



Effect of salinity and temperature on the expression of genes involved in branchial ion transport processes in European sea bass

Waliullah Masroor, Emilie Farcy, Eva Blondeau-Bidet, Alexander Venn, Eric Tambutte, Catherine Lorin-Nebel

► To cite this version:

Waliullah Masroor, Emilie Farcy, Eva Blondeau-Bidet, Alexander Venn, Eric Tambutte, et al.. Effect of salinity and temperature on the expression of genes involved in branchial ion transport processes in European sea bass. *Journal of Thermal Biology*, 2019, 85, pp.UNSP 102422. 10.1016/j.jtherbio.2019.102422 . hal-02439695

HAL Id: hal-02439695

<https://hal.science/hal-02439695>

Submitted on 20 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Effect of salinity and temperature on the expression of genes involved in branchial ion transport processes in European sea bass

Waliullah Masroor¹, Emilie Farcy¹, Eva Blondeau-Bidet¹, Alexander Venn², Eric Tambutté² & Catherine Lorin-Nebel^{1*}

¹Univ Montpellier, CNRS, IFREMER, IRD, UM, MARBEC, France

²Centre Scientifique de Monaco, Marine Biology Department, 8 Quai Antoine 1^{er}, Monaco 98000

*Corresponding author's address: Univ Montpellier, CNRS, IFREMER, IRD, UM, MARBEC, France

Place E. Bataillon, 34095 Montpellier cedex 05, France, Tel: +33 4 67 14 93 91
catherine.lorin@umontpellier.fr

Running title: Effect of temperature on sea bass gill ion transport

Declarations of interests: none

Abstract

The responses of European sea bass to temperature increase and salinity decrease were investigated measuring mRNA expression levels of main genes involved in ion transport. Juvenile fish were pre-acclimated to seawater (SW) at 18 °C (temperate) or 24 °C (warm) for two weeks and then transferred for two weeks to either fresh water (FW) or SW at the respective temperature. Unlike temperate conditions, there is no change in Na⁺/K⁺-ATPase α 1a (*nka α 1a*) and Na⁺/H⁺ exchanger 3 (*nhe3*) mRNA expression following FW transfer in warm conditions. This is linked to the high expression of these genes in warm SW compared to temperate SW. Na⁺/Cl⁻-cotransporter (*ncc2a*) expression however is increased following FW transfer in temperate and warm conditions. Main transporters involved in ion excretion (Na⁺/K⁺/2Cl⁻-1 cotransporter, *nkcc1* and cystic fibrosis transmembrane conductance regulator, *cfr*) as well as nitrogen excretion (Rh-glycoproteins, *rhcg1* and *rhbg*) and acid-base regulation (V-H⁺-ATPase, *vha-a* and *b*) are highly expressed in SW warm conditions vs FW warm. Overall, our results suggest a higher activation of ion transport processes in warm conditions and more strikingly in SW. This is linked to a strong interplay between diverse ion transporters in order to coordinate physiological responses at the gill level.

Keywords: *Dicentrarchus labrax*, ion transporter, gill, mRNA expression, thermal acclimation, salinity

1. Introduction

Temperature is considered a main factor affecting organism distribution, life-history traits and biological processes (Crockett and Londrville, 2006; Hutchison and Maness, 1979; Schulte, 2011). The effects of temperature on ion-regulatory mechanisms and other physiological processes have been shown in several fish species (Burton, 1986; Chou et al., 2008; Gibbons et al., 2018; Kreiss et al., 2015; Metz et al., 2003; Michael et al., 2016a; Morrison et al., 2006; Vargas-Chacoff et al., 2018) including the European sea bass *Dicentrarchus labrax* (Claireaux and Lagardère, 1999; Masroor et al., 2018). Ion homeostasis is mainly relying on ion pumps such as the branchial Na^+/K^+ -ATPase (NKA) and V- H^+ -type ATPase (VHA), both creating an electrochemical gradient for active ion transport across gill basolateral and apical membranes (Evans, 2008; Evans et al., 2005, 1999; Hwang and Lin, 2014; Vasić et al., 2008). It has been reported that active ion transporters represent a higher thermal sensitivity than carrier-mediated diffusive transporters (Moyes and Ballantyne, 2011) which could lead to imbalances between active and passive ion transport. Ion transport alteration at the gills due to high temperatures can also occur due to changes in membrane integrity and fluidity that could affect the proper insertion and function of ion transporters (Moyes and Ballantyne, 2011). As Na^+ and Cl^- are thought to be taken up by different uptake pathways, respectively in exchange for H^+ and HCO_3^- , imbalance in Na^+/Cl^- ratio likely affect acid-base status (Goss et al., 1998; Jensen et al., 1998). The fish gill is a plastic organ involved in gas exchange, ion regulation, acid-base balance and nitrogen excretion and significantly contributes to physiological homeostasis in changing environments (Evans et al., 2005). In response to temperature and/or salinity changes, fish gills are subject to significant morphological remodeling (Gibbons et al., 2018; Metz et al., 2003; Tzaneva and Perry, 2010) and changes in ionocyte density and distribution (Metz et al., 2003;

