

Effects of Salinity on Growth, Hormonal and Enzymatic Status in Fish: A Review

Maysa Enayatmehr

Phd student in Marine Biology, Department of Marine Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

Abstract: There has recently been an increasing interest in seafood products due to the growing awareness of their nutraceutical value. However, marine-based products are highly dependent on environmental conditions. Among other factors, many studies have reported an influence of water salinity on different aspect of fish physiology. In different studies, effect of different levels of marine salinity on growth factors, hormonal and enzymatic status, tissue histopathology and related gene expression have been investigated. Changes in growth rate, which depend on salinity, result from an action on: 1. standard metabolic rate; 2. food intake; 3. food conversion; and or 4. hormonal stimulation. Numerous studies reported the best range of salinity for better fish physiology performance but these domains in different species and different places are different. In this review, we considered all of these factors and discuss the effects of marine salinity on growth factors in different species.

Keywords: Salinity, Fish, Growth, Enzymatic Status

1. Introduction

Due to especially physiological function in each species and also environmental factors, development and growth in fish is different. Fish physiology is depending on internal and external factors such as nervous, endocrinological, neuro endocrinological and also ecological factors respectively (Katuli et al., 2014). Both of these factors control or synchronize many activities or functions, including growth capacity. In previous study ecological factors divided to two groups: 1- determining factors such as temperature, salinity and photoperiod which act directly through receptors to increase or decrease growth; and 2. limiting factors, which operate above ammonia or below such as oxygen and a specific threshold or within a tolerance range such as pH (Sharif et al., 2015).

Growth as a physiological function in fish is different from terrestrial animal. Growth pattern in terrestrial is discontinuous, this means that they just growth up to the limit age, but in fish is continuous, so that fish become larger the longer they live and they are much more dependent on external environmental conditions (Brett, 1979; Boeuf et al., 1999). Different external factors effect on growth in marine fish that temperature and environment salinity are most important of them (Brett, 1979). Many authors have demonstrated the influence of external salinity on growth capacities in fish (see Table 1). It is important to other species that have migration between different locations with different salinity considered because the fish that mentioned in Table 1 not influenced by salinity changes during their development and growth are rare. In other study it determined that many of fish prefer intermediary salinities especially in juvenile stage (Boeuf et al., 2001). So the question that can be asked is that, "how salinity influences growth?"

Table 1: Salinity and growth

Species	Tolerance	Best growth	Reference
<i>Salmo salar</i> (Atlantic salmon)	FW		Buckel et al. (1995)
	sW	17-19	Aristizabal Abud
<i>Salmo salar</i>	6 h SW	15	Woo and Kelly. (1992)
	10 d SW	8	Likongwe et al.
<i>Oncorhynchus mykiss</i> (rainbow trout)			Jonassen et al. (1997)
<i>Scophthalmus maximus</i> (turbot)		17.5	Chervinsky and Yashouv. (1971)
<i>Limanda limanda</i> (dab)		5-10	Suresh and Lin (1992)
<i>Pleuronectes platessa</i> (plaice)		10-15	Suresh and Lin (1992)
<i>Anguilla anguilla</i> (eel)		0	Suresh and Lin (1992)
<i>Merlangius merlangus</i> (whiting)		30-35	Suresh and Lin (1992)
<i>Morone chrysops</i> (White bass)		0-12	Heyward(1995)
<i>Chanos chanos</i> (Milkfish)		55	Swanson(1998)
<i>Chanos chanos</i> (Milkfish)		0	Alava(1998)

The influences of salinity on growth of different species of fish are indicated as follows: (positive +, or neutral =) tolerance (maximum salinity or range tested), the best salinity conditions for growth, and the reference (Brett, 1979).

2. Acclimation to Salinity and its influence on Growth

Salinity is due to the presence of salts, dissolved in water, which represent 60 of the 92 'basic' chemical elements (Riley, 1965). Chloride Cl⁻ (560 mM) and sodium Na⁺ (450 mM) are the most important in normal salinity sea water (SW, 35 ppt, 1050 mOsm l⁻¹). The most important aspect is that these elements are ionised in SW (19 g of Cl⁻, 10.5 g of

Na⁺ 1⁻¹). As a comparison, despite the fact that it is extremely difficult to specify what an 'average' composition of FW really is, Tchernia in 1969 tried to consider some data and proposed 'general values' for FW (0.32ppt), 35.1% of CO₃²⁻, 20.4% of HCO₃⁻, 12.1% of SO₄²⁻, 11.7% of SiO₂ and 5.8% of Na⁺. If chloride and sodium are predominant in SW, carbonates and HCO₃⁻ are most abundant in FW. The composition of FW can vary a lot, but it is important to remember that FW is not deionized water! Many physiological experiments have maintained fish in deionised water and the fish obviously rapidly face physiological disturbance, often die and, if they survive, are unable to grow. Some species are known for their ability to acclimate to very different salinity media (Griffith, 1974; Suresh and Lin, 1992), including extreme environments (> 100 ppt). Killifish (Cyprinodontiformes) are particularly interesting because of their capacities to acclimate to such environments and represent nice models in biology. An extreme situation is the persistence of the *Austrofundulus limnaeus* population, because diapausing embryos become embedded in pond sediments (Podrabsky and Hand, 1999).

