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A preliminary study on the effects of different salinities on some hormones level in serum of Yellow fin seabream *Acanthopagrus latus* fingerlings.

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Abstract – A laboratory experiment was performed on fingerlings of *Acanthopagrus latus* to estimate the effect of direct transfer from the controlled salinity 1.5 psu to different salinities of (1.5, 7.5, 15, 30 and 45 psu) for periods of (6, 24, 48 and 96) hrs. for short term effect experiments. The following hormones (cortisol, prolactin, thyroxin (T4) and triiodothyronine (T3)) were determined in the serum of these fishes by ELISA technique after exposed to different salinities. Results of T4 and T3 were correlated directly with salinity increase, the (96 hrs.) of time was not enough to reach the stable state of these hormones at all salinities. The salinity 45 psu gave the lowest level of T3 (0.468) ng/ml and showed a sharp drift of T4 level at the time 24 hrs. (0.368) µg/dl, which indicated a breaking down of these hormones, and *A. latus* fingerlings were unable to sustain at this salinity. Prolactin hormone was correlated inversely with the salinity increase which means that there was ability of this hormone to acclimate in freshwater. Cortisol level was high in high and low salinities indicating its activity in freshwater and seawater environments. The study concludes that the four hormones (cortisol, prolactin, T4 and T3) in the blood serum of *A. latus* fingerlings showed a clear variations upon salinity changes in the short term effect experiment as a transit status reflecting the capability of these fingerlings to adapt to wide range of salinities vary from 1.5 psu to 30 psu, and the salinity 45 psu was out of its tolerance range.

Key words: *A. latus*, Salinity, Cortisol, Prolactin, T3 and T4.

Introduction

Salinity stress is one of the most common stresses in aquatic animals that usually occurs and if prolonged, can lead to reduction of production efficiency or fish death (Sakamoto and McCormick, 2006).

Control of ion regulation is critical, and the neuroendocrine system is the major means for regulating these mechanisms. Osmoregulation in teleost fish is dependent on the interplay of a variety of hormones including Cortisol and pituitary hormones, Prolactin (PRL) and Growth hormone (GH), which play an important role in osmotic acclimation (McCormick, 1995, 2001; Sakamoto and McCormick, 2006).

Salinity changes can be stressful and may affect homeostasis of the fish. Numerous experiments on animals demonstrated that the blood concentrations of thyroid hormones are increased during early exposure to stressors such as high ambient temperatures and excitement stress (Guerrini and Bertchinger, 1983).

Environmental factors such as day length, lunar cycle events, temperature, pH, salinity and nutrition are all implicated in stimulating thyroid activity (Grau, 1988). In some fish species, thyroid hormones support the action of growth hormone and cortisol in promoting seawater acclimation (McCormick, 2001). Thyroid hormones (T3, T4) have marked effect on control growth, metabolism and osmoregulation in fish and often do these activities in association with other hormones such as cortisol and growth hormone (Evans, 1997).

Prolactin is an important regulator of multiple biological functions in vertebrates, and has been viewed as essential to ion uptake as well as reduction in ion and water permeability of osmoregulatory surfaces in freshwater and euryhaline fish (Sakamoto and McCormick, 2006). The major function of this hormone in fish is most likely the maintenance of permeability to water and ions in the epithelia of the skin, gill, intestine and renal tubules including the bladder at the low level that is required for survival of fishes in freshwater (Wendelaar Bonga, 1993).

Cortisol is one of the important fish hormones responsible in increasing the salinity tolerance during freshwater fish transfer to seawater through its effect on reducing body fluid concentration (hypo-osmotic) (Morgan and Iwama, 1991). Cortisol, regulate body fluid electrolytes of teleosts through its direct effect on cellular membrane permeability, increasing number and size of mitochondria and activating ATPase levels which control salt motion in gill chloride cells of teleosts (Uchida *et al*., 1997). In addition to the classical hypoosmoregulatory role of cortisol, and according to several evidences a new role of this hormone either in ion uptake in FW- or BW-adapted fish has been suggested (McCormick, 2001).