63 Mitrovic and Perry, 2009). We have previously shown that gill morphological parameters
64 differed in sea bass that have been pre-acclimated at two different temperatures, 24 °C and 18 °C
65 in seawater (SW) and fresh water (FW) with particularly a lower ionocyte density in warm FW
66 (Masroor et al., 2018). Plasma Na⁺ levels were decreased in warm (24 °C) compared to
67 temperate conditions (18 °C) at both salinities. On the contrary, plasma Cl⁻ levels were higher in
68 FW warm compared to temperate conditions and no effect of temperature was reported in SW.
69 These results may point out an effect of increased temperatures on enhanced FW Cl⁻ uptake and
70 decreased Na⁺ uptake (or enhanced Na⁺ loss) at the gill (Hwang and Lee, 2007) and/or kidney
71 levels (Hickman and Trump, 1969). Membrane transporters and channels, including Na⁺/H⁺
72 exchangers (NHE2/3), VHA, ammonia transporters (Rhesus (Rh) glycoproteins, mainly RHBG
73 and RHCG1), NKA and Na⁺/Cl⁻ cotransporters (NCC2) contribute directly or indirectly to ion
74 homeostasis (Hwang and Lin, 2014). Basolateral Na⁺/K⁺/2Cl⁻-1 (NKCC1) and apical cystic
75 fibrosis transmembrane conductance regulator (CFTR) are involved in ion excretion in SW
76 (Hwang and Lin, 2014). The mechanisms for ion uptake in FW have been reviewed in several
77 reports and involve a multitude of transporters working together to take up Na⁺ and/or Cl⁻ ions in
78 exchange of different counterions, mainly H⁺ and HCO₃⁻ (Dymowska et al., 2012; Hsu et al.,
79 2014; Hwang, 2009). Recently, Blondeau-Bidet et al. (2019) have shown that in sea bass *D.*
80 *labrax* maintained in FW for two weeks, gill NHE3 and NCC2 have been detected in different
81 ionocyte subtypes at the apical cell part (NHE3- and NCC2-type cell), coupled to basolateral
82 NKA in order to absorb Na⁺ or Na⁺, Cl⁻ (Hwang et al., 2011; Kumai and Perry, 2012; Tang and
83 Lee, 2011). CIC-3 is a volume-activated chloride channel involved in transepithelial Cl⁻ transport
84 (Bossus et al., 2013; Tang and Lee, 2011) and cell volume regulation (Duan et al., 1999, 1997;
85 Hermoso et al., 2002; Wang et al., 2000). Changes in expression of these and other transporters

86 have been reported in numerous species following salinity transfers, but the effect of increased or
87 decreased temperature on the expression of these genes in different salinity conditions is less
88 known (Chou et al., 2008; Gibbons et al., 2018; Hu et al., 2016; Kyprianou et al., 2010; Logan
89 and Buckley, 2015; Logan and Somero, 2011; Michael et al., 2016a; Morrison et al., 2006).
90 Several paralogs exist in fish for *nka α1* with potentially different functions and expression
91 patterns depending on the considered species (Hwang and Lee, 2007). Two paralogs have been
92 identified in sea bass, *nka α1a* and *nka α1b*, with *nka α1a* being the most expressed paralog in
93 sea bass gills whatever the considered salinity (Blondeau-Bidet et al., 2016). Temperature
94 generally increases NKA transcript expression and protein activity but the response highly
95 depends on the species and even the population (Michael et al., 2016a; Morrison et al., 2006).
96 Michael et al. (2016a) have reported population-specific differences regarding mRNA expression
97 levels of *nka* in cod *Gadus morhua* populations maintained at the same temperature but
98 originating from a different thermal niche. The European sea bass *Dicentrarchus labrax*
99 (Linnaeus 1758) is an important aquaculture species along the Mediterranean and Atlantic
100 coasts. It is considered as a highly eurythermal (tolerates temperatures from 4 to 35 °C) and
101 euryhaline species (tolerating from FW up to 70 ppt) (Barnabé, 1989; Dülger et al., 2012;
102 Madeira et al., 2013). In the wild, sea bass adults and juveniles are frequently exposed to
103 fluctuations of environmental parameters, notably during their stay in lagoons and estuaries
104 (Dufour et al., 2009; Newton and Mudge, 2003). In these habitats, temperatures can reach values
105 over 25 °C in the summer months. The Mediterranean basin is considered as one of the ‘Hot-
106 spots’ by Giorgi (2006) and in the context of climate change, temperatures will continue to rise
107 in the future. Temperature acclimation and preference have been studied in sea bass by
108 investigating oxygen consumption (Dalla Via et al., 1998), fish distribution (Trancart et al.,