Osmosensitivity and salinity detection are of the highest physiological interest (Katuli et al., 2014; Sharif et al., 2015). Fish have prolactin (PRL) cells which are directly osmosensitive (Grau et al., 1994). They also possess chemoreceptors, situated in the pseudobranch (Laurent and Dunel-Erb, 1984), providing information on water salinity. These are connected to the central nervous system (CNS) and participate in triggering water drinking in SW. Teleost fish in SW drink, at least in part, to compensate for the water lost by diffusion (to balance osmotic water losses occurring due to the osmotic difference between their milieu intérieur and the external medium), essentially through the gill. In summary, it appears that marine fish present higher developmental or growth rates at lower salinity and FW fish at higher salinity. Many species, often as juveniles, opt for intermediary salinity conditions of the brackish water (8-16 ppt) and grow optimally in estuaries and coastal systems (Sharifi et al., 2015). It was indicated that the salinity tolerance during early developmental stages depends on how internal fluids perform in various extents (Holliday, 1969; Alderdice, 1988). Salinity effects have been studied extensively in marine fish embryos and larvae (Yang and Chen, 2006; Jørgensen and Hansen, 2010), but they are limited in freshwater species (Fashina-Bombata and Busari, 2003; Albert et al., 2004; Bonisławska, 2009). It has shown that some freshwater teleost eggs can be incubated and hatched in 5ppt salinity but fertility and eggs hatching rate of freshwater teleost decreases in saline water (Gbulubo and Erundu, 1998). The other report showed lower than 5ppt salinity tolerance in Zebrafish (*Brachydanio rerio*) (Sawant et al., 2001). Rockwell (1956) reported 70-90 % mortality in Pacific salmon, *Oncorhynchus gorbuscha* and *O. keta* 19 to 40 days after incubation period in 12ppt salinity but, salinity less than 6ppt has not very lethal effect in these species. Rubin (1994) Also showed, (For the salinity range found in the Baltic Sea), a small negative influence on egg-to-

fry survival of sea trout eggs in 6ppt salinity (11% more compare to freshwater) but it caused a delay in hatching time and duration of the hatching period was increased. On the other hand some studies reported the salinity effects on hatching rate of freshwater teleost. For example Spined loach (*Cobitis taenia*) embryos developed successfully in the range of 0.12 to 4.80ppt salinity (Bohlen, 1999). But at 6.00ppt the hatching was strongly reduced, and development failed at or above 7.20 ppt. Froelich and Engelhardt (1996) reported that the low salinity incubation (as 2ppt) has control the fungus growth in koi carp (*C. caprio*) and Channel catfish (*Ictalurus punctatus*), also increases the hatching rate compare to control was reported but no effect on hatching time was reported in these species. Mortality occurs because of the embryo incapability to maintain osmotic pressure in normal rate in order to unbalanced ion gradient (Bunn et al., 2000). Holliday and Jones (1967) found that the egg salinity resistance was lowest in Blastula and gastrula in freshwater teleost eggs and Tylore (1971) found the most sensitivity just enclosing blastopore prior to hatch. However, there is limited information on how salinity affects developmental stages of sturgeons (Jian-Yi et al., 2006). Zotin (1965) defined five phases of water uptake by embryos of Beluga and sevruga during developmental stages. First between fertilization and gastrulation which differs as rapid uptake, the second stage continued up to yolk plug closure which ceases water uptake, third followed up to heart angle formation that water uptake was rapid. Forth related to heart pulsation beginnings and he reported some water lost in this stage and in fifth period to hatch that no water is absorbed. Khatooni et al (2013) was conduct a study investigate how salinity affects early developmental stages of the fertilized eggs and embryo of Persian sturgeon (*A. persicus*) during different developmental stages (Khatooni et al., 2013).