Acanthopagrus latus (Houttuyn, 1782) (Sub-Family: Sparidae) is a high value marine fish commonly found in the Arabian Gulf. The fish was distributed off Indo-West Pacific includes Arabian Gulf to Australia (Abou-Seedo *et al*., 2003). It is a carnivorous fish, with a moderately body size. Although, *A. latus* is highly economic value besides its high adaptations to different salinities and temperatures, it also plays an important role in fish aquaculture in ponds or cages (Leu *et al*., 1991; Leu and Chou, 1996). As no detailed study has been carried out on the thyroid, prolactin and cortisol patterns of *A. latus*, in the Arabian Gulf and inland waters, the present study was aimed to determine the effect of salinity changes as a short term effect on thyroid hormones (T_3 and T_4), cortisol and prolactin hormones levels in the serum of *A. latus* fingerlings.

Materials and Methods

Experimental Groups:

In this study, 360 *A. latus* fingerlings were divided randomly into four groups (24 fish in each group and three replicates per treatment). Three groups were held in five different salinities at concentrations of 1.5, 7.5, 15, 30 and 45 psu. The first group was considered as the control. Ten containers (200 l) were used in three replicates for each salinity. The containers were provided with aerators and were covered with nets to prevent fish from escape from the container. The containers were filled with tap water free from chlorine. The salinities of containers were corrected according to the designed experiment using marine salt from Aquamedic company (Bissendrof, Germany) with major elements Na^{+}, Mg^{+2}, Ca^{+2}, K^{+}, Cl^{-}, $So_4^{=}$, HCO_3^{-} and Sr^{+} (11000, 1200, 420, 350, 19700, 2200, 180 and 16) mg/l respectively. Twenty four fishes were transferred directly from control salinity 1.5 psu to the containers of different salinities.

Sampling and Measurements:

After direct transfer to the different salinities, fishes were taken and checked after (6, 24, 48 and 96) hrs. for measuring many physiological parameters. Six fishes were killed from each salinity at the times (6, 24, 48 and 96) hrs. after being anesthetized by clove oil by putting fish in container with water of the same salinity they taken from and adding clove oil to the water (Durvill and Collet, 2001). The total length (cm) and total weight (g) were recorded. Blood samples were collected from the heart for collecting plasma by a 3 ml syringe for measurements which will be described later. Blood samples were immediately transferred to sterile tubes and the serum was separated by centrifugation. Cortisol, prolactin, T3 and T4 were measured by enzyme linked immunosorbent assay (ELISA) method using a commercially available kits manufactured by (Monobined Inc., USA). Absorbance was read at 450 nm in ELISA reader (Humareader HS, Human, Germany).

Four environmental factors (temperature, dissolved oxygen, salinity and pH) were measured daily using the YSI instrument model (556 MPS). Survival rate was estimated from the following equation:

Survival Rate (%) = (N2/N1) × 100 (Teng *et al.*, 1985)
N1 = number of fishes at beginning of experiment.
N2 = number of fishes at end of experiment.

Statistical Analysis:

Values were compared using a one-way analysis of variance (ANOVA) and Revised Least Significant Difference (R.L.S.D.) to compare the variances between salinities at different times ($P< 0.05$) was set as the significance level using SPSS program. Values were expressed as the (Mean ± S.E.M.) (The standard error of the mean) (Steel and Torrie, 1960).

Results

Experimental Groups:

The total length and weight of fishes (10.41 ± 0.12 cm) and (22.97 ± 0.89 g) were used. Environmental factors of water containers such as temperature, D.O and pH were: (30.18 ± 0.47) °C, (4.70 ± 0.2) mg/l and (8.10 ± 0.1) respectively. *A. latus* fingerlings were survived (100 %) in the salinities (1.5, 7.5, 15 and 30) psu, while all fishes were die in the salinity 45 psu after 48 hrs. of direct transfer.

Cortisol Level:

Table (1) show the concentration of cortisol (µg/dl) in the serum of the *A. latus* after direct transfer from 1.5 psu to different salinities (1.5, 7.5, 15, 30 and 45) psu for the times (6, 24, 48 and 96) hrs. (short term effect). Results showed that the high and low salinities (45, 30, 15 and 1.5) psu gave high cortisol levels than the medium salinity 7.5 psu. The highest values of cortisol level were noted in the highest salinity 30 psu at the time 48 hrs. which was 3.847 µg/dl, cortisol level of this salinity started to decrease at the time 96 hrs. and reached 2.492 µg/dl. The second salinity in the cortisol level was the control salinity 1.5 psu which showed an increase in the cortisol level in the times 24, 48 hrs. and then the cortisol level was started to decrease at the end time 96 hrs. which was 3.073 µg/dl. The third salinity in the cortisol level was 15 psu which have an increase of cortisol concentration at the times (6, 24, 48) hrs. and then started to decrease at the end time 96 hrs. which was 2.007 µg/dl. The lowest values of the cortisol level obtained at the salinity 7.5

psu which have a decrease in cortisol level with time and reached the lowest value of cortisol level 0.768 µg/dl at the time 96 hrs. (Table 1).