2016), food intake (Dülger et al., 2012; Person-Le Ruyet et al., 2004) and swimming speed (Claireaux et al., 2006). *D. labrax* optimal growth rate was reported at 25 °C (Person-Le Ruyet et al., 2004) which is close to the warm temperature analyzed in our study.

In this study, we analyzed branchial ion regulatory mechanisms at the transcript level with a particular focus on selected genes involved in osmoregulation, acid-base regulation and ammonia excretion. We compared mRNA levels of *nka α1a* (*atp1a1a*), *nka α1b* (*atp1a1b*), *cfr*, *nkcc1* (*slc12a2*), *nhe3* (*slc9a3*), *ncc2a* (*slc12a3-like*), *clcn3* (*clc-3*), *vha-a* (*atp6v1a*) and *vha-b* (*atp6v1b2*), *rhbg* and *rhcg1* in gills from fish acclimated at two different temperatures (18 °C and 24 °C) and transferred from SW to FW.

2. Material and Methods

2.1. Experimental conditions

Experimental conditions have been previously described in Masroor et al. (2018). Briefly, juvenile sea bass *D. labrax* originating from a Western Mediterranean population were obtained from the Ifremer Station at Palavas-les-Flots (Hérault, France). Fish were brought to the Montpellier University at the age of 14 months and maintained for one week in 3,500 L tanks containing natural seawater (SW) from the Mediterranean Sea at 38 ppt and 18 °C, under a 12 h light/12 h dark photoperiod. Fish were transferred to 200 L tanks (14 fish/tank, density of 6-7 kg/m³, two replicates per conditions) to be acclimated either at 18 °C or 24 °C (with a temperature increase of 0.2 °C/h). After two weeks of temperature acclimation, fish were transferred directly either to dechlorinated tap water (fresh water, FW), or to SW (7 fish/tank, two replicates for each conditions) and maintained in this salinity two weeks before sampling.

Ionic composition (in mEq.L⁻¹) of the FW was Na⁺ (0.12), K⁺ (0.04), Ca²⁺ (5.70), Mg²⁺ (0.29), Cl⁻ (0.98), NO₃⁻ (0.06) SO₄²⁻ (0.61). Water was aerated and mechanically/biologically filtered (Eheim System, Germany). Temperature, salinity, oxygen and nitrogen levels were checked daily. A quarter of the water volume was changed every two days. Fish were fed *ad libitum* twice a week with fish granules (Aphymar feed, Meze, Herault, France) until 2 days before sampling. At the end of the experiment, fish were anesthetized in a solution of phenoxy-2-ethanol (240 ppm) prior to tissue collection. The fish used for the experiment had a length of 20.77±1.32 cm (mean ± SD) and average weight was 86.87±20.23 g. Four groups were compared: SW at 18 °C (temperate SW), SW at 24 °C (warm SW), FW at 18 °C (temperate FW) and FW at 24 °C (warm FW). These experiments respected the guidelines of the European Union (directive 86/609) and of the French law (decree 87/848) regulating animal experimentation.

2.2. Blood parameters, protein and cellular measurements

Following anesthesia, blood was sampled using a 1-ml syringe coated with heparin (Li-heparin, Sigma-Aldrich, France). Plasma was obtained following centrifugation of 8 min at 10,000g at 4 °C and frozen at -20°C until analysis. For pH measurements, plasma was thawed on ice then measured at 20 °C using a minimum of 15 µl of plasma with an InLab Ultra-Micro ISM pH probe coupled to a SevenMulti pH meter (Mettler Toledo, Ohio, USA) calibrated with National Bureau of Standards (NBS) buffers. It must be noted that samples were frozen and thawed twice before performing plasma pH measurements. Analysis of plasma Na⁺ and Cl⁻, gill Na⁺/K⁺-ATPase activities and gill ionocyte densities (in filaments and lamellae) have been performed on the same fishes and methods are described in Masroor et al. (2018).

