Journal of Animal Science and Products (JASP)
International peer-reviewed journal
http://www.trjasp.com
2 (1): 1-12, 2019

Influence of Transfer to High Salinity on Chloride Cells, Oxygen and Energy Consumption in Common Carp *Cyprinus carpio*

Mohammed S. Al-Khshali*, Hasan A. Al Hilali[1]
[1]Department of Animal Production, University of Baghdad, Directorate of Animal Resource, Ministry of Agriculture, Baghdad, Iraq

ARTICLE INFO

This study was presented at the 10th International Animal Science Conference in Antalya on 25-27 October 2018.

Research Article

Received : 09 March 2019
Accepted : 10 December 2019

Key Words

Salinity
Cyprinus carpio
Energy consumption
Aquaculture

***Corresponding Author**

alshaker64@yahoo.com

ABSTRACT

The present study was conducted to investigate the effect of high salinity on some stress parameters of common carp (*Cyprinus carpio*), which gradually exposed to salt concentrations of 5, 10 and 15 g/L, as well as tap water (control 0.1g/L) for 90 days. 80 fish were randomly distributed on eight glass tanks with 2 replicates as 10 fish /tank at average weight of 15 ± 3 g to study the effects of salinity on the number and percentages of the chloride cells in gills, beside the effect of salt concentrations on metabolism and the cost of the energy spent for osmoregulation through studying the oxygen consumption. Chloride cells in the gills were increased to 10.36, 14.80, 11.95 x 10^5 cell /g scraped matter. While the percentage of the chloride cells, increased to 11.34, 12.14 and 11.90% in the salt concentration of 5, 10 and 15 g/L, respectively, in comparison with the control treatment (8.42%). The rise in salinity was accompanied with an increase in the average of the oxygen consumed by common carp as it amounted 150,181.25 and 196.87 mg O_2 /kg/h when the salinity increased to 5,10 and 15 g/l respectively, in comparison with the control treatment (85.93 mg O_2 /kg/ hour), and this increase in the oxygen consumption resulted in an increase in the average of the energy consumed by fish that reached 0.50, 0.6 and 0.66 kcal (kg/h) with the increase of salinity to 5,10, and 15 g/L, respectively, in comparison to control (0.28 kcal/kg/h).

Introduction

Aquaculture plays an important role in solving famine and malnutrition nutrition problems in whole world through providing fish and other marine animals which are rich in protein, vitamins, minerals and amino acids. The rapid development in Aquaculture resulted in producing more than 123 of fish species in intensive and non-intensive culture systems. For achieving good growth rate, fish must have good health and resists diseases as well as the availability of good quality water (Fazio et al., 2013). The quantity and quality of the feed are insufficient to obtain high fish production, as the environmental factors also play a vital role to obtain a high production, salinity is one of the important factors beside the oxygen and the temperature, it has a direct effect on fish growth (Mommsen, 1998). The Iraqi internal water is exposed to continuous increase in salinity ratio for some reasons, for example; the lack of water scarcity in Tigris and Euphrates rivers, the effect of salty drains entering Iraq, the drying of vast areas of lakes, the decrease in rains and the regression of

Lütfen aşağıdaki şekilde atıf yapınız / Please cite this paper as following;
Al-Khshali, M. S., Al Hilali, H. A. 2019. Influence of transfer to high salinity on chloride cells, oxygen and energy consumption in common carp *Cyprinus carpio*. Journal of Animal Science and Products (JASP) 2 (1):1-12.