As result of Khatooni et al (2013) finding, the abnormalities were distinguished and classified in each of three developmental stages and the result showed in Fig. 1 to Fig.3. Result has shown that the effect of salinity on abnormality percent was different in each developmental stage (Table2). For example the abnormality percent increased significantly in gastrula stage only in 6 and 8ppt treatments. The abnormality rate in s- type heart formation stage was significantly higher in all treatments compare to control but it was still below 20%. The abnormality rate increased significantly in 4 and 6ppt treatments before hatch stage. Also our results showed that the salinity significantly affect abnormality percent during developmental stages of each treatment (P< 0.05). For example in 2ppt treatment the abnormality was increased significantly in s-type heart stage compare to gastrula stage but in 4ppt treatment the abnormality increased significantly in all three stages (P< 0.05). The significant decreased abnormality rate in 8ppt resulted from the high mortality of embryos in 8ppt treatment (Table2).

Table 2: The effects of different salinity treatments on abnormality percentage during three developmental stages of Persian sturgeon embryo Khatooni et al (2013).

Treatments	0.5	2	4	6	8
Gastrula	2.56±0.6 a	4.33±1.0 a*	5.76±0.6 a	18.26±5.6 b	28.23±8.9 c
S- type heart formation	5.56±2.4 a	11.8±3.0 b	15.73±2.0 bc*	19.63±3.6 c	15.16±7.1 bc

Before hatch	8.16±3.8 a	14.66±4.5 ab	21.73±3.0 b**	30.33±5.4 c*	7.16±2.5 *
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Three stages of Persian sturgeon embryonic developmental stages include in: Gastrula, S-type heart formation and before hatching (in developmental stage 35) in each treatment.* means significant difference between values of three stages in a treatment. Different superscript letters indicate significant difference between treatments in each stage ($P > 0.05$).

Hatching took place four days after fertilization (96h) and continued for three days more (7 d post-incubation) in all trials except 2ppt treatment which completed mostly in the day 6th. The hatchability of eggs incubated in 2ppt as well as control group ranged between $88.22 \pm 3.81\%$ and $88.51 \pm 2.60\%$, respectively ($P < 0.05$). The hatching rate was recorded $84.19 \pm 3.2\%$ in 4ppt treatment, while it was recorded 64.03 ± 1.8 in 6ppt treatments. Hatching did not occur in 8, 10 and 12ppt (Fig. 4). No Fungus was observed in salinities more than 2ppt.

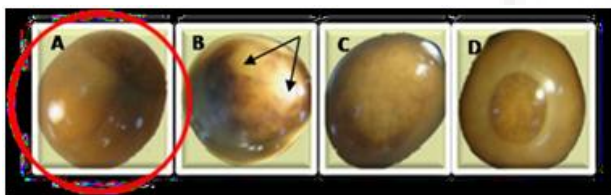


Figure 1: Different abnormalities samples observed during incubation period of gastrula stage in salinities treatment. A: normal embryo in gastrula stage. B: abnormalities in segmentation (arrows pointed the areas which the cells dose not made normal divisions or the cells has been damaged, this kind of abnormalities was mostly observed in salinities more than 4ppt salinity). C: the abnormal embryos with shortened yolk sac compare to normal stage(it was not numerous in salinities less than 6ppt). D: the developing retarded embryos compare to normal gastrula stage (this kind of abnormality was numerous in 8ppt treatment) Khatooni et al (2013).

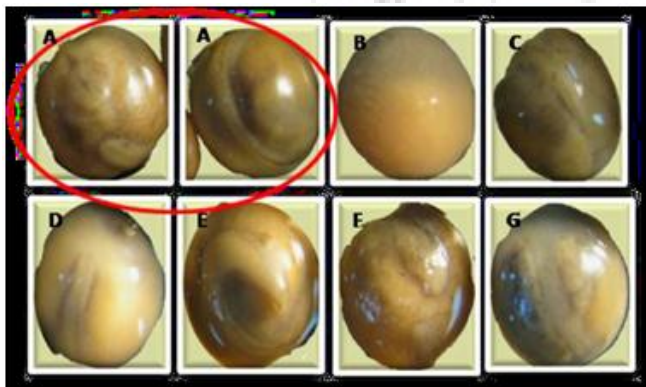


Figure 2: Different abnormalities samples observed during incubation period of s-type heart formation stage (35 embryonic developmental stages according to Dettlaff and Ginsburg, 1992). A: the normal embryo in s-type heart formation stage. B: retarded embryo. C: deficiency in notochord formation. D: deformity in head and tail formation, E: lack of head and fore part of body and deformed tail. E: deformity in head (lengthened than normal). G: deformed embryo with deficiency in back bone Khatooni et al (2013).

