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Adaptation of the sea-bass (*Dicentrarchus labrax*) to fresh water: Role of aquaporins and Na^+/K^+-ATPases

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Abstract:

Sea-bass (*Dicentrarchus labrax*) grow under different salinity regimes, from the open sea to lagoons and even rivers, but some mortality has been recorded in juvenile stages when exposed to low salinity water. Changes in water permeability of different osmoregulatory tissues could be the cause of reduction in blood osmotic pressure and death in some fish in fresh water (FW). In order to explore this condition, we have studied the changes of aquaporins (AQP1 and AQP3), α1 and α4 Na^+/K^+-ATPase transcript levels in the digestive tract, kidney and gills after a long-term exposure of juvenile sea-bass to sea water (SW) and FW fish able to survive in SW and FW are called SW-adapted fish (SWS), FW successfully-adapted fish (FWS) respectively, while fish that die in FW are called FW unsuccessfully-adapted fish (FWU). AQP1 was highly expressed in SWS digestive tract and kidney, suggesting its involvement in water absorption. In FWU, AQP1 transcript levels in the digestive tract were higher than in FWS, suggesting higher water absorption. AQP3 transcript levels in gills were higher in FWS compared to SWS, suggesting a role in FW adaptation. AQP3 transcript levels in gills were higher in FWU than in FWS, suggesting an increase in gill water permeability or other solutes. Transfer to FW was followed in gills by an increase in α1 and α4 Na^+/K^+-ATPase levels in FWS and FWU, supporting the current model of ion absorption through the gills.

Keywords: AQP1; AQP3; α1 Na^+/K^+-ATPase; α4 Na^+/K^+-ATPase; Differential mortality; Juvenile sea-bass; Salinity adaptation

1. Introduction

The European sea-bass (*Dicentrarchus labrax* L.) is a marine teleost whose adults tolerate salinities ranging from fresh water (FW) to hypersaline sea water (Jensen et al., 1998). In the wild, some populations move seasonally between the open sea and lagoons/estuaries or even migrate up rivers to fresh water (Barnabé et al., 1976; Kelley 1988). They are known to mate only in sea water (Pickett and Pawson 1994). Different studies have shown that this species is able to live in low salinity environments (Barnabé 1980; Pickett and Pawson 1994) and to successfully adapt to FW under experimental conditions (Cataudella et al., 1991; Venturini et al., 1992; Varsamos et al., 2002; Nebel et al., 2005b), although in other experimental studies, mortality rates have been shown to be important (Dendrinos and Thorpe 1985; Allegrucci et al., 1994; Jensen et al., 1998). It has also been shown that sea-bass juveniles have a 15 ppt salinity preferendum (when compared to 37 ppt in sea water) (Saillant et al., 2003) which corresponds to the conditions they may encounter during their juvenile ecophase in some lagoons.

Lagoons are considered to be very suitable feeding grounds, but at the same time stressful for juvenile fishes (Bruslé and Cambrony 1992) because numerous environmental parameters fluctuate, in particular salinity, temperature and oxygen levels (Kierner 1978). When adult sea-bass leave the lagoons to mate in the sea, their pool of gametes is integrated into that of individuals having spent their early life in sea, thereby homogenizing gene frequencies and braking up an eventual disequilibrium between sea and lagoon populations. A new pool of larvae is produced and the process of selection is repeated for those sea bass entering the lagoons (Allegrucci et al., 1997; Lemaire et al., 2000). A hypothesis explaining this phenomenon has been proposed. If adults leaving the lagoons have a higher probability to mate between them than with marine residents, then the genetic load will be reduced to some extent by creating differentiated larval cohorts and only certain populations would retain the genetic capabilities to colonize lagoons (Lemaire et al., 2000). Therefore, gene expression and/or gene regulation in osmoregulatory organs during the course of development at the time of transition between low salinity water and fresh water might reveal differences in ecological requirements of this species at specific stages. The determination of differentially regulated genes in these lagoon populations might be very useful in order to establish better marine fish stocks differentiation programs based on more appropriate genetic markers (Allegrucci et al., 1997). The understanding of the genetic variation in wild sea bass populations could likewise be improved.

The involvement of aquaporins (AQP1 & AQP3) (Giffard-Mena et al., 2007b) and α1 and α4 Na^+/K^+-ATPase (Nebel et al., 2005b; Boutet et al., 2006) in salinity adaptation has been reported in different organs of *D. labrax*. The expression level of AQP3 and α4 Na^+/K^+-ATPase is high in FW and it is possible that their abundance also differs between successfully- (FWS) and unsuccessfully- (FWU) fish adapted to fresh water. A lower renal Na^+/K^+-ATPase activity and tubular density was recorded in FWU fish compared to FWS fish (Nebel et al., 2005b). One of the hypothesis raised by the authors was that the FWU fish have a decreased ability to reabsorb ions leading to a failure to produce hypotonic urine to blood and a consequent reduction in blood osmolarity from 316 ± 20 to 214 ± 14 $mOsm.kg^{-1}$. Also, an increased permeability of the collecting ducts to water was suggested. In what appears as a compensatory reaction to the low blood osmolarity, ionocytes tend to proliferate along the lamellae of the FWU fish gills (Nebel et al., 2005b) and the branchial Na^+/K^+-ATPase activity is significantly higher. The high gill surface covered by the ionocytes seems to negatively interfere with gas exchanges and to reduce the respiratory effectiveness (Perry 1998a; Marshall and Grosell 2005). Following these observations, questions concerning hydro-mineral regulation arise regarding FWU fish: Is the water absorption mechanism of the digestive tract altered in FWU fish? Is there an increase of permeability in the kidney collecting ducts in these fish? Are gills involved in FWU mortality?_In order to answer these questions, the expression of AQP1, AQP3, α1 and α4 Na^+/K^+-ATPase was studied in juvenile European sea-bass (*Dicentrarchus labrax*) maintained in sea water (SW fish) and fresh water, where successfully- (FWS) and unsuccessfully- (FWU) adapted fish were identified.