2.3. RNA extraction and reverse transcription

Gill tissues were collected from gills of SW- and FW-exposed sea bass in temperate and warm conditions. The epithelium of the first gill arch was scraped with a sterile scalpel, immersed in Trizol[®] reagent and flash frozen in liquid nitrogen. Tissues were then stored at -80 °C until analysis. Total RNA was extracted using Trizol[®] reagent according to the manufacturer's instructions. RNA quantity and purity were assessed by measuring the A260/A280 ratio using the NanoDrop[®] ND-1000 V3300 spectrometer (Nanodrop Technology Inc., Wilmington, Delaware, USA). RNA quality was checked using Agilent bioanalyzer (Agilent) using electrophoretic trace method. One microgram of the total RNA was treated with DNase I amplification grade (Invitrogen[™], Life Technologies). Reverse transcription was performed using 200 U M-MLV reverse transcriptase (Invitrogen[™]) and first strand of complementary DNA (cDNA) was generated using 12.5 ng/μl of random primers (Invitrogen[™]), dNTPs (10 mM) and 40 U of RNase OUT (Invitrogen[™]), following manufacturer's instruction.

2.4. Quantification at the transcript level

Specific primers (forward and reverse) for different transporters are listed in Table 1. Quantitative real-time PCR analyses (qRT-PCR) were performed using the LightCycler[®] 480 Real-Time PCR System (Roche, Mannheim, Baden-Württemberg, Germany) with 2X LightCycler-FastStart DNA Master SYBER-Green I[™] Mix (Roche), forward and reverse primers (at a final concentration of 0.5 μM) and cDNA. The qRT-PCR conditions were: denaturation at 95 °C for 10 min, followed by 45 cycles of repeated amplification (95 °C, 10 s), hybridization (60 °C, 10 s) and elongation (72 °C, 10 s), and a final step at 40 °C for 30 s. A melting curve program was performed to control the amplification specificity, and the amplification products were sequenced. *Eflα* (encoding elongation factor 1α) was used as reference gene as in previous studies performed on salinity challenged sea bass (Blondeau-Bidet

et al., 2016; Lorin-Nebel et al., 2006) and as recommended by Mitter et al. (2009). Ultra-pure water was used as a no-template control in the qRT-PCR. Efficiencies were determined and given in Table 1. The relative expression ratio of each target gene was calculated using the ΔC_t method with the formula: Efficiency (E)^{- ΔC_t} and the efficiency of each primer pair.

2.5. Statistical analysis

Statistical analyses were performed using Graphpad Prism (version 6, GraphPad Software Incorporated, La Jolla, CA, 268 USA). Outliers were identified using the ROUT method based on the False Discovery Rate (with a Q set at 1%). Normality and homogeneity of variance were respectively checked using D'Agostino-Pearson test and Bartlett test. For *nka ala*, *cfr*, *clc-3*, *rhcg1*, *nkcc1*, *nhe3*, *ncc2a* and *vha-a*, data were square root transformed to fit homogeneity of the variance assumption. Two-way factorial analysis of variance with temperature and salinity as the main factors was performed. Critical differences between groups were appraised using the Fisher's least-square difference test. Linear correlations were determined using the Spearman correlation (Two-tailed). Data are presented as box and whisker plots showing median, minimum and maximum values. Level of statistical significance was set at $p < 0.05$. Linear correlation analysis was carried out with data from relative mRNA expression levels from this study and physiological parameters (plasma Na⁺ and Cl⁻ levels, plasma Na⁺/Cl⁻ ratio) recently reported in Masroor et al. (2018) obtained in the same fish (Table 3).

3. Results

3.1. Quantitative gene expression

197 For *nka α1a* (*atpl1a1a*) and *nka α1b* (*atpl1a1b*), there was a significant salinity effect (Table 2,
198 two-way ANOVA, $p < 0.01$). Temperature and interaction between both parameters (salinity and
199 temperature) exerted a significant effect only on *nka α1a* expression (Table 2, two-way ANOVA,
200 $p < 0.01$). In temperate conditions (18 °C), fish challenged to FW exhibited a 2.2 fold significantly
201 higher expression of *nka α1a* compared to SW controls (Fig. 1A, Table 4). In warm conditions
202 (24 °C), *nka α1a* expression was not significantly different between salinities (Fig. 1A).
203 Moreover, a significant higher *nka α1a* expression was measured in SW warm compared to SW
204 temperate.