rivers. The salinity of the lakes in the southern areas of Iraq is about 2.20 to 3.82 g/L and it's in a continuous increase as well as the increase in vaporization rates due the increase in temperature rates and the increase in global warming rates (Al-Faiz et al., 2009). As we became in a due need to exploit the water which suffer a continuous change in salt concentrations in fish culture because of the lack of fresh water and the availability of brackish water sources which has few uses so the fish must be adapted to raise its ability to bear the salinity in this kind of water as most of the fish living in the fresh water including *Cyprinus carpio* do not bear the high salinity because they are Stenohaline fish and suffer from failures when moving to saline water due to what's known by Osmosis Shock (Jackson, 1981). It was found that it's possible to raise the level of salt durability for fish through the gradual moving to water with gradual salt concentrations (Bardach et al., 1972), The chloride cells present in the gills are responsible for the process of ion transmission and ion equilibrium as well as their participation in the process of acid and base equilibrium (Kaneko and Hiroi, 2008). Thus, chloride cells play an important role in the adaptation process in environments with different osmoses as they have evolved in a way that allows them to obtain large amounts of energy in order to complete the transfer of sodium and potassium ions with the help of ATPase (Shikano and Fujio, 1999),where the change in respiratory rate of fish is one of the common physiological responses to face the salt stress. The change in oxygen consumption is usually used to estimate the change in metabolic rate under environmental imbalance conditions (Dube and Hosetti, 2010) Since the *Cyprinus carpio* fish is considered the first rearing fish in Iraq and due to the increase of salinity rates in the southern areas and some of the middle areas which threaten the survival and the growth of the fish. This study aims to investigate the effect of the increase in the salinity of the osmoregulation of the *Cyprinus carpio* through the study of some physiological indicators which is considered an indication of the negative effects of salinity increase like stress and deficiency of ionic and osmoregulation process. This is reflected in both consumption of the feed and growth, which is the main objective of fish cultivation.

Material and methods

Common carp at the average weight of 15 ± 3 g were obtained from a local fish farm, south of Iraq. They were safely brought to the laboratory and acclimatized for 14 days in a large plastic tank (80×40×55 cm) prior to the experiment. During the acclimatization period, fish were fed on artificial diet once daily (Table 1), fish were distributed on four different salinity treatments (0.1, 5, 10 and 15 g/L) and tested in two replicates for each salinity treatment. There were 10 fish in each tank, fed on a diet with 31% protein content (Table 1) at a ratio of 4% of body weight, twice a day. Salt concentrations were prepared by

dissolution of specific of salt in litter of tap water, and fish were exposed to gradual salt concentration of 5, 10 and 15 g/L. while the tap water concentration (0.1 g/L) was represented control treatment.

Table 1. Chemical analysis of diet, %

Chemical parameters	Values
Crude protein	31.00±0.90
Ether extract	9.00±0.20
Moisture	8.66 ± 0.21
Crude ash	11.40 ± 1.20
Crude fiber	40.88 ± 0.12

Chloride cell

To determine the ratio and number of gill chloride cells, the fish gills were dissected and fixed in 10% formalin for 48 hours. After dehydration, clarification, and paraffin immersion, gill paraffin blocks were prepared, and then 5 µm sections were prepared and stained with hematoxylin-eosin. The number and ratio of chloride cells were observed using light microscopy and photographed (Sargent et al., 1978).

Oxygen consumption

Oxygen consumption of common carp at various salinities and oxygen concentrations was measured in closed, opaque chambers as in (Nordlie and Leffer, 1975). A single, post-absorptive fish which had been acclimated to the initial test salinity and temperature was placed in a test chamber containing air-saturated water. The chamber was sealed and placed in a water-bath at 25°C. Approximately 5 min later (the fish became quiescent almost immediately in the dark chamber) water samples were drawn from the chamber. Additional samples were taken at 1 h intervals for 3 h. Oxygen concentrations in the samples were determined using a Radiometer p0, electrode. Following metabolic determinations, the fish were removed and weighed. The mean weights of fish run at each salinity were maintained at between 15 and 18 g so that salinity effects would not be confounded with body size effects.

Statistical analysis

Experiment was conducted in a completely randomized block design.

Data were analyzed by Windows version of SPSS software (Release 21.0). Results were analyzed using a t-test for comparison between treatment. Results were shown as means ± standard error. Significant differences between means were calculated using least significant differences (LSD). Statistical significance was set at $p < 0.05$.