2. Materials and Methods

Fish and rearing conditions

Juvenile European sea-bass *Dicentrarchus labrax* (Linné, 1758) were obtained from the fish hatchery "Les Poissons du soleil" located in south-west France (Balaruc, Hérault, France). They were transported to the "Station Méditerranéenne de l'Environnement Littoral" (SMEL) aquaculture facility (Sète, Hérault, France) located along the Thau lagoon, which communicates with the Mediterranean Sea. The salinity challenge was conducted using 3 month-old juvenile sea-bass hatched in December. The culture parameters and conditions were similar to those previously described (Nebel et al., 2005b). Briefly, the fish were divided into eight 200L-tanks, each of them containing 250 fish. In four tanks, fish were progressively transferred from SW to FW over a period of three weeks. The ionic composition of FW (0.3 ppt ~ 10 mOms.kg^{-1}) was in mEq l^{-1}: Na^+ (0.12), K^+ (0.04), Ca^{2+} (5.7), Mg^{2+} (0.29), Cl^- (0.98), NO_3^- (0.06) and SO_4^{2-} (0.61) (F. Persin, pers. comm.). The other four tanks were used for SW-acclimated fish (34 ppt, ~ 1000 mOms.kg^{-1}). Fish were fed throughout the study on Aphymar granulates (Aphytec, Mèze, France) at a daily ration of 3% of the estimated wet weight with an automatic 24h clockwork belt feeder (Aquaculture Technology, Kitzbühel, Austria) and they were subjected to a natural photoperiod and temperature regime. Fish were sampled for further studies when different patterns of behavior appeared in FW, leading to the distinction of FWS and FWU fish (Nebel et al., 2005b). SWS fish were sampled simultaneously. All animals were treated in agreement with the French regulation concerning the handling of experimental animals. Fish were anaesthetized with 300 µg.l^{-1} phenoxy-2-ethanol; they were weighed, measured (from mouth to tail) and sacrificed. Organs were quickly dissected under sterile conditions.

Reconditioning of FWU to SW

The identified FWU fish, still alive, were kept in individual cages in separate 20 L aquaria filled with the same FW at 0.3 ppt. The salinity of the water was increased progressively up to SW value over a four-day period by incremental additions of SW. As a parallel test, FWU fish were also put in individual cages immersed in 20 L FW aquaria at 0.3 ppt. The behavior of all fish (n = 10 by salinity) were thereafter recorded.

Muscle water content

Muscle water content was determined as weight loss after drying in an oven at 105°C for 3 days. Values are expressed as percent wet weight.

Expression of Aquaporins and ATPases following salinity adaptation

Six samples for each salinity group (SWS, FWS, FWU) were analyzed. Total RNA was isolated from the digestive tract (from esophagus to rectum), kidney and gills (lamellae and filaments were separated from the first to fourth gill arch from the right side of the fish) after homogenization in TRIzol^{TM} reagent (Invitrogen, Carlsbad, CA, USA). A treatment with DNAse I (Invitrogen) was applied to all RNA samples to prevent genomic DNA contamination. The total RNA concentration was determined by OD_{260} measurements in an Eppendorf BioPhotometer (Eppendorf, Hamburg, Germany), and its purity was verified using the 260/280 absorbance ratio. The integrity and relative quantity of total RNA were checked by electrophoresis. 3.5 µg of total RNA extracted from each salinity group were reverse-transcribed into cDNA using 500 µg.ml^{-1} of Oligo (dT) primer and 200 units of M-MLV RT (Invitrogen) following the manufacturer's instructions.

Specific primers were designed to amplify the sea-bass AQP1, AQP3, α1 and α4 Na^+/K^+-ATPase (Table I). The PCR reaction was performed with a Light Cycler^{TM} system version 3.5 (Roche, Mannheim, Germany) in a final volume of 10 µl as previously described (Giffard-Mena et al., 2007a). Briefly, 2 µl of Lightcycler-FastStart DNA Master SYBR-Green I^{TM} Mix (Roche), 1 µl of each forward and reverse primers (5 µM) and 0.5 µl of transcribed cDNA were used. The relative expression of each gene in each tissue at each salinity condition was calculated for 100 copies of the housekeeping gene (EF1α) using the formula:

$N=100 \times 2^{(Ct\ housekeeping\ gene\ -\ CtXgen)}$ (Rodet et al., 2005). The PCR fragments were then cloned into a pCR4 vector (TOPO TA cloning™ kit, Invitrogen) and sequenced with an ABI PRISM 3130X1 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using the BigDye™ Terminator Sequencing kit (Applied Biosystems) as previously described (Giffard-Mena et al., 2007b).

Histomorphology of the digestive tract and kidney

The SWS, FWS and FWU fish (3 individuals per group) were fixed in a Bouin solution over 48 h. The fixative penetration was facilitated by removing the caudal fin from the animal and dividing the animal in two sections at the stomach level. They were washed and dehydrated in an ascending series of ethanol, and finally embedded in Paraplast (Sigma). Histological sections of 6 µm were stained according to the Masson-trichrome procedure for tissular topography. Mounted slides were observed on a Leica Diaplan microscope (Leitz Wetzlar, Germany). The different digestive tract segments were determined and 3 slides per animal were used for further analyses. Sections from the kidney were similarly selected. Photographs were taken with a Leica DC300F digital camera adapted to the microscope, and a Leica FW4000 I software (Leica Microsystems, Rueil-Malmaison, France).

Statistical analysis of data

Differential mRNA expression levels between SWS, FWS and FWU fish were analyzed by Student's t-tests and Kruskal-Wallis non parametric test. Statistical comparisons of the mean expression values ($p < 0.05$) were determined for each tissue, from 6 individual fish.

3. Results

Juvenile sea-bass successfully and unsuccessfully adapted to fresh water

Juvenile sea-bass successfully (FWS) and unsuccessfully (FWU) adapted to fresh water were recognized according to previously observed criteria (Nebel et al., 2005b). These include erratic and slow swimming behavior and decreased reactions to external movements in comparison to the FWS fish, leading to the isolation of the FWU fish from the shoal of FWS fish. We additionally observed that when FWU fish were not swimming any more, they were on the bottom with the trunk slightly curved and that they exhibited an abnormal movement of the mouth and opercula, showing deep red-colored gills. FWU fish died within 48 h of the occurrence of this specific behavior. FWU fish were sampled alive as soon as the first signs of abnormal behavior were detected. Each time FWU fish were sampled, fish displaying standard behavior in FW (FWS fish) and fish kept in SW (SWS fish) were also sampled. At the time of sampling, the fish were about 130 days post-hatch (D130). Three weeks after the FW challenge, FWU fish started to occur and die, and high mortalities occurred during the two following weeks, leading to a total mortality of 28% at the end of the experiment. A low mortality (3%) was recorded in the SW fish tank and the FWS fish survived over a period of 24 months with an annual mortality of 7%.

