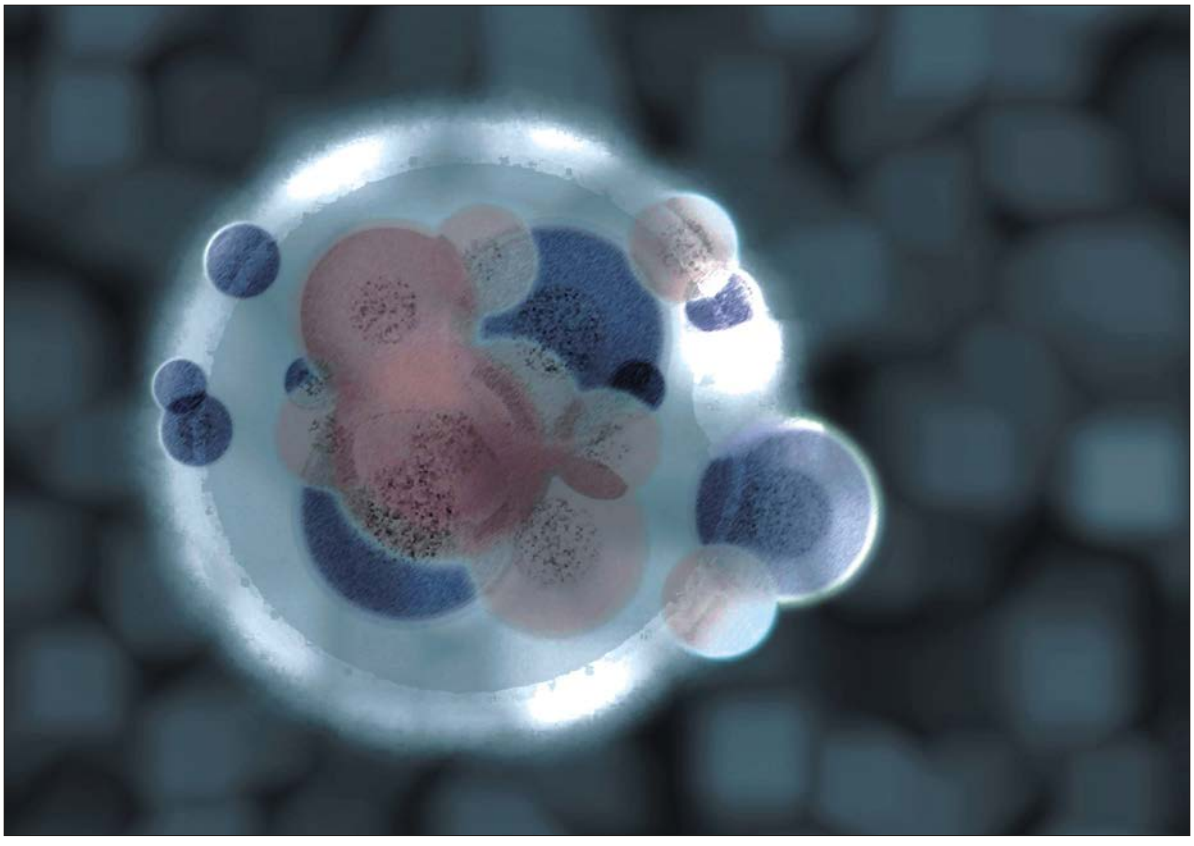

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# Adaptation to Sea Water and Growth Performance of Rainbow Trout, *Oncorhynchus mykiss*