Results

Table 2 shows that the number of chloride cells in gills of common carp was increased to 10.36 x 10^5, 12.14 x 10^5 and 11.95 x 10^5 cells/g scraped matter in salt concentration of 5,10 and 15 g /L compared to control treatment (8.18 x 10^5 cells / g scraped matter). The decrease in the number of chloride cells is observed at the concentration of 15 g / L compared with the saline concentration of 10 g / L. Results of the statistical analysis showed significant differences ($P \geq 0.05$) in the number of chloride cells between the control

sample and saline concentrations of 5, 10 and 15 g / L, as well as among salt concentration 5, 10 and 15 g /L.

Table 2. Shows that the number of chloride cells in gills in different salt concentrations of common carp (mean ± standard error)

Salt concentrations (g/L)	Number of chloride cells in gills (cells/g scraped matter)
0.1	$8.18 \times 10^5 \pm 1.08 \times 10^5$ b
5	$10.36 \times 10^5 \pm 1.05 \times 10^5$ b
10	$12.14 \times 10^5 \pm 1.03 \times 10^5$ a
15	$11.95 \times 10^5 \pm 1.06 \times 10^5$ a

g, gram; L, litter

On the other hand, Table 3 shows that the ratio of chloride cells from the total cells of the common carp was increased to 11.34%, 12.14% and 11.90% at the saline concentrations of 5, 10 and 15 g / L respectively, compared with the control sample (8.42%). A decrease in chloride cell ratios is observed at salt concentration of 15 g / L, compared with saline concentration of 10 g / L. The statistical analysis showed significant differences (P≥0.05) in chloride cell ratios between control treatment and saline concentrations of 5, 10 and 15 g / L and between saline concentrations 10 and 15 g / L.

Table 3. Shows the ratios of chloride cells in different salt concentrations of common carp (mean ± standard error)

Salt concentrations (g/L)	The ratio of chloride cells (%)
0.1	8.42 ± 0.86 c
5	11.34 ± 0.90 b
10	12.14 ± 1.30 a
15	11.90 ± 1.12 a

Effect of salinity on oxygen and energy consumption rate

Table 4 shows the increase oxygen consumption in common carp to 150, 181.25 and 196.87 mg / kg / h with increase in salinity to 5, 10 and 15 g / L, respectively, compared with the control sample (85.93 mg / kg / h) Results of the statistical analysis showed significant difference (P≥0.05) in the oxygen consumption rate between the control sample and each of the saline concentrations 5, 10 and 15 g / L, among all of saline concentrations.

Table 5 shows the amount of energy consumed (kcal/kg/h) in common carp, resulting from the conversion of the amount of oxygen consumed to energy. with an increase in energy consumption to 0.50, 0.61 and 0.66 kcal per kg with the increasing in salinity to 5, 10 and 15 g / L respectively, compared to the control treatment (0.28 kcal / kg / h). There were significant differences (P≥0.05) in the amount of

energy consumed between the control sample and each of the concentrations 5, 10 and 15 g / L, and among all saline concentration.

Table 4. Shows the oxygen consumption in different salt concentrations of common carp (mean ± standard error)

Salt concentrations (g/L)	Oxygen consumption rate (mg / kg / h)
0.1	85.93 ± 3.26 d
5	150.00 ± 4.06 c
10	181.25 ± 4.46 b
15	196.87 ± 5.06 a

g, gram; L, litter; mg, milligram; Kg, kilogram; h, hour

Table 5. Shows the amount of energy consumed (kcal / kg / h) in different salt concentrations of common carp (mean ± standard error)