Reconditioning in sea water of unsuccessfully-adapted sea bass to fresh water

Twenty fish identified as FWU fish were captured. Ten of these FWU fish were kept in FW and 10 were reconditioned to SW in individual cages. All 10 FWU fish kept in FW died within 48 h. They did not eat and no faeces were observed. One of the 10 FWU fish reconditioned to SW died at 48 h, while the other nine were alive 10 days after the transfer. They displayed an active swimming, and a notable recovery had been detected 24 h after the transfer to 10 ppt. They started to eat when salinity was at 15 ppt (48 h after the transfer).

Sea-bass characteristics at different salinities

SWS and FWS fish length (mean 5.4 ± 0.15 cm) and wet weight (mean 1.75 ± 0.15 g) were not significantly different, while FWU fish were 20% smaller and 77% lighter than SWS and FWS fish (Fig. 1A,

B). Muscle water content was significantly lower in SWS fish (71 ± 1.5%) than in FWS (74 ± 1.7%) and FWU fish (75 ± 1.7%) (by 4 and 5% respectively), while values in FWS and FWU fish were not significantly different (Fig. 1C).

Transcript levels of AQP1, AQP3, α1 and α4 Na^+/K^+-ATPase during successful and unsuccessful adaptation of sea-bass to fresh water.

Quantitative PCR expression patterns were performed for 4 genes (AQP1, AQP3, α1 and α4 Na^+/K^+-ATPase) in the main osmoregulatory organs of SWS, FWS and FWU fish. Transcript levels of the housekeeping gene EF α1 did not change for each organ in SWS, FWS and FWU fish (results not shown).

In the digestive tract (Fig. 2A), the number of AQP1 transcripts was highest in SWS fish. It was lower (by 4.7-fold) in FWS fish. In FWU fish, it was significantly higher (by 1.9-fold) than in FWS fish. In the kidney (Fig. 2B), the number of AQP1 transcripts was also highest in SWS fish. It was much lower (by 6.5-fold) in FWS fish. In FWU fish, no significant difference was found compared to FWS fish. In the gill (Fig. 2C), the level of AQP1 transcripts was low in all three fish categories, without any significant difference between them.

The abundance of AQP3 transcripts was low in the digestive tract and kidney in all salinity-acclimated groups (Fig. 3A, B) without significant inter-group difference. In the gill, the abundance of AQP3 transcripts increased significantly (by 3.7-fold) in FWS fish (Fig. 3C) comparatively to SWS fish. In FWU fish, the abundance of AQP3 transcripts was 2.4-fold higher than in FWS fish (Fig. 3C).

Concerning the numbers of α1 Na^+/K^+-ATPase transcripts, they were low in the digestive tract and no inter-group significant difference was observed (Fig. 4A). The highest level of expression for α1 Na^+/K^+-ATPase was detected in the kidney, and although an apparent increase was observed in FWU fish kidney, the difference was not significant in respect to SWS and FWS fish (Fig. 4B). In gills, α1 Na^+/K^+-ATPase transcript abundance was 2.9-fold higher in FWS than in SWS fish without significant difference between FWS and FWU fish (Fig. 4C).

The numbers of α4 Na^+/K^+-ATPase transcripts were low in the digestive tract and without inter-group significant difference (Fig. 5A). The highest level of expression for α4 Na^+/K^+-ATPase was observed in the kidney, and the slightly higher value in FWU fish was not significantly different (Fig. 5B). In gills, α4 Na^+/K^+-ATPase transcripts was 2.8-fold more abundant in FWS than in SWS fish without significant difference between FWS and FWU fish (Fig. 5C).

Histomorphology

In FWS and FWU fish, FW exposure was not followed by any observable change in the morphology of the anterior intestine, posterior intestine and rectum (not illustrated). In the kidney sections, the urinary tubules and collecting ducts of FWU fish (Fig. 6C) were larger and less abundant than in SWS and FWS fish kidney (Fig. 6A, B). In the posterior sections of the three FWU fish, renal bodies were observed in some urinary tubules, in the collecting ducts (Fig. 6C, D) and in the urinary bladder (Fig. 6E). Similar structures were also detected in one of three FWS fish, and they were absent in the three observed SWS fish (Fig. 6A).

4. Discussion

The behavior of FWU fish recorded during our experiments was in agreement with previous observations (Nebel et al., 2005b). FWU fish occurrence has now been observed over 3 consecutive years and is not dependent on their time of hatching. In this study, fish hatched in December were three month-old when exposed to FW, and mortality occurred in April at D130 (~5.4 cm, ~1.75 g). In earlier studies, fish hatched in November and January were at D100 and D140 when differential mortalities started, by March and June (Nebel et al., 2005b). Mortality rates of 28% reported in this work are similar to those of 26%

reported previously (Nebel et al., 2005b) for FWU juvenile sea-bass. FWU fish were smaller and lighter than FWS and SWS fish, probably due to a reduced feeding as observed in isolated animals. This lower size and growth in FWU fish may correspond to an energy reallocation to osmoregulation. The histology of kidney structure revealed tissue differences and the presence of renal bodies in the collecting ducts and urinary bladder of FW fish. FWU fish recovered when they were transferred into saline water. An important distinction must be done between FWU fish described in this work and the fish affected by the swim bladder stress syndrome (SBSS) (Jonhson and Katavic 1984). The SBSS symptoms that occur around the transition from yolksac depletion to exogenous feeding (5.5 – 6.5 mm) in 3% of the total population, include distended swim bladder, abundance of mucus, opaque and edematous tissues, spinal abnomalies and calculi in the urinary bladder (Jonhson and Katavic 1984; Peruzzi et al., 2007).

Compared to FWS fish, FWU fish present higher levels of AQP1 transcripts in the digestive tract and of AQP3 transcript in the gills. These findings suggest an increased water permeability in the digestive tract and an increase in gill permeability (Isaia 1984; Cutler and Cramb 2002; Cutler et al., 2006; Tse et al., 2006; Giffard-Mena et al., 2007b; Hill et al., 2007). A higher water permeability in the digestive tract of FWU fish may contribute to an increased water uptake in addition to gill and tegumentary osmotic water uptake to which all FW fish are exposed. The water absorption through the digestive tract would contribute to the low blood osmolarity found in FWU fish (Nebel et al., 2005b). However no significant difference was found in the muscle water content between FWS and FWU fish, despite the decrease in plasma osmolarity reported in FWU fish and attributed in part to a decrease in renal ion reabsorption (Nebel et al., 2005b). Similar results have been reported in the sea-bass and other euryhaline fish (Dendrinos and Thorpe 1985; Jensen et al., 1998; Nielsen et al., 1999) and could be explained through elimination of water by urine (Marshall and Grosell 2005).