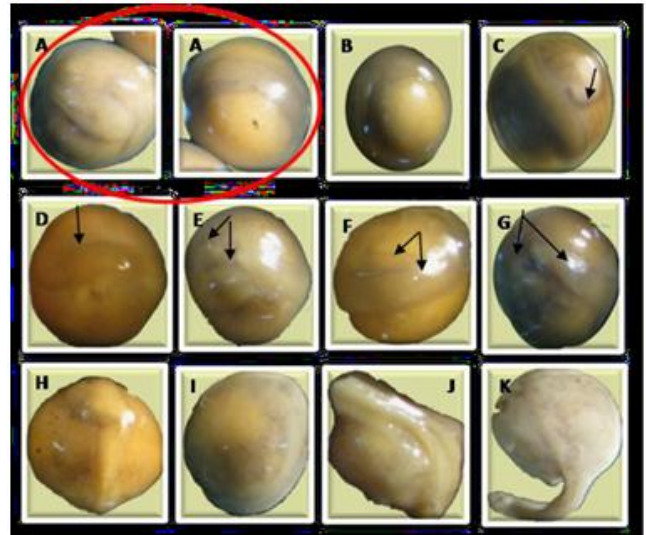


Figure 3: Different abnormalities samples observed during incubation period of before hatching stage. A: normal embryo in before hatch stage, B: Yolk sac Vol. decreasing more than normal mood in this stage, C: the arrow pointed to presence of abnormal assemblage of cells on the yolk sac (the larvae which hatched with this kind of abnormality does not have any problem in their activity but the assemblage become thinner than compare to control in salinities more than 4ppt), D: the abnormal embryo without head and the for head parts. E: the abnormal embryos which have two heads. F: the abnormal embryo with 2 spinal cords. G: the embryo with sever deformities on both head and tail. H: deformed retarded embryos which both head and tail parts are not form clearly. I: the retarded embryos which is not deformed yet in this stage. J and K: this samples are included the sever deformity in retarded embryos which was mostly seen in 8ppt salinity that no hatch was obtained Khatooni et al (2013).

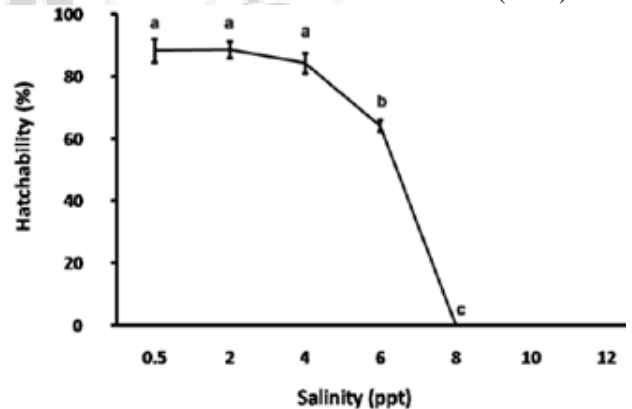


Figure 4: The egg hatchability percent incubated in different salinities. Different superscript letters indicate significant difference between treatments in each stage ($P > 0.05$) Khatooni et al (2013).

Zotin (1965) reported that the water absorption will cease in gastrula stage and it will start to absorb rapidly in heart formation stage again in Beluga (*Huso huso*). So the increasing of abnormality and mortality rate in all brackish water treatments comparing to control in heart formation stage confirm the presence of the same mechanism in Persian sturgeon. However, Holliday and Jones (1967) found that the egg salinity resistance was lowest in blastula and gastrula in freshwater teleost eggs and Tylore (1971) found the most

sensitivity just enclosing blastopore prior to hatch. Sawant, et al. (2001) also reported increasing salinity potential along with developmental stage in zebra fish in low salinity as 2ppt (from embryonic cleavage up to gastrula stage) and cytological examinations indicated that higher salinity mainly impaired the nuclear division of the embryonic cells. In Persian sturgeon it was shown that its embryos demonstrates a little adaptability during incubation period in higher salinity than freshwater (Sharif et al., 2015). It seems that there wouldn't be another ion regulatory mechanism (chloride cell) like some of tolerable teleost in early developmental stages (Kaneko et al., 2002). Decrease of hatching rate in salinities more than 6ppt and significant increase in mortality percent of *Acipenser persicus* embryo only in this treatment shows that the eggs could not tolerate salinities more than 6ppt during incubation stages. The same salinity potential was reported in some freshwater teleost eggs. For example Gbulubo and Erondu (1998) found the optimal salinity ranges for incubation 0.5 ppt and they found that hatching was significantly low above 5‰, no hatch was observed in 8ppt salinity in African catfish (*Heterobranchus longifilis*).