Statistical analysis showed no significant differences (P>0.05) between the salinity 30 psu and the control salinity 1.5 psu at the time 48 hrs., but there were significant differences (P<0.05) between all other salinities. This reflect the cortisol activity in high and low salinities (Table 1).

Prolactin Level:

The Table (2) show the Prolactin level (ng/ml) in the serum of the *A. latus* after direct transfer from 1.5 psu to different salinities (1.5, 7.5, 15, 30 and 45) psu for the times (6, 24, 48 and 96) hrs. (short term effect). Results stated that the prolactin level had a reverse proportion with the salinity increase. The highest values of the prolactin level showed in the control salinity 1.5 psu at the time 24 hrs. which was 9.722 ng/ml. Prolactin level of this salinity reached 8.208 ng/ml at the end time 96 hrs. of the experiment.

The second salinity in the prolactin level was the salinity 7.5 psu. The prolactin level was 5.410 ng/ml in the first times 6 hrs. and then started to increase at the time 24 hrs. and reached 5.751 ng/ml at the end time 96 hrs. of the experiment. The third salinity in the prolactin level is the salinity 15 psu which increased for the prolactin concentration in the times 24 hrs. and 48 hrs. and started to decrease at the time 96 hrs. which was 3.944 ng/ml.

The fourth salinity was 30 psu which have a decrease in prolactin level with time and reached to 2.661 ng/ml at the end time 96 hrs. The lowest value of the prolactin level was obtained at the highest salinity 45 psu which reached to 2.3 ng/ml at the time 24 hrs. of the experiment (Table 2).

Statistical analysis showed no significant differences (P>0.05) in prolactin level between the salinity 7.5psu and the salinity 15 psu at the times (6, 24, 48) hrs., and there were significant differences (P< 0.05) between all salinities at the end time 96 hrs. of experiment (Table 2).

Table 1. Cortisol concentration (µg/dl) in the serum of *A. latus* in the short term effect experiment of different salinities (Mean ± S.E.).

Time	1.5 psu	7.5 psu	15 psu	30 psu	45 psu
6 hrs.	2.876 ± 0.06 b	1.586 ± 0.03 D	2.247 ± 0.007 c	3.724 ± 0.002 a	3.783 ± 0.03 a
24 hrs.	3.616 ± 0.1 b	1.354 ± 0.06 D	2.462 ± 0.005 c	3.871 ± 0.05 a	3.648 ± 0.01 b
48 hrs.	3.805 ± 0.01 a	0.960 ± 0.01 C	3.690 ± 0.006 b	3.847 ± 0.05 a	----
96 hrs.	3.073 ± 0.01 a	0.768 ± 0.008 D	2.007 ± 0.01 c	2.492 ± 0.06 B	----

- Similar letters means that there was no significant differences between salinities for each time.
- Different letters means that there was significant differences between salinities for each time.

Table 2. Prolactin concentration (ng/ml) in the serum of *A. latus* in the short term effect experiment of different salinities (Mean ± S.E.).

Time	1.5 psu	7.5 psu	15 psu	30 psu	45 psu
6 hrs.	8.452 ± 0.51 a	5.410 ± 0.19 b	4.486 ± 0.33 bc	3.686 ± 0.15 cd	3.00 ± 0.42 d
24 hrs.	9.722 ± 0.65 a	5.65 ± 0.16 b	5.127 ± 0.05 b	3.285 ± 0.02 c	2.300 ± 0.21 c
48 hrs.	8.672 ± 0.56 a	5.310 ± 0.18 b	5.26 ± 0.06 b	3.056 ± 0.03 c	----
96 hrs.	8.208 ± 0.57 a	5.751 ± 0.37 b	3.944 ± 0.02 c	2.661 ± 0.11 d	----

- Similar letters means that there was no significant differences between salinities for each time.
- Different letters means that there was significant differences between salinities for each time.