The transcription pattern of aquaporins described in the present study, together with mRNA variations described previously in sea-bass preadults (Giffard-Mena et al., 2007b), suggest that the body water regulation in FWU fish is effected by eliminating the water excess in large urine volumes (Marshall and Grosell 2005). However, the inability of the urinary system of FWU fish for net ion reabsorbtion leads to the production of urine which is isotonic to the blood compared to the hypotonic urine to blood in FWS. The resulting low osmolarity of the blood, which is not compensated despite the higher number of actively pumping gill ionocytes, is most probably the cause of the death of the FWU fish (Nebel et al., 2005b). High levels of AQP3 have been also related to high excretion rates of urea and/or ammonia across the gills (Cutler and Cramb 2002; Lignot et al., 2002a; 2002b), and they may facilitate water flux to prevent swelling (Cutler et al., 2006; Giffard-Mena et al., 2007b).

If water permeability increases in urinary tubules of FWU fish as suggested earlier (Nebel et al., 2005b), this increase is probably not related to AQP1 or AQP3 transcript levels since no difference has been found between FWU and FWS fish. The high level of AQP1 in the kidney of SWS fish is related to water re-absorption in these fish submitted to dehydration in SW (Nebel et al., 2005a; Giffard-Mena et al., 2007b). Indeed, teleosts in marine environment are constantly loosing water to the external hyper-osmotic sea water across permeable body surfaces such as the gills. Therefore they absorb water across the intestinal epithelium and reabsorb water across urinary tubules (Giffard-Mena et al., 2006) following the osmotic gradient created by the Na^+/K^+-ATPase (Marshall and Grosell 2005). In mammals, another aquaporin, AQP2, is involved in kidney water balance (Bichet 1997; Nielsen et al., 2007). The loss of expression of AQP2, particularly in the nephrogenic diabetes insipidus, is characterized by an inability to concentrate urine (Nielsen et al., 2002; Nejsum 2005). To date, fish AQP2 homologues have been described in the zebra-fish only (Strausberg et al., 2002; Vihtelic et al., 2005), but the possibility of its existence in other marine teleosts, including sea-bass, and its possible involvement in FWU fish mortality cannot be excluded. In addition, a lack of oxygen in human blood is known to cause an ischemic cascade that can trigger kidney damage (Perry 1998b; Nielsen et al., 2002). It is thus possible that the abundance of chloride cells on the gills of FWU fish reduces the surface available to gas exchanges and negatively affects the oxygen availability to the blood (Nebel et al., 2005b) causing injury to the kidney by ischemia (Nielsen et al., 2002).

In this study, before and after the exposure of the sea-bass to FW, the lowest expression of both α1 and α4 Na^+/K^+-ATPases was found in the digestive tract, suggesting that none of these isoforms participate in

salinity acclimatation at the gut level. Differences between the fish groups occurred in the gills, where both isoforms increased significantly after transfer to FW, suggesting ion uptake. These results correlate with high levels of Na^+/K^+-ATPase activity (Nebel et al., 2005b) at low salinity and mRNA levels of α4 Na^+/K^+-ATPase (Boutet et al., 2006) in lagoon-caught sea-bass. The higher abundance of the two isoforms in the kidney compared to the gills and digestive tract suggests their participation in ion reabsorption, but with no apparent difference between all fish groups. Several α- and β-subunit isoforms of Na^+/K^+-ATPase (α_1 to α_7, β_1, β_3, β_{185}, β_{233}) are known in teleost fish (Cutler et al., 1996; 1997; 2000; Cutler and Cramb 2001; Richards et al., 2003). Exposure to high salinities increases the mRNA levels of α_1, α_3, β_1, β_{233} in gills, kidney and intestine in some fish species (Cutler et al., 1995a; b; 2000; Shin-Huey et al., 2002; Hirose et al., 2003; Scott et al., 2004; Tse et al., 2006). More recently, some α-isoforms (α1a and α1b) have been identified; the α1b and the α1a isoforms would be respectively involved in ion secretion (in SW) and ion uptake (in FW) (Richards et al., 2003; Bystriansky et al., 2007). The isoforms studied in this work (α1 and α4) seem to be involved in ion uptake in FW.

In summary, the results from the present investigation and from previous research can be put together to explain the inability of about a quarter of a sea-bass population to adapt to FW at the juvenile stage. The digestive water gain in FWU fish, suggested by comparatively higher levels of AQP1 in the digestive tract (this study), must be evacuated to prevent whole body water accumulation. Since the density of urinary tubules and the renal activity of Na^+/K^+-ATPase are low in FWU fish, there is a net ion loss in the urine (Nebel et al., 2005b). All together, the higher water absorption (through the digestive tract) and the lower net ion reabsorption (through the kidney) contribute to a decrease in the blood osmolarity (Nebel et al., 2005b), ultimately causing death of the FWU fish. Ion uptake through more numerous gill ionocytes does not compensate this hydro-mineral inbalance (Nebel et al., 2005b). The low prolactine levels detected in the gill and intestine (Boutet et al., 2007) of FWU fish and the increase of their gill membrane permeability (suggested by higher AQP3 levels; this study) can even more compromise the maintenance of the blood osmolarity.