Salt concentrations (g/L)	The amount of energy consumed (kcal / kg / h)
0.1	0.28 ± 0.001 d
5	0.50 ± 0.004 c
10	0.61 ± 0.007 b
15	0.66 ± 0.012 a

g, gram ; L, litter ; kcal, kilocalorie; Kg, kilogram ; h, hour

Discussion

Chloride cells are considered the main key for the osmoregulation success, where the increase in their numbers, in the current study is due to the exposure to high salt concentrations, for the fish to be able to adapt to the gradual increase in salinity, It increases the number of chloride cells to raise the ability of the gills to regulate the ionic exchange process and reach internal stability, the decrease in their numbers in the concentration of 15 g/L compared to 10 g/L is due to the decrease in the fish ability to complete the ionic regulation process and just tried to survive. The increase in Sodium ions may support these results as the ATPase/Na/K enzyme exists mainly in gills and the chloride cells play an important role in its secretion, which is responsible for the exchange of sodium, potassium and chlorine ions. The increase in the number of chloride cells in the gills is an evidence of an increase in the activity of the enzyme due to the increase in the number and size of the chloride cells that secrete it. The chloride cells present in the gills are responsible for ions transmission and ions equilibrium, as well as the participation in the process of acid and base equilibrium (Kaneko and Hiroi, 2008). Therefore, chloride cells play an important role in the adaptation process in different environments with different osmoses. As it evolved in a way that allows it to obtain large amounts of energy to complete the transfer of sodium and potassium ions

with the help of ATPase (Shikano and Fujio, 1999), and that exposure to high salinity leads to an increase in the number of chloride cells as well as an increase in the activity of ATPase to transfer Sodium and potassium ions (Carmona et al., 2004). Freshwater fish contain less chloride cells than marine fish, although they have a high ability to absorb salts from the surrounding environment (Fielder et al., 2007). The changes in the shape and size of chloride cells and their increasing numbers are part of the adaptation phase which the freshwater fish follow in response to the increase in environmental salinity (Ghahremanzadeh et al., 2014). Several studies have shown that the increase in salinity is usually accompanied by an increase in the number, size, and ratio of chloride cells in the gills (Evans et al., 2005). Numerous studies have been conducted on Tilapia *Oreochromis mossambicus* to identify the changes that occur during the process of osmoregulation. Fish is used as an important example in the study of salinity endurance for fish and endocrine studies (Hiroi et al., 2005). The location of ATPase enzyme has been frequently diagnosed in chloride cells and is indirectly responsible for chloride secretion by these cells (Epstein et al., 1980). An increase in number of chloride cells in freshwater fish was observed during their adaptation to salt water (Mangum and Towle, 1977). The differences in shape, size and number of chloride cells reflect the responses by fish to adapt to different saline environments (Gulácsi et al., 2003). The present study showed that the exposure of *Cyprinus carpio* to a gradual increase in saline concentrations resulted in an increase in the numbers and proportions of chloride cell in the gills. This shows the effect of the increase in salinity on stimulating the increase in the number and proportions of chloride cells as they play an important role in the necessary effective transmission process to eliminate the salt accumulation that occurs by diffusion due to increased salinity of the external ambient and thus maintaining the internal stability. The ionic equilibrium process is associated with the chloride cells which is rich in mitochondrial for its ability to extract ions against concentration gradient by active transport process which requires energy, obtained from Na + / K + ATPase enzyme (McCormick et al., 1991). Mancera and McCormick (2000) showed that the Na + / K + ATPase enzyme increases in gills exposed to sea water, also Mylonas et al. (2009) mentioned an increase in the effectiveness of Na + / K + ATPase in gills by decreasing environment osmosis. Chloride cells have been shown to be the effective location of ionic exchange and their increase is a physiological response to saline stress which causes ion loss. Eddy (1982) observed that Freshwater Salmo gerdnairy has few chloride cells and low levels of Na + / K + ATPase, and cells number are increasing and their shape changes with increasing salinity. This is done under the control of cortisol, prolactin and growth hormone (McCormick et al., 1991). The result of this study is matching with many previous studies which noted an increase in numbers and proportions of chloride