Level of Thyroxin (T4):

The Figure (1) shows the level of the thyroxin (T4) (µg/dl) in the *A. latus* serum after direct transfer from 15 psu to different salinities (1.5, 7.5, 15, 30 and 45) psu for the times (6, 24, 48 and 96) hrs (short term effect). Results showed that the hormone level correlated directly with salinity increase the highest values of thyroxin level were noted in the salinity 30 psu at the time 24 hrs. which was 1.377 µg/dl. Thyroxin level of this salinity was started to decrease at the time 48 hrs. and reached 2.034 µg/dl at the end time 96 hrs. The second salinity in the thyroxin level was the salinity 15 psu. The thyroxin level was 1.044 µg/dl in the first times 6 hrs. and then it levels started to increase with time and reached 1.468 µg/dl at the end time 96 hrs. of the experiment. The third salinity in the thyroxin level was the salinity 7.5 psu which had a thyroxin concentration of 0.836 µg/dl in the first time 6 hrs. of the experiment. The T4 activity started to increase with time and reached 1.022 µg/dl at the end time 96 hrs. of the experiment. The lowest values of the thyroxin level obtained at the control salinity 1.5 psu which have the lowest value of thyroxin level 0.286 µg/dl at the time of 24 hrs. and then started to increase with time and reached to 0.375 µg/dl at the end time 96 hrs. The salinity 45 psu showed a high value of T4 level which was 1.274 µg/dl at the first time 6 hrs. but then showed a sharp drift of the hormone level at the time 24 hrs. which was 0.368 µg/dl (Fig. 1).

Statistical analysis suggested the significant differences ($P<0.05$) in thyroxin level between all salinities at all times of experiment excepting the salinities 1.5 psu and 45 psu had no significant differences ($P>0.05$) at the time 24 hrs., and this reflect the hard drift of T4 level in the salinity 45 psu at the time 24 hrs. (Fig. 1).

Level of Triiodothyronin (T3):

The Figure (2) shows the level of Triiodothyronin (T3) (ng/ml) in the *A. latus* serum after direct transfer from 1.5 psu to different salinities (1.5, 7.5, 15, 30 and 45) psu for the times (6, 24, 48 and 96) hrs. (short term effect). Results found that the T3 level correlated directly with salinity increase.

The highest values of triiodothyronin level was in the salinity 30 psu at the time 24 hrs. which was 7.955 ng/ml; the T3 level of this salinity started to decrease after this time and reached 7.188 ng/ml at the end time 96 hrs. of the experiment. The second salinity in the triiodothyronin level was the salinity 15 psu, the T3 level was 5.352 ng/ml at the first time 6 hrs. and then the hormone level started to increase with time and reached 7.731 ng/ml at the end time of 96 hrs. of the experiment. The third salinity in the triiodothyronin level is the salinity 7.5 psu which has a T3 concentration 3.093 ng/ml at the first time of 6 hrs. of the experiment. The T4 level started to increase with time and reached 6.71 ng/ml at the end time 96 hrs. of the experiment. The fourth salinity was the control salinity 1.5 psu which have a hormone level of 3.061 at the time 24 hrs. and then started to decrease with time and reached to 0.905 ng/ml at the end time 96 hrs. The lowest values of the triiodothyronin level was obtained in the salinity 45 psu which reached to the lowest value of the triiodothyronin level 0.468 ng/ml at the time of 24 hrs. of the effect experiment (Fig. 2).

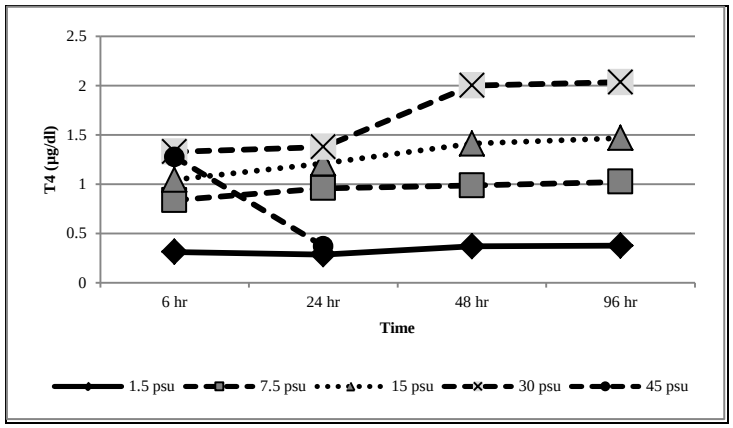

Figure 1. Thyroxin (T4) concentration (µg/dl) for the *A. latus* serum in the short term effect experiment of different salinities.