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**Abstract:** The $Na^+$-$K^+$-ATPase enzyme activity in gill tissues, changes of plasma ions level and, survival and growth performances of rainbow trout, *Oncorhynchus mykiss*, were investigated after gradual and direct acclimatization to high saline water in the Aegean Sea. Five experiments were performed with rainbow trouts weighing 120, 140, 160, 200 and 225 g. In this experiment, $Na^+$, $Cl^-$, $K^+$, $Ca^+$ and $Mg^{+2}$ ion levels in blood plasma, $Na^+$-$K^+$-ATPase enzyme activities in gill tissues of rainbow trouts weighing 160 g in both freshwater and saltwater were measured. After adaptation to seawater, fish were held in marine cages of 64 $m^3$ volume to grow up at Island of Urla-Karantina. Rainbow trouts weighing 120, 140, 200 and 225 g were reared in these cages for 80 days. Parallel to that, fish weighing 225 g were stocked in freshwater for comparison. The best survival percentage among directly acclimatized rainbow trouts was seen in 200-225 g fish, whereas the survival percentage among gradually acclimatized trouts presented similar results in every group. It can be concluded from these studies that, rainbow trout in certain seasons (Autumn-Winter) can adapt to the Aegean Sea condition (3.6-3.7%) and show good growth performance.

**Key words:** *Oncorhynchus mykiss*, aegean sea, adaptation, osmoregulation, gill ATPase activity, growth rate

## INTRODUCTION

The rainbow trout farming in Turkey has been carried out since 1969[1]. Nowadays, there are 989 freshwater trout farms and 30 seawater trout farms with an annual production of approximately 39.674 and 1.194 tons, respectively[2]. In freshwater growth of rainbow trout to marketable size which is about 200-300 g takes about 10 to 18 months. Mariculture farms of rainbow trout are concentrated on the Black Sea and are geared towards rearing fish weighing a kilo or more in certain periods of the year. Although there are suitable conditions for rearing rainbow trout on the Aegean Sea, any culture facility about mariculture of rainbow trout has not been established yet.

As a euryhaline fish, rainbow trout can be adapted to and reared in seawater[3]. Some species of trout have the smoltification process[4,5] so they can develop appropriate physiological mechanisms to adapt to and live in seawater. Rainbow trout has no smoltification process, but they can be acclimatized to seawater (3.6%) directly under convenient conditions. Increasing $Na^+$-$K^+$-ATPase enzyme activities are used as an indicator to show adaptation capability of fish in hypertonic media. After adaptation to seawater, there is a significant correlation between alterations of physiological functions and survival and growth[5,6]. Rainbow trouts of definite sizes (>120 g) develop physiologic accommodation to seawater (3.5-3.6%) directly at appropriate temperature (<14°C), so they can tolerate high saline water[7]. However, gradually acclimatization to high saline water is more favorable than direct adaptation and also it is convenient for physiological accommodation of rainbow trout[8]. The aim of this study was to investigate adaptation to seawater and growth performances of rainbow trout groups which were different from each other in weight, on the Aegean Sea.

## MATERIALS AND METHODS

The experiment was carried out at EÜ, Fisheries Faculty, Aquaculture Department, Karantina Island, Urla, İzmir, Turkey, using rainbow trout in 1995. At the begining of the study, rainbow trout weighing 120±2.4, 140±2.9, 160±2.5, 200±2.6 and 225±3.1 g were used. Each group of the study had 100 fish. To monitor growth, 30 fish were sampled from each group and anaesthetized with quinaldine, weighed and measured. A month before the adaptation, fish were vaccinated with Vibrio by injection method. During this period fish were fed with a diet

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Table 1: Increases of salinity in gradual transfer to sea water

| Days | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Salinity (%) | 0 | 1.8 | 2.0 | 2.3 | 2.6 | 2.7 | 2.8 | 2.8 | 2.9 | 3.2 | 3.2 | 3.3 | 3.6 | 3.7 |

Table 2: Mean temperatures of fresh water and sea water

| | November | Desember | January | Febmary | March | April | May |
|---|---|---|---|---|---|---|---|
| Fresh water | 10.6 | 10.5 | 11.5 | 12.0 | 12.0 | 14.0 | 15.0 |
| Sea water | 14.3 | 13.5 | 13.8 | 14.2 | 14.0 | 15.5 | 22.3 |

containing 5% sodium chloride. Fish were held in rectangular tanks for a week before adaptation. Methods of direct and gradual transfer (Table 1) to seawater (3.6-3.7%) were carried out with each group. Every experiment was repeated only once. Moreover, in this experiment, $Na^+$, $Cl^-$, $K^+$, $Ca^{+2}$ and $Mg^{+2}$ ion levels of blood plasma, $Na^+$-$K^+$-ATPase enzyme activities in gill tissues of rainbow trouts weighing 160 g were measured in both freshwater and saltwater.