In summary, according to study that conducted by Mardaneh Khatooni et al., (2013), the mortality and abnormality of non-teleost species such as Persian Sturgeon embryos increased a long with increase in salinity but the embryos showed more tolerance during blastula and gastrula stages in lower salinity and larvae could nothatch in salinities more than 6 ppt. Results suggest that as the salinity increases more than 6ppt the embryonic development will be retarded or make it useless in Persian sturgeon. All in all, the salinity tolerable range during incubation period in Persian sturgeon is between 0.5- 6 ppt.

3. Energetic Cost

In previous study measurements of oxygen consumption in fish further showed a reduction in metabolic rate (2&28%) in isotonic salinity relative to fresh water and seawater (Rao 1968; Sharif et al., 2015). These studies support the hypothesis that the energetic cost of ion regulation is lowest in an isotonic environment, where the ionic gradients between blood and water are minimal, and that this energy savings is substantial enough to increase growth. Other studies, however, fail to show isotonic salinity as the point of maximal growth in salmonids; growth rates were highest in fresh water in studies by Shaw et al. (1975), Clarke et al. (1981), McKay and Gjerde (1985). A theoretical estimate of the energetic cost of osmoregulation in salmonids suggests that it would be very low, being less than 1 % of resting metabolism (Eddy 1982). Accordingly, changes in metabolic rate of that magnitude would be very difficult to measure accurately. Studies such as that of Bullivant (1961), who reported no significant differences between the metabolic rates of yearling chinook salmon (*Oncorhynchus tshawytscha*) reared in fresh water, half strength sea water, and full-strength seawater, support those theoretical estimates.

Few studies have made simultaneous measurements on growth and metabolic rates of salmonids in relation to salinity. Here, we focus on a study that Morgan et al. (1991) conducted. John et al. (1991) examined growth, metabolic rates, and ionic regulation in juvenile rainbow and steelhead

trout and fall chinook salmon acclimated to a range of salinities, with one near isotonic. These species were chosen to investigate the possible effects of different life history patterns on growth and metabolic responses to environmental salinity. The steelhead trout is an anadromous form of the rainbow trout, usually spending 2 or in fresh water before migrating to the sea (Scott and Crossman 1973). Fall chinook salmon are also anadromous, but spend a shorter period of time in fresh water than steelhead trout, migrating seaward within 3 months of emergence (Lister and Walker 1966). Their results showed that the mortality was less than 5% in all treatments except for chinook salmon fry reared in 28 ppt, which suffered a loss of 24 fish (Table 3). The majorities of the mortalities were less than 5 cm in fork length and weighed less than 0.5 g. The mortality data indicate that the small fry stages (approximately 8.5-10 g) of these species can adapt to salinities of up to 28 ppt when a gradual acclimation procedure is followed.

Growth rates declined with increasing salinity for all three species (Fig. 5; Table 3). Rainbow trout fry growth decreased. Growth of Chinook salmon fry was significantly higher in fresh water than all other treatments and showed a steady decline with increasing salinity to full strength seawater (fig. 6). Specific growth rate values for Chinook salmon fry in fresh water and seawater were similar to those reported by Clarke et al. (1983). Final condition factors varied among growth experiments, showing no consistent pattern between species (Table 3). Rainbow trout fry in fresh water and isotonic salinity had significantly higher final condition factors than the hypertonic treatment, but the condition factor for steelhead trout fry in fresh water was significantly lower than the other treatments (Sharif et al., 2015). Differential growth rates between salinity treatments, however, resulted in size differences which masked the anticipated dehydration effect of salinity on moisture content (Table 3).

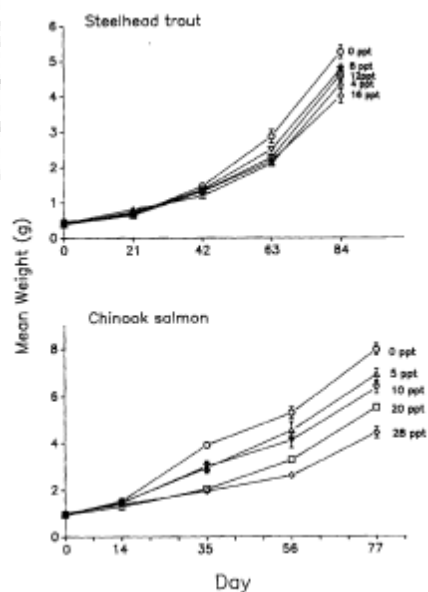


Figure 5: Growth curves for steelhead trout and chinook salmon fry reared in five different salinities. Absence of standard error bars indicates that the SE was smaller than the symbol (Morgan et al. 1991).