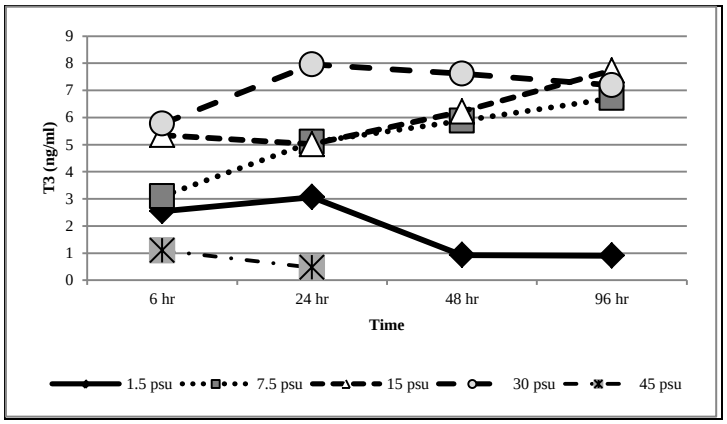

Figure 2. Triiodothyronin (T3) concentration (ng/ml) for the *A. latus* serum in the short term effect experiment of different salinities.

Statistical analysis found significant differences ($P<0.05$) in triiodothyronin level between all salinities at all times of experiment excepting the salinities (15 and 30) psu and (15 and 7.5) psu had no significant differences ($P>0.05$) at the times 6 hrs. and 24 hrs. respectively (Fig. 2).

Discussion

Living organisms especially aquatic animals are facing many types of stress under natural and artificial conditions. Fishes are sensitive to stress in their natural environment which are resulting from changing in abiotic environmental factors such as temperature, salinity and pollution or biotic factors such as food and space competition, predation, migration and reproduction, whereas in laboratory (artificial environment) stress source would be transportation, crowding and vaccination (Iwama *et al.*, 2006). Any stress factor may create an internal physiological imbalance in fish appears through disorder hormones and enzymes functions and changing in same blood picture characteristics, which need systematic physiological response by fish against stressors returning to homeostasis (Eddy, 2006; Al-Kashali and Al-Shawi, 2013). The present results of cortisol indicated a crucial role of this hormone in osmoregulation of the *A. latus* fingerlings in both the high and low salinities. This was in agreement with the resent theory of the dual function of cortisol in the osmoregulatory activities in both saltwater and freshwater fishes, Cortisol play an important role in increasing ions uptake and it acts with prolactin hormone during acclimatization to freshwater (Pelis and McCormic, 2001). Whereas, the hormone itself shares growth hormone in increasing fish capability to salinity stress (Hyde *et al.*, 2004; McCormick, 2011).

The results of prolactin level in *A. latus* fingerlings showed that it was decreased with salinity increase indicating its role in freshwater adaptation. Evidence for prolactin as a freshwater adapting hormone in fish comes from studies on exogenous prolactin treatment and prolactin dynamics in freshwater and euryhaline fish, including salmonids, gold fish, cichlid fishes (tilapia) and mudskipper (Sakamoto and McCormick, 2006). Manzon (2002) stated that plasma levels of prolactin increased following freshwater exposure. Metabolic clearance rate of prolactin in salmonids are also increased following freshwater acclimation (Sakamoto *et al.*, 1991; Ogasawara *et al.*, 1996). Jeanette *et al.* (2007) found that plasma PRL levels were markedly reduced in SW and 200 % SW-acclimated fish compared to those in FW-acclimated fish at all temperatures (20, 28, 35) ˚C.

This present study showed that the activity of T4 and T3 correlated directly with salinity increase, i.e. the highest levels of T4 and T3 showed in the highest salinity 30 psu followed by the salinities (15, 7.5 and 1.5) psu respectively in all times, and this in agreement with many studies showed an increase in thyroid hormones with salinity increase like the study of McCormick and Saunders (1990) showed that in juvenile Atlantic salmon (*Salmo salar*) following acute exposure to seawater (30 ppt), plasma T4 increased 80 % in the first 6 hrs., declined to initial levels after 24 hrs., and remained stable for 18 days, there-after. Peyghan *et al.* (2013) results showed that increasing water salinity can have significant effect on thyroid activity by decreasing T3 and increasing T4 level in serum of grass carp in experimental condition. Results of Movahedinia *et al.* (2010) on *A. latus* held in different salinities indicated that thyroid hormone levels in plasma (T3) showed a small rise that quickly return to normal after 12 hrs. of salinity change compared to the control samples.