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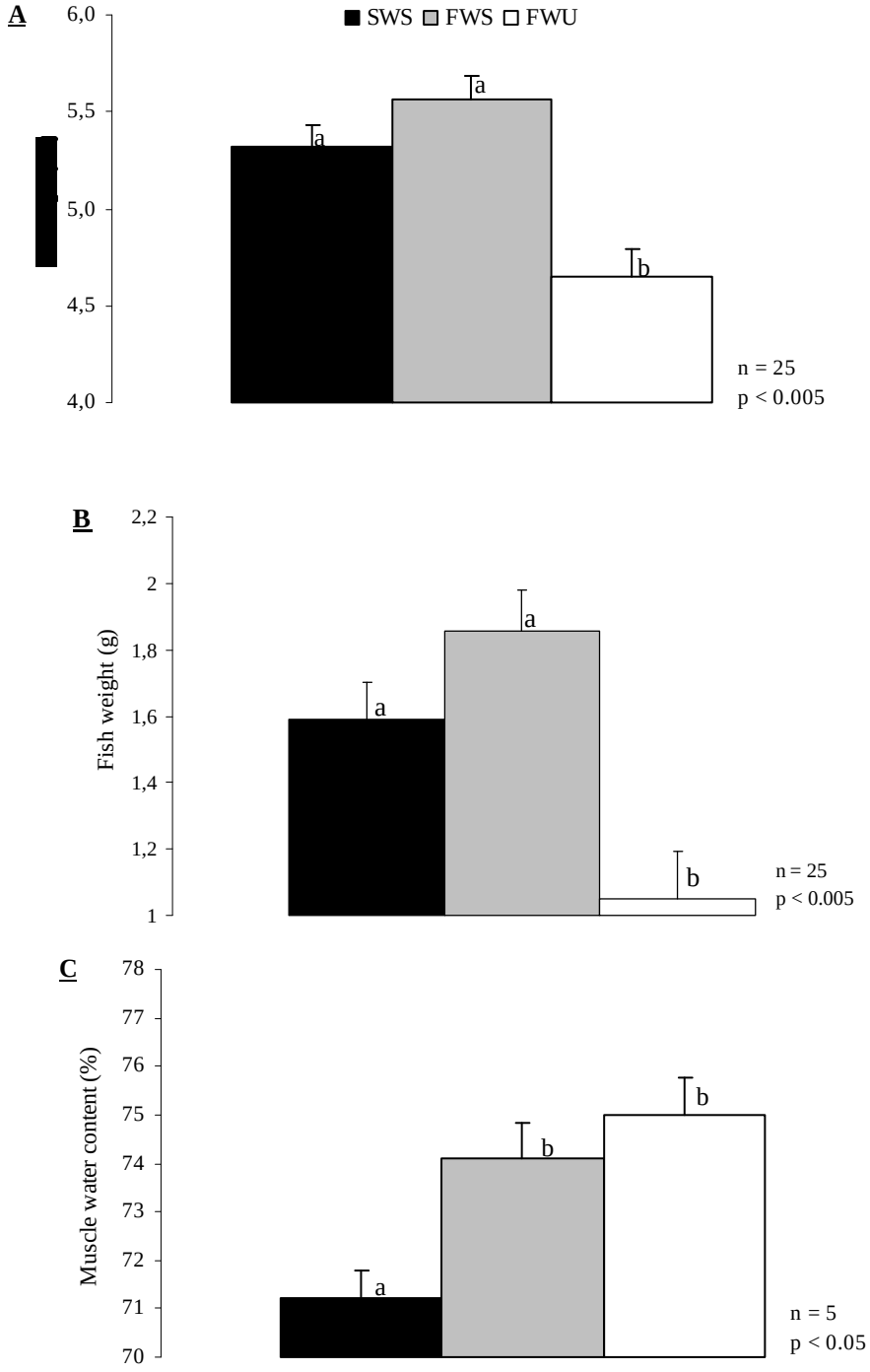
Tables

Table I. Primer sequences used for the amplification of *Dicentrarchus labrax* AQP1 and ATPase Na^+/K^+ fragments for quantitative PCR. Primers were designed based on sea-bass AQP1 (NCBI **DQ924529**), AQP3 (NCBI **DQ647191**), α1 Na^+/K^+-ATPase (FishShellfish **C-CL1885**), α4 Na^+/K^+-ATPase (NCBI **CX660460**). Nucleotides are identified by a single letter code (A=adenine, T=thymine, C=cytosine, G=guanine, W= A or T).

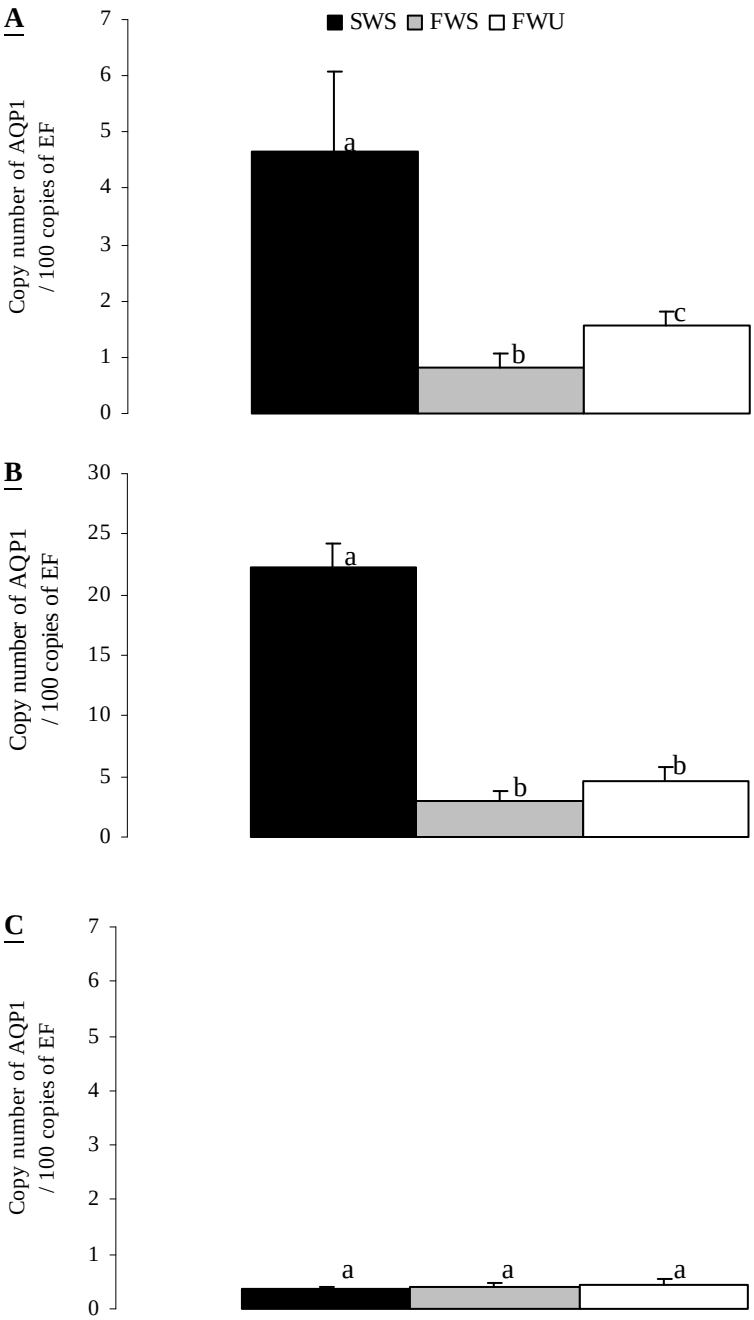
Specific primers	Nucleotide sequence 5' – 3'	Position
AQP1F	(Giffard-Mena et al., 2007a)	298-317
AQP1R	(Giffard-Mena et al., 2007a)	388-405
AQP3F	CTCTTTCAGACAATCGGTGC	383-402
AQP3R	CTGTGGCATTAGGTCCAGTCA	474-494
ATPase Na^+/K^+ α1 F	CTGGAGTGGAAGAAGGTC	94-111
ATPase Na^+/K^+ α1 R	GATGAAGAGGAGGAAGG	181-197
ATPase Na^+/K^+ α4 F	(Boutet et al., 2006)	48-73
ATPase Na^+/K^+ α4 R	CTGGGCTGTCTCTTCATGATGTC	432-454