cells once they exposed to an increase in salinity levels. Azizi et al. (2010) mentioned an increase in the number of chloride cells in *Cyprinus carpio* exposed to a gradual increase in salinity to 9 g/L. Al-Khshali (2011) also observed an increase in the number of chloride cells to 12 x 10^5, 7.8 x 10^5, and 6.55 x 10^5 cells / g of scraped matter with a gradual increase in salinity to 4, 8 and 12 g / L respectively, compared with its control sample (2.88 x 10^5 cells / g scraped matter in *Carassius auratus*. Lee et al. (2000) mentioned an increase in the number of chloride cells in *Oreochromis mossambicus* exposed to salinity of seawater. An increase in the number and proportion of chloride cells in gills of *Liza abu* was recorded after 14 days of salinity elevation to 7 and 15 g / L (Ahmed et al., 2004). This indicates a strong correlation between the number of chloride cells with the salinity of the external ambient (Pereira and Caetano, 2009) showed an increase in the number of chloride cells when fish were exposed to inappropriate environmental conditions causing stress.

Effect of salinity on oxygen and energy consumption The increase in oxygen consumption showed in the present study is resulting from the increase in the metabolic rate to confront the high energy requirements corresponding to the osmosis situation due to exposing the fish to high levels of salinity. This is a natural condition due to saline stress and increased demand for energy by fish for the purposes of osmoregulation. That is can prove through the increase in the number of chloride cells, which is the main location of ionic exchange, whose increase indicates in energy need for the active transport of ions. Oxygen has the largest role in energy production by breaking down the bonds in the food and converting them into energy, and because the body needs amounts of energy in order to reach the state of internal stability, fish need more oxygen to provide greater energy and as fish get farther away from the stable osmosis environment, this led to an increase in energy needs trying to get back to the state of the original equilibrium it was before the exposure to the stress factor. Metabolism includes, chemical reactions occurring in living organisms and the measurement of oxygen consumed is the most common method of indirectly detecting metabolic rate. The energy needed by the body to adapt to changes in saline concentrations can be identified by changing in the oxygen consumption rate (Tseng and Hwang, 2008) which change in respiratory rate of fish is one of the common physiological responses to face saline stress. The change in oxygen consumption rate is usually used to estimate the change in metabolic rate under conditions of environmental imbalance (Dube and Hosetti, 2010). The reason for the change in the oxygen consumption rate following the change in salinity levels is due to increased activity in the active transport of ions and the activity of the Na / k / ATPase in gills, which increases the energy needs for the completion of osmoregulation process (Sangiao-Alvarellos et al., 2003). Morgan and Iwama (1991) Showed increased metabolic rate in *Oreochromis mossambicus* with higher

