalmus maximus*) reduced gill NKA activity at 15‰ salinity levels would lead to reduced energy expenditures [65]. In another experiment, gill NKA activity of Caspian roach decreased with salinity in the short term with activity being the lowest in fish kept 48 h at 15 ppt although with longer acclimation (+96h) returned to control levels [10]. Furthermore, the results of this study might be indicating that Caspian roach are a bit intolerant of salinity. Some studies revealed that the source of changing NKA activity upon salinity challenge might be alterations on mRNA level [43,77], or protein level (*O. mossambicus*: [78,79], or both levels [80]; *O. mossambicus*: [69]). In general, related to the effect of abrupt transfer to different salinities on physiologic /osmoregulatory functions more studies are required. Perhaps, Caspian roach like salmonids, anadromous species, [45] activation of gill NKA takes place 3-7 days after transfer to higher salinity or that 15 ppt is just not enough salinity to induce increases. It would be of interest to see if the fish can survive long term exposure (2 weeks or more) to 15 ppt (or more) and what the levels of plasma ions and gill NKA activity are after this acclimation.

Heat Shock (HS)

The ambient temperature can effect on the internal ionic and osmotic balance of fish [17,24,81,82]. Rapid temperature changes, heat or cold shocks, are among the stressors with a high physiological impact on fish [83,84]. Changes in plasma osmolality and electrolytes in HS treatment were more less similar to NHS treatment which might be occurring of initial dehydration. A reduction was observed in plasma K^+ level accompanied by salinity increase in the second stage. It has been shown that in SW the fish gills are permeable to K^+ that efflux is greater than influx [85]. This would indicate that reduced uptake, rather than increased loss of K^+, is the more important factor contributing to the poor performance of fish [10,86]. Also, change in gill NKA activity [45] and passive efflux of K^+ from kidney segments [87] can be potential reasons for such reduction. After exposure of *R. caspicus* to short term increasing temperature condition, ascending trend in first stage and significant increase of plasma osmolality at second stage were found (Figure 2d and 3d). Even though the gill NKA was not affected (Figure 2f). Moreover, NKA activity was assayed at the higher level than exposure temperature of the fish that might show the apparent NKA activity not provide a physiological interpretation of our results. The protein conformation, kinetic properties, and assembly can be affected by influencing of temperature on the reactivity of molecules [88]. The activation of ion transporter system is energy-required while the main process for energy providing, the rate of cellular respiration, is temperature-dependent [89]. Therefore, detected higher gill NKA at 10 ppt at first stage might be related to the stress of HS and requiring stabilizing the energy consuming then reduced to the half in second stage as regulatory period (Figure 2f) which potentially reflected that metabolically-dependency of ion transporter proteins to temperature change rather susceptible to passive ion diffusion [89-92]. Furthermore, inhabitation of specific activity of NKA by temperature was found in the Mozambique tilapia and common carp [88]. It was found that a lower apparent NKA activity was compensated by strongly enhanced NKA expression [17,24]. The present study was difficult to rule out the possibility of heat shock having much of an impact on overall ion regulation although clear responses to salinity can be found. Study on the heat shock proteins (HSP70, 90) by suing immunoblotting and gene expression (PCR-qPCR) considering response to the environmental stress such as heat shock or salinity changes, measurement the plasma cortisol, lactate and glucose might be interest for future works.

Conclusion

It seems that Caspian roach juveniles need to complete ion-osmo regulation systems for adaptation with BW and biochemistry-physiologic parameters of juveniles are determinant their adjustment to the natural conditions. According the management point of view, it seems that HS and salinity changes have some physiological effects concern ion regulation in this fish. Although for more confidence, study various salinities, different time of sampling and other environmental tolerances such as temperature, culture density, diet and also focus on the expression patterns of ion transporters such as NKA, $Na^+/K^+/2Cl^-$ (NKCC), cystic fibrosis transmembrane conductance regulator (CFTR, chloride channel), V-ATPase proton pump in the gills, kidney and digestive tract [24,55] are needed.

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