Cages were 4x4 m in size and moored in Urla-Karantina Island. After adaptation they were used to rear experimental fish that weighing 120, 140, 200 and 225 g (this group was added later to cage) and rearing period lasted for 60-80 days. Parallel to these experiments, fish weighing 225 g were stocked also in freshwater. The size of pellet was 4 mm (commercial feed). During the experiments, water temperature ranged from 13 to 14°C (December-January) to 20-22°C (May) (Table 2).

**Blood sampling and serum chemistry:** Blood sample was collected (1.5 mL) separately from hearts of 3 fish by heparinized injection. Plasma samples were separated by centrifugation and stored at –30°C until analysis. Serum $Na^+$ and $K^+$ contents were measured using Radiometer (FLM3, Denmark); Serum $Cl^-$ and $Ca^{+2}$ contents were determined by Autoanalyzer (with Biomerieux kits, tool: Coulter, Dacos, Amerika); serum $Mg^{+2}$ content was assessed via manuel spectrophotometer (with Biocan kit, tool: Shimadzu, CL, 750, Japan).

**Gill $Na^+$, $K^+$-ATPase enzyme activity:** After cutting fish head behind operculum, 20 mL heparamized sucrose (0.3 M) solution was injected from bulbus arteriosus to eliminate eryhtrocytes. Then gills were removed from fish to dry them with filter paper and the gills were dissected and placed into 5ml non-heparanized sucrose (0.3 M) solution. They were stored at –30°C until analysis. Gill $Na^+$-$K^+$-ATPase enzyme activity was measured by estimating PNpp ase activity, using Para-Nitro-Fenil-Fosfat (PNpp) substrate[9].

**Statistical analysis:** All data were presented as means±SE Specific Growth Rate (SGR) and Food Conversion Ratio (FCR) were calculated. SGR, expressed as percentage body weight per day, was calculated from SGR= 100 x $(\ln W_f - W_i)/t$, where, $W_f$ = mean weight at the end of the period, $W_i$ = mean weight at the beginning of the period and t=time in days. Statistical analysis was done by paired, two tailed student t-test using Microsoft exel 7.0 computer program. The values of $p< 0.05$ were accepted as statistically significant.

## RESULTS

$Na^+$, $K^+$-ATPase enzyme activities of rainbow trout in freshwater were determined to be quite low. No significant alteration in gill ATPase enzyme activity was observed in groups that were transfered gradually until the salinity reached 2.9%. After salinity reached to 3.7%, the ATPase enzyme activity increased to the maximum level in 16-17 days. For several days, levels of ATPase enzyme activity remained at 0.25 mM PN pp $mg^{-1}$ protein. However, significantly increased ATPase enzyme activity on fourth day was determined at 0.21 mM PN pp $mg^{-1}$ protein level in groups which were transfered directly. Afterwards, the level increased gradually to 0.25 mM PN pp $mg^{-1}$ protein in 21 days. Gill ATPase enzyme activity levels in fish about to die were determined to be between 0.12-0.22 mM PN pp $mg^{-1}$ protein (Fig. 1 and 2).

**Ion levels of blood plasma:** $Na^+$, $Cl^-$, $K^+$, $Mg^{+2}$, $Ca^{+2}$ ion levels of blood plasma had changed little relatively at direct groups until sea water salinity level increased to 3.0%. Then in comparison to freshwater groups they increased significantly ($p<0.05$). Plasma ion levels at directly transfered groups generally increased after 24 h.