salinity levels in the fish environment to meet energy requirements for osmoregulation process. the reason for increasing the need for energy is the entry of some hormones in some interactions, as well as the increase in the need of some organs, which are not responsible of the osmoregulation process of energy, such as the brain, (Sangiao-Alvarellis et al., 2007). Thus, the measuring the rate of oxygen consumption at different concentrations of salinity is an approach to determine the cost of energy for osmoregulation (Morgan et al., 1997). Also, oxygen consumption on different metabolic processes has been described previously (Peck et al., 2005). Studies indicate that the difference in the rate of oxygen consumption due to salinity change varies depending on the fish species and duration of exposure to salinity, the design of the experiments and the details of the measurement method used (Gracia-Lopez, 2006). The results of several previous studies of different species fish have shown that an increase in oxygen consumption is corresponding to the increase in the salinity of the environment which is in line with the results of the current study. Al-Khshali (2011) reported that the average oxygen consumption in *Ctenopharyngodon idella* was 132.11, 197.08 and 241.77 mg / kg / h at saline concentrations 4, 8 and 12 g / L, respectively, compared to control sample (102.72 mg of O_2 / kg / h), while the amount of energy consumed was 0.44, 0.66 and 0.81 kcal / kg / h at saline concentrations of 4, 8 and 12 g / L, respectively, compared to control sample (0.34 kcal/kg/h). Ahmed (2005) showed an increase in the oxygen consumption rate to 256.5 and 262.0 mg of O_2 / kg / h in *Liza abu*, with salinity increase to 7 and 15 g / L respectively, compared to fish in freshwater (151.0 mg of O_2 / kg / h). Wang et al. (1997) showed that the average oxygen consumption in *Cyprinus carpio* decreased in fresh water, which was 134.1 mg / kg / h and salinity of 2.5 g / L was 123.8 mg of Oxygen / kg / h compared to salinity 8.5 and 10.5 g / L whose the consumption rate was 175.2 and 183.0 mg oxygen / kg / h, respectively. In a study conducted by (McKenzie et al., 2001) on *Acipenser Naccarii*, an increase in oxygen consumption was observed with an increase in salinity from 0-11 g / L, so the consumption rose from 112. to 146.4 mg / kg / h then rose to 216 mg / kg / h during 70 days. Other studies indicated high metabolic rate when fish were subjected to high saline concentrations, resulting in an increase in routine oxygen consumption (Fischer, 2000; Keddy, 2001). The effect of different salinity levels (1.5, 7.5, 15 and 30 g / L) was studied on *Liza carinata*, it was found that fish in salinity 1.5 and 30 g / L consumed 15.5% and 20.4% more oxygen respectively, fish in 15 g / L salinity. Some results were different from the current study as Grøtan et al.(2012) reported no change in the oxygen consumption rate and metabolic rate of *Acanthopagrus latus* when exposed to three environments (fresh water, brackish water and saline water) during rapid exposure to high or low salinities when transported from marine water to fresh and from salty water to

brackish water, this was attributed to the fact that these fish have genes that are responsible for conserving energy when the saline concentration changes over a short period of time as part of the adaptation phase to the saline concentration. Awal et al. (2012) reported that the decrease in the oxygen consumption of Tilapia was 2.14, 0.71, 1.43, and 1.42 mg O_2 / kg / h when exposed to salinity 0, 10, 20 and 30 g / L, respectively.

Conclusion

The researcher attributed the decrease in oxygen consumption to the decrease in fish activity due to increased salinity and so their exposure to stress.

Acknowledgment

This study has been supported by University of Baghdad. I appreciate the invaluable technical support of the Ministry of Science and Technology in Iraq.

References

Ahmed, S. M. 2005. Bioenergetics of osmoregulation in *Liza abu* Juveniles during salinity acclimation. Bas. J.Vet. Res. 4(1): 9- 16.

Ahmed, S. M., Al-Dubiakel, A. Y., Mohamed, F. A. 2004. Changes in alkaline phosphatase activity in the intestine of *Liza abu* Juveniles during salinity acclimation. Iraq.J. Aquaculture 11:17-27.

Al-Faiz, A.F., Jabir A., Yesser, A. 2009. Variance salinity concentrations effects on survival, growth and feeding of common carp (*Cyprinus carpio*) fingerlings. Iraqi J. Aquaculture 6(2):59–70.

Al-Khshali, M. S. 2011. Effect of different salt concentrations on some physiological and nutritional aspects of grass carp *Ctenopharyngodon idella* and gold fish *Carassius auratus*, University of Baghdad, Ph.D Thesis p. 120.

Awal, M. A., Kuri, K. C., Sarker, S. 2012. Effect of Salinity on the Oxygen Consumption of Tilapia Fingerlings. Daffodil International University Journal of Science and Technology 7(1):12-14.

Azizi, S., Kochanian, P., Peyghan, R. 2010. Chloride cell morphometrics of Common carp, *Cyprinus carpio*, in response to different salinities. Comp. Clin. Pathol. 4: 363-367.

Bardach, J. E., Ryther, J. H., Mclarney, W. O. 1972. Aquaculture: The Farming and husbandry of fresh water and marine organisms. New York NY, Willey.