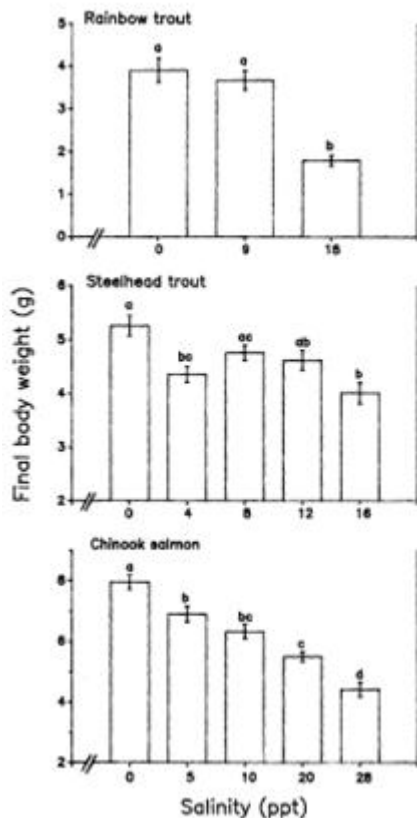


Figure 6: Effect of salinity on growth of juvenile rainbow and steelhead trout and chinook salmon (starting weights = 1.8, 0.4, and 1.0 g, respectively). Means (4 1 SE) with a common letter are not significantly different by Tukey's test (Morgan et al. 1991)..

Table 3: Mortality specific growth rates, condition factors and moisture content of juvenile rainbow and steelhead trout and chinook salmon reared in various salinities. Mean values followed by the same letter are not significantly different ($P < 0.05$) by Tukey's test (Morgan et al. 1991).

Salinity treatment (ppt)	Percent mortality	Specific growth rate (% wt. d-1)	Final mean condition factor (\pm SE)	Final mean percent moisture content (\pm SE)
Rainbow trout				
0	2	3.35	1.13 \pm 0.01 a	
9	0	3.25	1.17 \pm 0.01 a	
18	4	1.57	1.07 \pm 0.01 b	
Steelhead trout				
0	3	3.27	1.12 \pm 0.01 a	
4	0	2.64	1.15 \pm 0.01 b	
8	0	2.98	1.19 \pm 0.01 c	
12	0	3.11	1.17 \pm 0.01 bc	
16	0	2.75	1.17 \pm 0.01 bc	
Chinook salmon				
0	0	2.62	1.15 \pm 0.01 a	78.0 \pm 0.02 a
5	0	2.44	1.06 \pm 0.01 a	78.1 \pm 0.02 ab
10	0	2.32	1.06 \pm 0.01 a	78.6 \pm 0.02 ab
20	0	2.30	1.05 \pm 0.01 a	79.1 \pm 0.02 b
28	24	1.84	1.05 \pm 0.01 b	81.6 \pm 0.02 c

As results, salinity had a negative effect on the growth of all three species, particularly at salinities above the isotonic level. These findings are in agreement with Shaw et al. (1975), Clarke et al. (1983) and McKay and Gjerde (1985),

who also found that isotonic salinity was not the point of maximal growth in juvenile salmonids. It is possible; therefore, that confinement stress affected the study results. In the experiments conducted by Otto (1971), growth rates of coho salmon fry were actually higher in fresh water from June to September and increased in 18 ppt salinity only from October to February. This shift in salinity for optimal growth during the fall is consistent with other studies on the behaviour and physiology of coho salmon psmolts. As mentioned previously, activity of gill Na^+ , K^+ -ATPase, the enzyme located in chloride cells which is indicative of ion excretion capacity, increased during mid-October in coho salmon under yearlings in fresh water. This resulted in improved growth and survival of the fish when they were transferred to seawater netpens during autumn compared with other times of the year (Haache et al. 1980). These results are all consistent with the observation that coho salmon are sometimes found in estuaries as under yearlings Tschaplowski (1987) and have therefore evolved physiological mechanisms to optimize growth in changing environments. Based on these data, we can say, even at the fry stage, anadromous steelhead trout and chinook salmon adapt and grow better at hypertonic salinities than the freshwater resident rainbow trout.

John et al. (1976) found that oxygen consumption rates for all three species of salmonids were not lowest in isotonic salinity; they were lowest in fresh water. The higher metabolic rates at higher water salinities apparently reflected a significant energetic cost, as growth rates declined with increasing salinity, and correlated very well with the changes in oxygen consumption. Assuming that these increased energy demands were related solely to ionic and osmotic regulation (= ion-osmotic regulation), the metabolic rate data suggested that the energetic cost of ion-osmotic regulation for fry of these salmonid species was lowest in fresh water and increased to 12-18% in isotonic salinity.