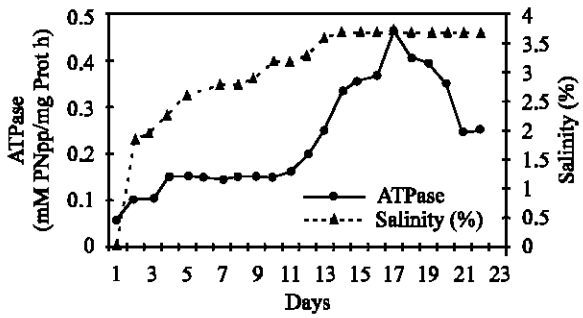


Fig. 1: Changes of $Na^+$, $K^+$-ATPase enzymes at gradual acclimatization

Table 3: Ratios of ion levels between plasma and sea water

| Ions | In plasma (FW) (mM $L^{-1}$) | In sea water (mM $L^{-1}$) | Ratio |
|---|---|---|---|
| $Na^+$ | 168±1.2 | 534±2.3 | 3.1 |
| $Cl^-$ | 181±1.9 | 518±2.9 | 2.8 |
| $K^+$ | 1.19±0.03 | 12±0.17 | 10.0 |
| $Ca^{+2}$ | 6.9±0.06 | 67±0.58 | 9.7 |
| $Mg^{+2}$ | 3.52±0.06 | 123±0.3 | 34.9 |

FW: fresh water

Table 4: Ion and $Na^+$, $K^+$-ATPase enzyme levels of acclimated and non-acclimated fish

| | $Na^+$ (mM $L^{-1}$) | $Cl^-$ (mM $L^{-1}$) | $K^+$ (mM $L^{-1}$) | $Ca^{+2}$ (mg $dL^{-1}$) | $Mg^{+2}$ (mg $dL^{-1}$) | ATPase (mM PNpp/mg prot h) | $Cl^-/Na^+$ | $Cl^-/Na^+$ (in FW) | $Cl^-/Na^+$ (in SW) |
|---|---|---|---|---|---|---|---|---|---|
| Acclimatized Fish | 245.8±8.5 | 222.9±3.9 | 3.5±0.61 | 12.1±0.39 | 7.2± 1.2 | 0.23-0.38 | 0.9 | 0.8 | 0.9 |
| Non-acclimatized Fish | 285.4±7.3 | 291.1±5.2 | 4.3± 0.5 | 13.2±0.2 | 16.7± 1.5 | 0.12-0.22 | 1.01 | - | - |

SW: Sea Water, Fw: Fresh Water

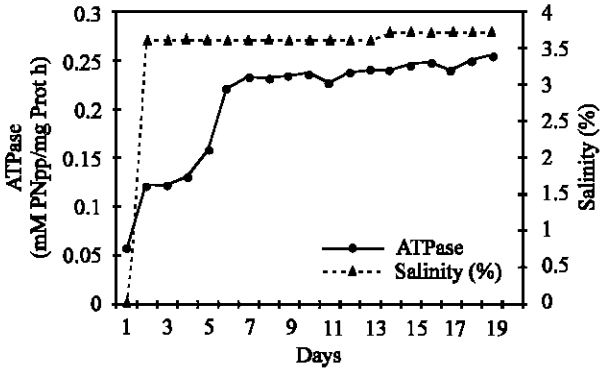


Fig. 2: Changes of $Na^+$, $K^+$-ATPase enzymes at direct acclimatization

In subsequent days, they reached new levels increasing significantly compared to that of freshwater ones (Table 3 and 4).

**Survival:** Mortality rates were different considerably among groups depending on the adaptation techniques. In groups which were transferred gradually no osmotic stress responses observed until salinity increased to 3.2%. In these groups, there was no differentiation significantly ($p>0.05$) between final mean weight and survival rates. In experimental direct group, when fish were exposed to 3.7% salinity directly, they showed osmotic stress responses. High mortality was observed for first 7 days. Survival rates were different significantly ($p<0.05$) among treatment groups transfered directly (Table 5). In gradual and direct transfer trials, there was a significant difference among sea adaptation survival rates of fish that had different mean weights.

The mortality increased during cageculture when temperature reached 17°C. Most probably, pathogenic agents caused mortality in adaptation and post-adaptation periods (Table 5).

**Growth:** Growth performances of rainbow trouts reared on the Aegean Sea (3.6-3.7%) were higher than that of the control group reared in fresh water. When temperature rose above 15°C, lower growth performances were observed. Growth performance significantly differed ($p<0.05$) at different temperature (13, 15 and 18°C) (Table 6).