Carmona, R., García-Gallego, M., Sanz, A., Domezaín, A., Ostos-Garrido, V. 2004. Chloride cells and pavement cells in gill epithelia of *Acipenser naccarii*: ultrastructural modifications in seawater-acclimated specimens. J. Fish Biol. 64(2): 553–566.

Dube, P. N., Hosetti, B. B. 2010. Behavior, surveillance and oxygen consumption in the fresh water fish *Labeo rohita* (Hamilton) exposed to sodium cyanide. Biotech. Anim. Husbandry 26(2-1): 91- 103.

Eddy, F.B. 1982. Osmotic and ionic regulation in captive fish. Comp.Bio.Physiology 73(1):125-141.

Epstein, F. H., Silva, P., Kormani, G. 1980. Role of Na-K-ATPase in chloride cell function. Am. J. Physiology 238 (3):R246-250.

Evans, D. H., Piermarini, P. M., Choe, K. P. 2005. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol Rev. 85(1): 97-177.

Fazio, A., Marafioti, S., Arfuso, F., Piccione, G., Faggio, C. 2013. Influence of different salinity on haematological and biochemical parameters of the widely cultured mullet, *Mugil cephalus*. Mar. Freshwater Behav. Physiol. 46(4): 211–218.

Fielder, D. S., Allan, G. L., Pepperall, D., Pankhurst, P. M. 2007. The effects of changes in salinity on osmoregulation and chloride cell morphology of juvenile Australian snapper, *Pagrus auratus*. Aquaculture 272: 656–666.

Fischer, P. 2000. An experimental test of metabolic and behavioural responses of benthic fish species to different types of substrate. Canadian J. Fish. Aqua. Sci. 57: 2336–2344.

Ghahremanzadeh, Z., Namin, J. I., Bani, A., Hallajian, A. 2014. Cytological comparison of gill chloride cells and blood serum ion concentrations in kutum (*Rutilus frisii kutum*) spawners from brackish (Caspian Sea) and fresh water (Khoshkrood River) environments. Polish Fisheries 22(3): 189–196.

Gracia-Lopez, V., Rosas-Vazquez, C., Brito-Perez, R. 2006. Effects of salinity on physiological conditions in juvenile common snook *Centropomus undecimalis*. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 145(3): 340-345.

Grøtan, K., Østbye, K., Taugbøl, A., Vøllestad, L. A. 2012. No short-term effect of salinity on oxygen consumption in threespine stickleback (*Gasterosteus aculeatus*) from fresh, brackish, and salt water. Can. J. Zool. 90(12): 1386-1393.

Gulácsi, A., Lee, G. R., Sík, A., Viitanen, T., Kaila, K., Tepper, J. M., Freund, T. F. 2003. Cell type-specific differences in chloride-regulatory mechanisms and GABAA receptor-mediated inhibition in rat substantia Nigra. The Journal of Neuroscience 23: 8237-8246.

Hiroi, J., McCormick, S. D., Ohtani-Kaneko, R., Kaneko, T. 2005. Functional classification of mitochondrion-rich cells in euryhaline Mozambique tilapia (*Oreochromis mossambicus*) embryos, by means of triple immunofluorescence staining for Na +/K+-ATPase, Na+/K+/2Cl− cotransporter and CFTR anion channel. J.Exp. Biol.208:2023–2036.

Jackson, A. G. 1981. Salinity tolerance and osmotic behavior of European carp (*Cyprinus carpio L.*) from the river Murray. Australia Trans. R.Soc.Aus. 103 (7): 185- 189.

Kaneko, T., Hiroi, J. 2008. Osmo and ion regulation. In R. N. Finn, & B. G. Kapoor, Fish Larval Physiology. Enfield: Science Publishers.

Keddy, P. A. 2001. Competition, 2nd Edition. London, Kluwer Academic Publishers.