Figures



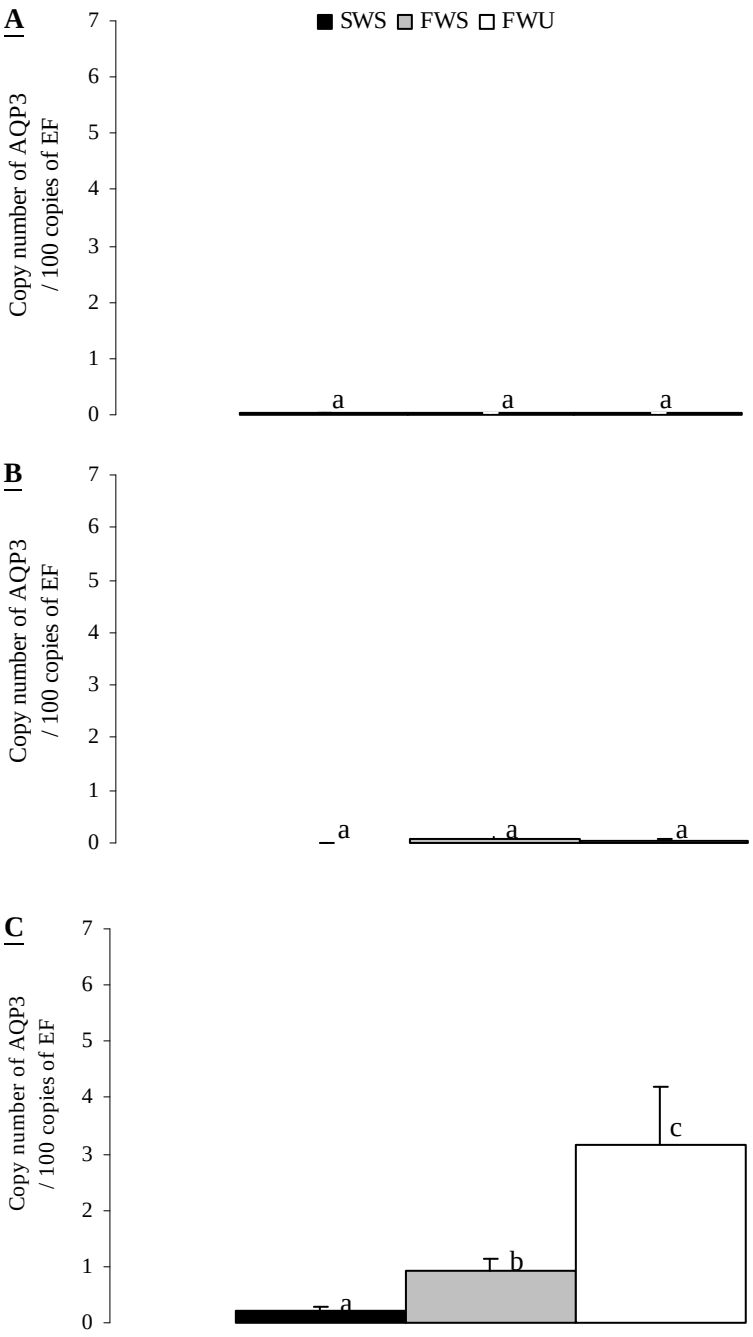
(Fig. 1)

Figure 1. *Dicentrarchus labrax* juveniles exposed to SW and FW. Length (A), weight (B) and muscle water content (C). FW, fresh water; FWS, fresh water successfully adapted fish; FWU, fresh water unsuccessfully adapted fish; SW, sea water; SWS, sea water-adapted fish. Different letters indicate significant differences ($p < 0.05$).



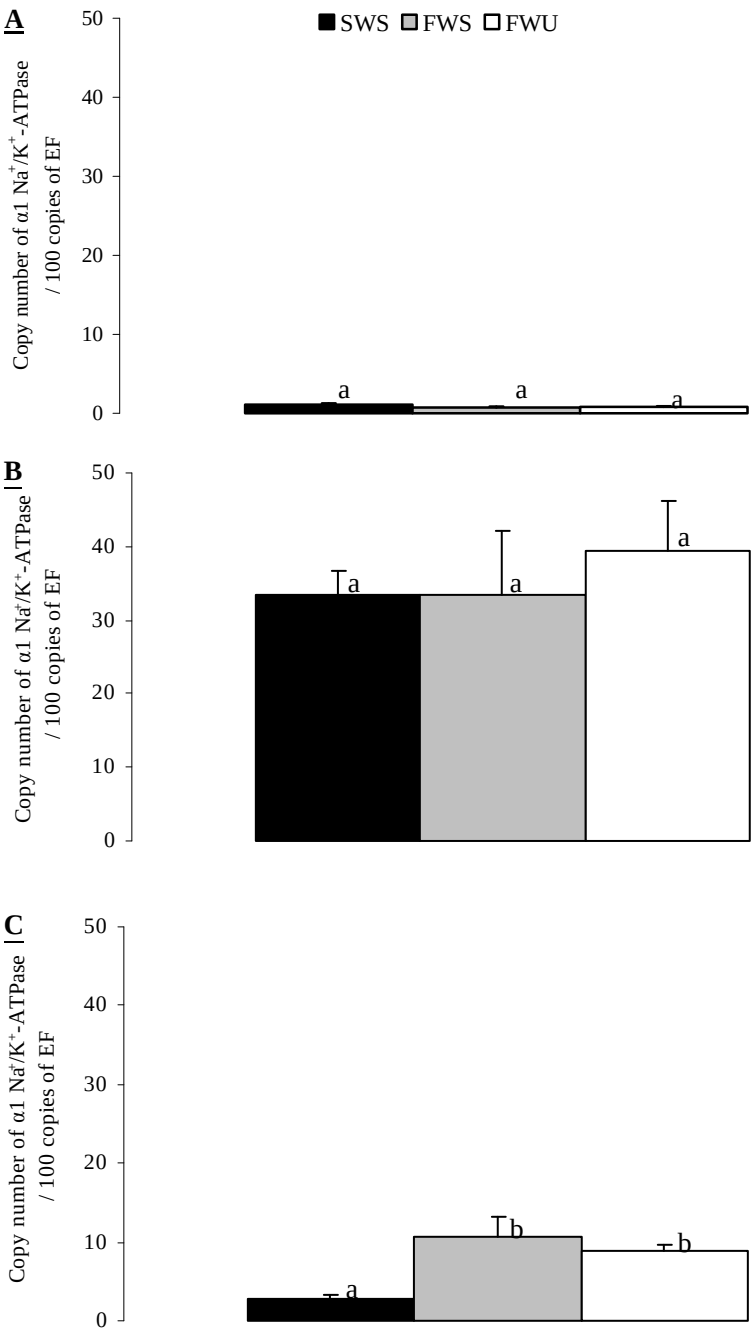
(Fig.2)