Hematocrit values for rainbow trout fry increased with salinity and were significantly higher in 18 ppt than in fresh water. The measured values for both Na^+ and Cl^- in fresh water are in good agreement with normal values for all three species (Conte and Wagner 1965; Wagner et al. 1969). Na^+ and Cl^- in rainbow and steelhead trout rose slightly when reared in salinities above the isotonic level, while plasma ion concentrations in chinook salmon fry did not change across all salinities (the ionic status of mortalities in 28 ppt could last be determined). Despite these slight increases (8-10%) in the trout, plasma ion concentrations for all three species were maintained within acceptable levels. To justify this phenomenon, some authors previously mentioned that the fish were regulating blood ions competently against concentration gradients (Table 4). It is likely, therefore, that the observed growth and metabolic rates reflect steady state in fish with respect to ionic status. The increase in Ca^{2+} content of the external media at higher salinities may have assisted in regulating the passive movements of Na^+ and Cl^- , as Ca^{2+} is known to alter the permeability of gill membranes to these ions (Eddy 1975). Similar with these findings, Katuli et al., 2014 found the number of chloride cell (or mitochondria rich) along with rise of salinity, increased in Caspian Roach fingerling (Kauli et al., 2014). These cells through their ATPase activity can create more ion exchange in gill tissue and

this followed by change in electrolytes values (Kauli et al., 2014). The variable responses of hematocrit to salinity observed in the present study have been reported previously (Bath and Eddy 1979) and may be explained by differential changes in red cell and plasma volumes which appear to be species specific.

Table 4: Chemical composition of water samples collected from the steelhead trout and chinook salmon fry rearing tanks (FW = fresh water; SW -- seawater) (Morgan et al. (1991)).

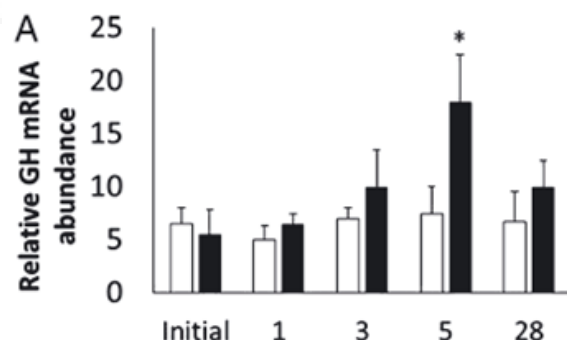
Salinity treatment (ppt)	Na ⁺ (meq.E ⁻¹)	Cl ⁻¹ (meq.L ⁻¹)	Ca ²⁺ (meq.L ⁻¹)	pH
<i>Stellhead trout</i>				
0 (FW)	3	<1	>1	6.0
4	71	63	1	7.1
8	146	120	1	7.3
12	189	170	1	7.4
16	213	240	1	7.5
28 (SW)	386	450	6	7.7
<i>Chinook scklmoat</i>				
0	3	<1	<1	6.0
5	81	71	1	7.1
10	163	156	3	7.3
20	273	288	5	7.5
28	931	456	7	7.6

In another study, external relation factors investigated. McCormick et al (1987). They concluded, inhibition of increases in gill Na⁺, K⁺-ATPase activity and salinity tolerance by exposure to continuous Bight did not preclude gradual adaptation of the L24 group to 3mo seawater. Although survival and capacity to maintain plasma osmolality after 5 mo in seawater was not significantly different from the other groups, the growth of the fish during this period was halved. This reduction in growth is due to salinity, since they found that poor growth does not result if L24 fish are maintained in freshwater. It seems likely that endogenous and environmental factors which affect changes in osmoregulatory physiology during the pan-smolt transformation will also affect growth in seawater (McCormick et al., 1987).

In summary, growth and metabolic rates of euryhaline fish species, such as rainbow trout, steelhead trout, and chinook salmon fry were optimum in fresh water, their natural habitat at this life stage. Isotonic salinity did not provide metabolic or growth advantages in the present study, despite the hypothesis that it would provide the lowest energetic demands for ion-osmoregulation. Comparison with other studies indicated that optimal salinities for growth and metabolic rates were influenced by species, life stage, and season. Although the oxygen consumption data suggested that the energetic cost of ion-osmotic regulation increased with salinity, attempts to quantify this cost were probably affected by other metabolic processes which respond to changes in salinity.