Lee, T. H., Hwang, P. P., Shieh, Y. E., Lin, C. H. 2000. The relationship between `deep-hole' mitochondria-rich cells and salinity adaptation in the euryhaline teleost, *Oreochromis mossambicus*. Fish Physiology and Biochemistry 23:133-140.

Mancera,, J. M., McCormick, S. D. 2000. Rapid activation of gill Na+,K+-ATPase in the euryhaline teleost *Fundulus heteroclitus*. J. Exp. Zool. 287: 263-274.

Mangum, C., Towle, D. 1977. Physiological adaptation to unstable environments. Am. Sci. 65: 67-75.

McCormick, S. D., Sakamoto, T., Hasegawa, S., Hirano, T. 1991. Osmoregulatory actions of insulin-like growth factor-I in rainbow trout (*Oncorhynchus mykiss*). Journal of Endocrinology 130: 87-92.

McKenzie, D. J., Cataldi, E., Romano, P., Taylor, E. W., Cataudella, S., Bronzi, P. 2001. Effects of acclimation to brackish water on tolerance of salinity challenge by young-of-the-year adriatic sturgeon (*Acipenser naccarii*). Canadian Journal of Fisheries and Aquatic Sciences 58: 1113–1121.

Mommsen, T.P. 1998. Growth and metabolism. In D. Evans, The Physiology of Fishes, Second Edition. Boca Raton: CRC Press.

Morgan, J. D., Iwama, G. K. 1991. Effects of salinity on growth, metabolism, and ion regulation in juvenile rainbow trout (*Oncorhynchus mykiss*) and fall Chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquacult. Sci. 48 (11): 2083-2094.

Morgan, J. D., Gordon Grau, T. E., Iwama, G. K. 1997. Physiological and respiratory responses of the Mozambique Tilapia (*Oreochromis mossambicus*) to salinity acclimation. Comp. Biochem. Physiol 117(3): 391-398.

Mylonas, C. C., Pavlidis, M., Papandroulakis, N., Zaiss, M. M., Tsafarakis, D., Papadakis, I. E., Varsamos, S. 2009. Growth performance and osmoregulation in the shi drum (*Umbrina cirrosa*) adapted to different environment salinities. Aquaculture 287: 203-210.

Nordlie, F. G., Leffer, G. W. 1975. Ionic regulation and the energetic of osmoregulation in *Mugil cephalus* Lin. Comparative Biochemistry and Physiology Part A: Physiology 51(1): 125-131.

Peck, M. A., Buckley, L. J., Bengtson, D. A. 2005. Effects of temperature, body size, and feeding on rates of

metabolism in young- of the year haddock. J. Fish Biol. 66:911-923.

Pereira, B. F., Caetano, F. H. 2009. Histochemical technique for the detection of chloride cells in fish. Micron 40(8): 783- 786.

Sangiao-Alvarellos, S., Miguez, J. M., Soengas, J. L. 2007. Melatonin treatment affects the osmoregulatory capacity of rainbow trout. Aquacult Res. 38: 325–330.

Sargent, J. R., Pirire, B. D., Thompson, A. J., George, S. G. 1978. Structure and function of chloride cells in the gills of Anguilla Anguilla. Physiology and Behavior of Marine Organisms 123- 132.

Shikano, T., Fujio, Y. 1999. Relationships of salinity tolerance to immunolocalization of Na +K+ ATPase in the gill epithelium during seawater and freshwater adaptation of the Guppy, *Poecilia reticulata*. Zool. Sci. 15(1): 35-41.

Tseng, Y. C., Hwang, P. P. 2008. Some insights into energy metabolism for osmoregulation in fish. Comp. Biochem. Physiol. C-Toxicol. Pharmacol. 148(4): 419–429.

Wang, J. Q., Lui, H., Po, H., Fan, L. 1997. Influence of salinity on food consumption, growth and energy conversion efficiency of common carp (*Cyprinus carpio*) fingerlings. Aquaculture 148: 115–124.