The results of Rejitha *et al.* (2009) on short-term salinity acclimation demands of thyroid hormone action in the climbing perch *Anabas testudineus* (Bloch) suggested that the following transfer of fish to 20 ppt salinity for a day after transient salinity changes, plasma T4 was elevated and plasma T3 concentration was decreased whereas plasma cortisol remained unchanged. The levels of these hormones, however, returned to basal levels when these fish were kept for a prolonged acclimation of three weeks. Their results indicate that salinity acclimation in climbing perch demands thyroid hormone secretion and its action and not cortisol as part of coordinating the acclimation processes in the early phase of salinity acclimation. The results of Klaren *et al.* (2007) showed that the plasma free T4 concentration increases 2.5 fold, and T3 in the gills are reduced by 20-32 % in the effect of acclimation to low salinity water on the activities of thyroid hormone-metabolizing enzymes in gills, kidney, and liver of gilthead seabream (*Sparus auratus*). The salinity 45 psu showed a sharp drift of T4 level at the time 24 hrs. which was 0.368 µg/dl and but the lowest level of T3 was 0.468 ng/ml and had no effect in level of prolactin which was 2.300 ng/ml, which indicates the breaking down of these hormones and *A. latus* fingerlings were unable to sustain at this salinity. While cortisol level at this salinity still high after 24 hrs. indicating that fingerlings were continue undergoing the hard stress at this time.

In conclusion, the four hormones (cortisol, prolactin, T4 and T3) in serum of *A. latus* fingerlings showed a clear co-relation to salinity changes in the short term exposure as a transit status reflecting the capability of these fingerlings to adapt to a wide range of salinities which was ranged from 1.5 psu to 30 psu while the salinity 45 psu was out of its tolerance range.

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دراسة اولية لتأثيرات ملوحات مختلفة على مستويات بعض الهرمونات في مصل اصبعيات اسماك الشانك البحري *Acanthopagrus latus*

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المستخلص - أجريت تجربة مختبرية على أصبعيات أسماك الشانك البحري *Acanthopagrus latus* لبيان تأثير النقل المباشر من ملوحة السيطرة 1.5 psu إلى ملوحات مختلفة (1.5, 7.5, 15, 30, 45 psu) للفترات الزمنية (6, 24, 48, 96 hrs.) كتأثير قصير الأمد. أختبرت بعض الهرمونات (الكورتيسول، البرولاكتين، الثايروكسين و التراي ايودو ثايرونين) في مصل دم هذه الأسماك باستخدام تقنية الألايزا. أشارت النتائج بأن هرموني الثايروكسين والتراي ايودوثايرنين قد أمتلكا تناسبا طرديا مع زيادة الملوحة وأن الوقت 96 ساعة غير كافي للوصول إلى حالة الأستقرار في قيم تلك الهرمونات في كل الملوحات هذا وأعطت الملوحة 45 psu أقل قيمة لفعالية التراي ايودوثايرونين وبلغت 0.468 ng/ml وإنحدارا شديدا في فعالية هرمون الثايروكسين عند الزمن 24 ساعة إذ بلغ 0.368 µg/dl مما يدل على تحطم هذين الهرمونين وعدم قابلية تحمل أصبعيات أسماك الشانك البحري لتلك الملوحة. يمتلك هرمون البرولاكتين علاقة عكسية مع زيادة الملوحة مما يشير إلى قابلية تكيف هذا الهرمون للماء العذب وعدم فعاليته في الماء المالح. أما هرمون الكورتيسول فقد أمتلك قيما عالية في الملوحات العالية والواطئة مما يشير إلى فعالية هذا الهرمون في بيئات المياه العذبة والمالحة. نستنتج من هذه الدراسة أن الهرمونات الاربعة (الكورتيسول، البرولاكتين، الثايروكسين والتراي ايودوثايرونين) في مصل دم أصبعيات الشانك البحري قد أظهرت تأثيرا واضحا من التغيرات الملحية في تجربة التأثير القصير الأمد كحالة مؤقتة تعكس قابلية تحمل تلك الأصبعيات لمدى واسع من الملوحة يتراوح بين 1.5 psu إلى 30 psu وأن الملوحة 45 psu هي خارج مدى تحمل تلك الأصبعيات.