As one contributing factor in salinity adaptation, the role of growth hormone (GH) and insulin-like growth factor 1 (IGF-1) has been frequently emphasized in teleost fish such as *S. trutta* and rainbow trout, *Oncorhynchus mykiss* (Madsen and Bern 1992; Shimizu et al. 2007), and several non-salmonid species such as tilapia, *Oreochromis mossambicus* (Sakamoto

et al. 1997; Riley et al. 2003), naked carp, *Gymnocypris przewalskii* (Cao et al. 2009), and zebrafish, *Danio rerio* (Almeida et al. 2013). However, little information is available on the specific functional roles and regulation of each GH hormone (i.e., GH1 and GH2) during growth and salinity adaptation. GH and its modulator, IGF-1, are the main components of the somatotrophic axis, which is involved in a wide range of physiological mechanisms in mammals (Le Roith et al. 2001) and fish (Rousseau and Dufour 2007; Beckman 2011). Sharif et al., (2015) showed that the fish reared in brackish water exhibited a significantly higher SGR than did their counterparts in freshwater (Fig. 7) (Sharifi et al., 2015). Also they found qPCR analysis revealed that the messenger RNA (mRNA) abundance of GH from the fish reared in brackish water exhibited an increasing trend over time and was significantly higher ($P < 0.05$) than that of their counterparts in freshwater after 5 d (Fig. 8A) (Sharif et al., 2015). The relative transcript abundance of IGF-1 revealed a trend comparable with that of GH, with a significant increase observed at 5 and 28 d in the fish kept in brackish water (Fig. 8B) (Sharif et al., 2015). In anadromous fish, the activation of GH/IGF-1 during seawater acclimation has frequently been reported. GH and IGF-1 are mitotic factors that trigger cell division and cell growth (Cao et al. 2009). It has been shown that one of the active sites for the action of GH/IGF-1 is the gill. Although the effects of brackish water on GH and IGF-1 gene expression and/or somatic growth remain unknown, salmonid fish exhibit wide variation in the growth response to a salinity challenge depending on the stage of the life cycle (Shrimpton et al. 2005; Bystrjanski et al. 2006). For example, transferring young salmon before smoltification to sea water produces severe somatic growth retardation and abnormal development, most likely because of down regulation of hepatic GH receptor and low levels of plasma IGF-1, whereas post-smolt salmon respond positively to the salinity challenge and show a high growth rate and large body size (Duan 1997; Handel and Stefansson 2002). In their study, a significant increase in SGR was observed in brackish water (Sharifi et al., 2015). This phenotypic trait was supported by a significant increase in the GH and IGF-1 transcript levels. It is unclear whether the increase in IGF-1 mRNA levels is a result of increases in GH or represents a direct effect of salinity on IGF-1 gene expression (Sharif et al., 2015).



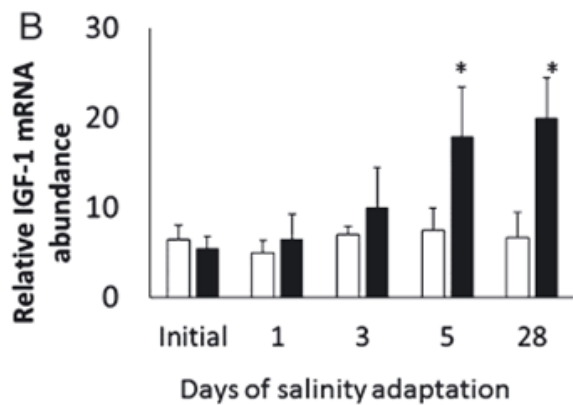


Figure 8: A-B. Transcript abundance of growth hormone (A) and insulin-like growth factor 1 (B) of Caspian trout, *Salmo trutta caspius*, after 1, 3, 5 and 28 d of adaptation in brackish water. Open bars represent freshwater trials, and closed bars represent brackish water trials. Data are shown as the mean \pm SEM. The asterisks indicate significant differences between freshwater and brackish water trials (Sharifi et al., 2015).

In conclusion, GH and IGF-1 genes are highly conserved in teleost fish. Brackish water adaptation of juvenile teleost fish such as Caspian trout in the early stage of smoltification is associated with the stimulation of somatic growth, and it is possible that this effect is mediated through the GH/IGF-1 axis (Sharifi et al., 2015). Therefore, it appears that the early stage of smoltification is the most suitable stage for introducing juvenile Caspian trout to Caspian Sea water. This result is in agreement with the findings of a recent study (Toorchi et al. 2012). Note also that the mineral composition and osmolarity range of brackish water relative to growth and smoltification stages need to be examined in future research to ensure the optimum conditions for the culture of other teleost species.

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