## DISCUSSION

Survival and growth of rainbow trouts which are transferred to saline media depend upon development of ion regulation mechanisms at hyper osmotic environment. Increase in $Na^+$ and $Cl^-$ ions in blood plasma results in stimulation of ion excretion mechanisms and increasing of $Na^+$-$K^+$-ATPase enzyme activity that causes decreasing of $Na^+$ and $Cl^-$ ions levels. Consequently, acclimatization to saline environment can be achived[10,11].

In this study, increases in ion levels in blood plasma were tolerated better by rainbow trout which were acclimatized to sea water gradually, even though between gradual and direct adaptation no significant differentiations, in ion levels of plasma and $Na^+$, $K^+$-ATPase enzyme values of gill tissues were found during acclimatization of rainbow trout to sea water, on the Aegean Sea (3.6-3.7%). Rainbow trout directly transferred to sea water faced some pathogen agents which damaged resistance of fish immune system and osmoregulation problems. Myxobacteria agents were diagnosed by mycrobiologic diagnosis. Boeuf[8] reported that gradual transfer slowed down the hyper ion increase, whereas direct transfer caused sudden increase. Same author indicated that there was a correlation between increased osmotic pressure which occurred between 24-48 h in rainbow trout blood plasma and mortality. Furthermore, the author reported that after direct acclimatization of trouts to saline water, $Na^+$, $K^+$-ATPase

Table 5: Survival rates of experimental groups (%)

| Experimental groups | Adaptation periods | | Periods of cageculture | |
|---|---|---|---|---|
| | Gradual (21 days) | Direct (21 days) | Growing (60 days) | Growing (80 days) |
| 1 | 95 | 10 | 75 | 47 |
| 2 | 85 | 11 | 70 | - |
| 3 | 80 | 44 | - | - |
| 4 | 91 | 79 | 88.3 | 62 |
| 5 | 92 | 80 | 85 | 73 |
| 6* | - | - | - | 100 |

*In fresh water

Table 6: Growth performances of rainbow trouts on sea water

| Experimental groups | Media | Initial W (g) | Final W (g) | Days | Growth rate (%/day) |
|---|---|---|---|---|---|
| 1 | SW | 120±2.4 | 300±5.4 | 80 | 1.14 |
| 2 | SW | 140±2.9 | 260±5.1 | 60 | 1.03 |
| 3 | SW | 200±2.6 | 555±1.4 | 80 | 1.25 |
| 4 | SW | 225±3.2 | 600±2.1 | 80 | 1.23 |
| 5 | FW | 225±3.7 | 506±5.7 | 80 | 1.02 |

SW: Sea water, FW: Fresh water

enzyme activity started to increase about 4 to 7 days (6-7 μm Pi.mg.prot.h) reaching highest level (25 μm Pi mg protein h) on 14 to 21 days, while $Na^+$, $K^+$-ATPase enzyme activity of trouts that were in fresh water media, was lower (<8-9 μm Pi mg protein h). In the present study, it was found that 12 to 13 days were needed for regulation of excessive ions concentration in rainbow trouts which were transfered to sea water by direct and gradual methods (Fig. 1 and 2). Enzyme levels of acclimatized rainbow trouts and non-acclimatized rainbow trouts to saline water varied from 0.23 to 0.38 mM PNpp $mg^{-1}$ protein h and from 0.12 to 0.22 mM PNpp $mg^{-1}$ protein h, respectively. $Cl^-/Na^+$ ratio of acclimatized fish (0.9) was found to be equal to those of reared directly in sea water (0.9), while it was higher than found in those reared in fresh water (0.8). Non-acclimatized fish that did not survive had 1.01 ratio which was even higher than sea water ratio (Table 4). Bardou *et al.*[12] found similar values. Boeuf and Harache[3] reported that $Na^+$-$K^+$-$Cl^-$ ion levels of blood plasma did not change considerably up to 3.0% salinity; however, $Na^+$, $K^+$-ATPase enzyme activity increased significantly at 3.5% salinity. Bardou *et al.*[12] reported that when concentration of ion plasma reached external levels, it caused an increase in osmoregulation problem. According to the authors this problem particularly existed for the ratio of concentration of external environment/concentration of plasma. In this study, $Na^+$, $Cl^-$, $K^+$, $Ca^{+2}$ and $Mg^{+2}$ plasma ions ratios were measured as 3.1, 2.8, 10, 9.7 and 34.9,(Table 3), respectively while Bardou *et al.*[12] found these values as 3, 4.1, 2.4, 4 and 70, respectively. Boeuf and Harache[7], Boeuf[8] reported acclimatization methods had no significant effect on survival of rainbow trouts weighing more than 120 g which were transfered to sea water by direct and gradual adaptation methods. Rainbow trouts which have definite sizes can be transfered to high saline water at certain seasons in France, although rainbow trouts are not anadromous species[7]. In this study, however, it was observed that methods of adaptation had important effects on survival of rainbow trouts weighing of 120, 140, 160, 200 and 225 g. Only one of the experimental groups weighing approximately 200-225 g was able to tolerate high salinity when transfered directly to sea water of the Aegean Sea in winter season (Table 5). Nevertheless, all of trial groups were able to adapt to sea water. High mortality appeared in this study during the direct transfer because of mainly pathogenic agents which were Myxobacteria.