Figure 2. AQP1 transcript abundance in sea-bass juveniles exposed to sea water and fresh water in the digestive tract (A), kidney (B) and gill (C). FW, fresh water; FWS, fresh water successfully adapted fish; FWU, fresh water unsuccessfully adapted fish; SW, sea water; SWS, sea water-adapted fish. Different letters indicate significant differences ($p < 0.05$, with $n = 6$).



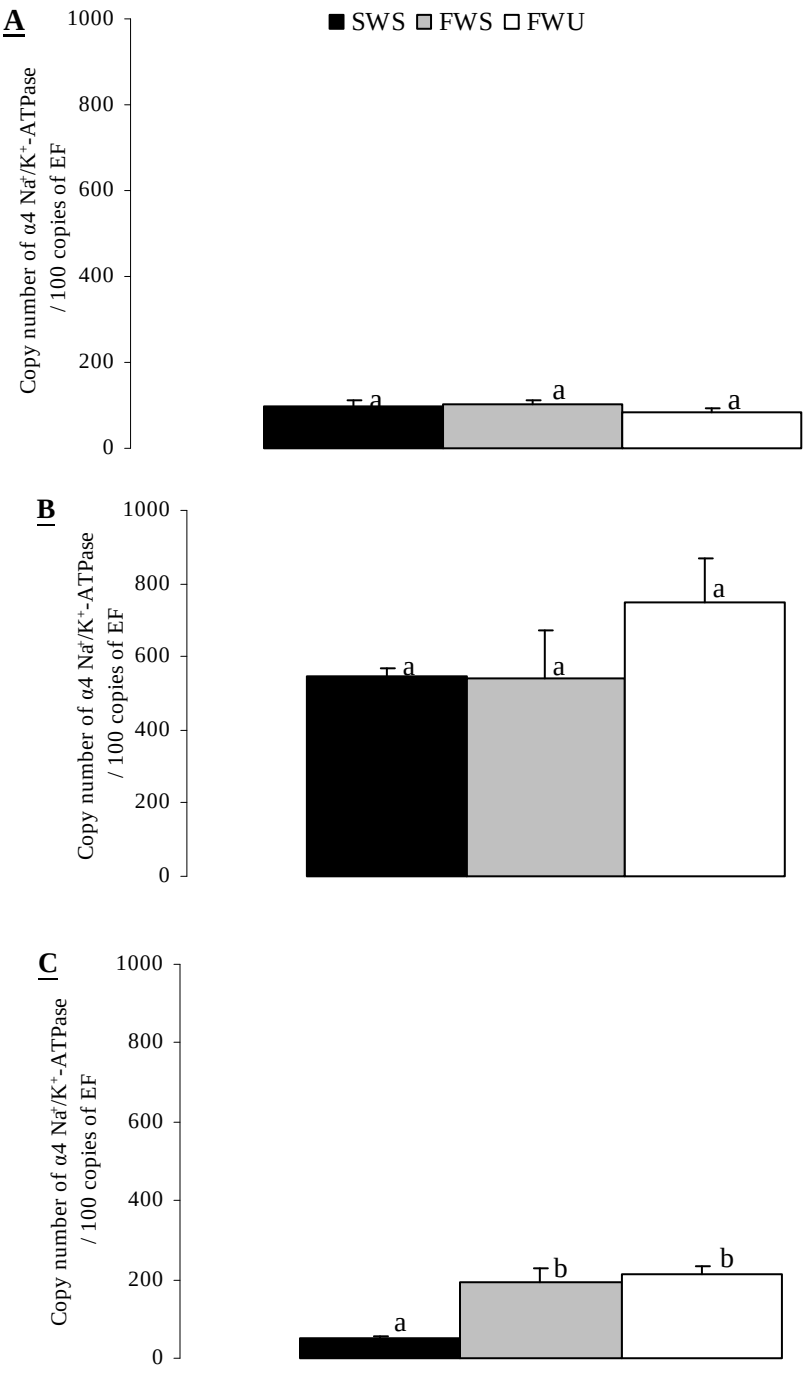
(Fig. 3)

Figure 3. AQP3 transcript abundance in sea-bass juveniles exposed to SW and FW, in the digestive tract (A), kidney (B) and gill (C). FW, fresh water; FWS, fresh water successfully adapted fish; FWU, fresh water unsuccessfully adapted fish; SW, sea water; SWS, sea water-adapted fish. Different letters indicate significant differences ($p < 0.05$, with $n = 6$).