Boeuf *et al.*[13], reported that rainbow trouts were reared from 155 to 528-548 g mean weight at 35.5 ppt for 5 months. A later study in which rainbow trouts weighing 150-250 g that reached 400-650 g mean weight in 4 months were cultivated at marine cages for 240 days until June in France[14]. Growth of rainbow trouts weighing 186 and 212 g was reported by Güner[15] that were reared on the Aegean Sea in 4 months and they reached 575 and 608 g mean weights, respectively (Table 6). The values of weight were obtained at the final of 80 days during the present study, showed similarity with results. Food consumption, growth performance and survival of fish were affected negatively when temperature raised to 18°C (15 April) on marine environment. An excessive increase in ions of blood plasma reduced food consumption[8]. In addition, higher temperatures of sea water resulted in some osmoregulation problems and caused larger amounts of ions from saline environment to pass through fish tissues[12].

Harache[16], Boeuf[17,18] and Harache reported osmoregulation problems at high salinity, particularly above 3.4% and at high temperatures above 16°C. The critical limit of salinity for all trout species was 30 ppt salinity. Many researchers[19,20] claimed that $Na^+$-$K^+$-ATPase enzyme activities of rainbow trouts are inhibited in sea water at temperatures above 15°C some pathologic problems appear when rainbow trouts are kept in sea water during summer season[21], owing to the fact that rising salinity (>3.4%) and temperature (>16°C) cause

increasing mortality[22]. Furthermore, marine aquaculture of rainbow trouts in summer results in high mortality without any pathogenic agents such as vibrio, myxobacteria, parasit[14]. Most probably, mortality occurs due to lack of physiological accommodation of fish to high salinity and temperature. In the present study, with the increase of water temperature, external lesions were observed as clinical etiologies of summer syndrom, just as Aldrin[21] reported. Fish should be harvested before temperatures reach 19°C, which results in decreases in food consumption and resistance to diseases[23]. Alexis *et al.*[24] reported also that marine culture of rainbow trouts on Mediterreanen Sea should be done in winter periods because of high mortality at warm sea water. In conclusion, rainbow trout can be adapted and cultured, in certain periods (Autumn-Winter), on the Aegean Sea (3.6-3.7%). However, mariculture of rainbow trout on the Aegean Sea exactly has not been facilitated yet because of these mentioned reasons: Adaptation sizes of fish should be above 120 g which is not economical, insufficient mechanisms for adaptation facilities and mariculture of rainbow trouts can be done in a limited season on the Aegean Sea. On the other hand, it was reported in France that rearing of Brown trout, *Salmo trutta fario* in seawater is more advantageous in terms of protection from summer temperatures and smaller size as well (30-35 g)[3]. In conclusion, mariculture of brown trout on the Aegean Sea, Marmara Sea and Black Sea seems quite reasonable economical.

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