(Fig. 4)

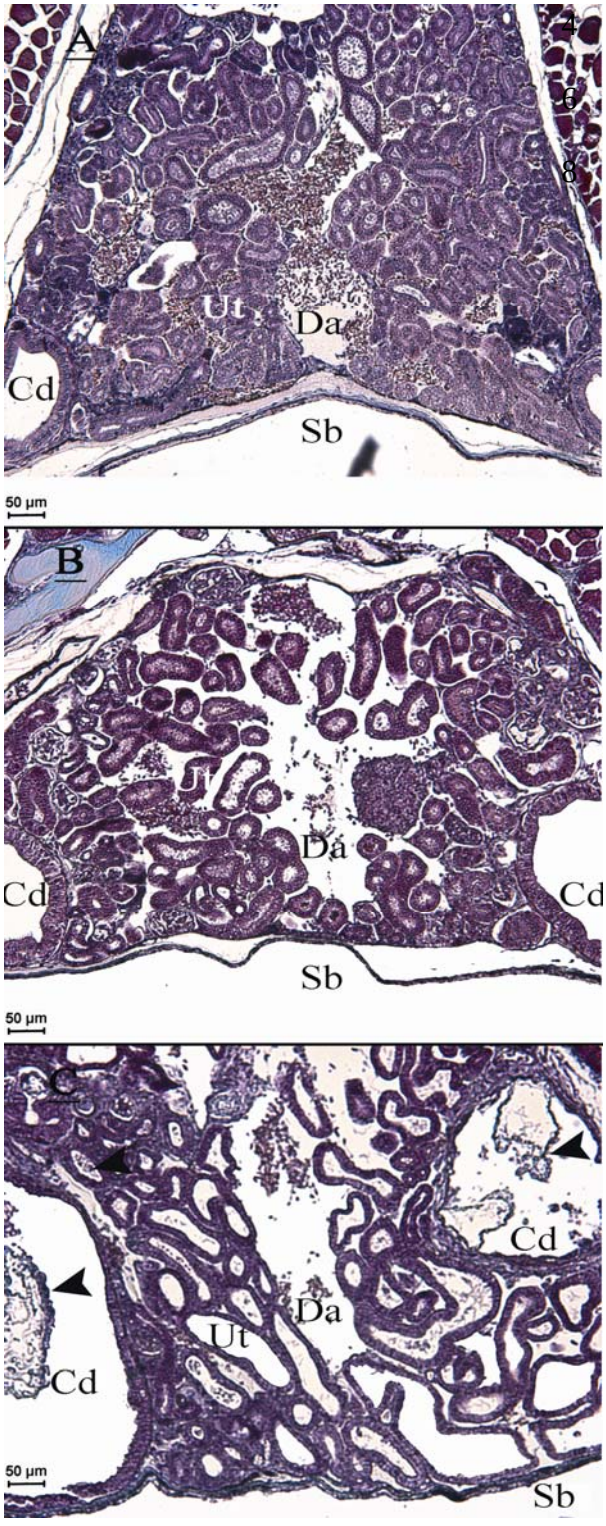
Figure 4. α1 Na^+/K^+-ATPase abundance in sea-bass juveniles exposed to SW and FW, in the digestive tract (A), kidney (B) and gill (C). FW, fresh water; FWS, fresh water successfully adapted fish; FWU, fresh water unsuccessfully adapted fish; SW, sea water; SWS, sea water-adapted fish. Different letters indicate significant differences ($p < 0.05$, with $n = 6$).



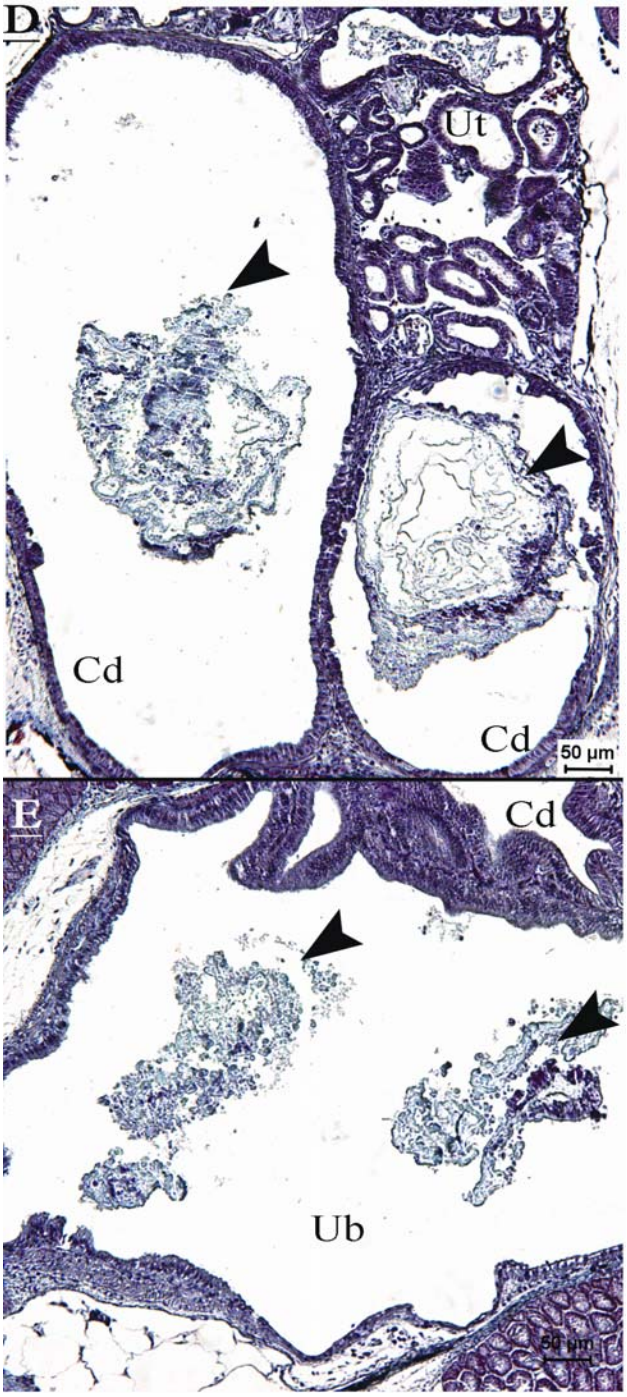
(Fig. 5)

Figure 5. α4 Na^+/K^+-ATPase abundance in sea-bass juveniles exposed to SW and FW in the digestive tract (A), kidney (B) and gill (C). FW, fresh water; FWS, fresh water successfully adapted fish; FWU, fresh water unsuccessfully adapted fish; SW, sea water; SWS, sea water-adapted fish. Different letters indicate significant differences ($p < 0.05$, with $n = 6$).

2



(Fig. 6)



(Fig. 6)

Figure 6. Kidney sections of SWS (A), FWS (B) and FWU (C, D, E) sea-bass juveniles stained with Masson's trichrome. (D) Collecting ducts and (E) urinary bladder. The presence of renal bodies is indicated by arrows. FW, fresh water; FWS, fresh water successfully adapted fish; FWU, fresh water unsuccessfully adapted fish; SW, sea water; SWS, sea water-adapted fish. Cd, collecting duct; Da, dorsal aorta; Sb, swim bladder; Ub, urinary bladder; Ut, urinary tubule.