205 On the other hand, *nka α1b* expression did not change in temperate conditions between SW and
206 FW (Fig. 1B). Conversely, a significant higher *nka α1b* expression was recorded in warm
207 conditions in SW vs FW- exposed fish (Fig. 1B).

208 For *cftr* and *nkcc1* (*slc12a2*), there was a significant salinity (*cftr*, $p < 0.0001$; *nkcc1*, $p < 0.001$) and
209 temperature effect (*cftr*, $p < 0.0001$; *nkcc1*, $p < 0.05$) but the interaction between temperature and
210 salinity did not exert an effect on both of these genes (Table 2, two-way ANOVA). *Cftr*
211 expression was significantly lower in FW than in SW at both temperatures (Fig. 1C). In both
212 salinities, fish acclimated to 24 °C had a significantly higher expression of *cftr* than at 18 °C.
213 Regarding *nkcc1*, fish challenged to FW had a significantly lower expression than SW fish, at
214 both tested temperatures. *Nkcc1* expression did not change significantly between both tested
215 temperatures (Fig. 1D) in the FW- and SW-exposed groups.

216 For *ncc2a* (*slc12a3-like*) and *nhe3* (*slc9a3*), there was a significant salinity (*ncc2a*, $p < 0.0001$;
217 *nhe3*, $p < 0.01$) and temperature effect ($p < 0.01$). Interaction between both parameters did not exert
218 a significant effect (Table 2, two-way ANOVA). In temperate conditions, fish exhibited a

219 significantly higher expression of *nhe3* in FW compared to SW. In warm conditions however, no
220 differences were observed in the expression level of *nhe3* between both salinities (Table 4). In
221 SW warm conditions, fish showed a significantly higher expression of *nhe3* compared to fish
222 acclimated to SW temperate conditions (Fig. 1E). In temperate and warm conditions, *ncc2a*
223 expression was significantly higher in FW compared to SW (Fig. 1F). In SW, *ncc2a* expression
224 was higher in warm compared to temperate conditions (Fig. 1F, Table 4).

225 For *clc-3* (*clcn3*), the temperature ($p < 0.0001$) was the only factor exerting a significant effect
226 (Table 2, two-way ANOVA). *Clc-3* expression was 2 and 1.6 fold higher at 24 °C compared to
227 18 °C in SW and FW, respectively (Fig. 1G).

228 Regarding expression of *vha-a* (*atp6v1a*), there was a significant salinity ($p < 0.01$) and
229 temperature effect ($p < 0.0001$) but the interaction between temperature and salinity was not
230 significant (Table 2, two-way ANOVA). In the case of *vha-b* (*atp6v1b2*), the temperature
231 ($p < 0.05$) is the only factor exerting a significant effect (Table 2, two-way ANOVA). In
232 temperate conditions, no differences were observed between salinities. In warm conditions
233 however, significantly lower *vha-a* expression was detected in FW compared to SW. *Vha-a*
234 expression was 2 and 1.6 fold higher at 24 °C compared to 18 °C in SW and FW respectively
235 (Fig. 1H). *Vha-b* expression was significantly higher in fish challenged to 24 °C in SW,
236 compared to all other conditions (Fig. 1I).

237 For *rhbG*, there is no effect of neither salinity nor temperature (Table 2, two-way ANOVA, Fig.
238 1J) and no difference in *rhbG* expression was observed between all analyzed conditions. In case
239 of *rhcgl*, there was a significant salinity ($p < 0.05$) and temperature effect ($p < 0.0001$) but the
240 interaction between temperature and salinity did not exert an effect on *rhcgl* expression (Table

2, two-way ANOVA). No difference in *rhcg1* expression was observed between salinities in temperate and warm conditions. Mean *rhcg1* expression was significantly increased by 4 and 3.3 fold at 24 °C compared to 18 °C in SW and FW, respectively (Fig. 1K). In warm conditions and notably in SW at 24 °C, a high variability was observed.

3.2 Plasma pH

Mean plasma pH are shown in Fig. 1S. There was a significant salinity ($p<0.05$) and temperature ($p<0.0001$) effect as well as a strong interaction between the two parameters ($p<0.001$) (Table 2, two-way ANOVA). A significant 5% increase from 8.25 ± 0.23 to 8.66 ± 0.13 (mean $\pm$ SD) in plasma pH was observed in temperate conditions following FW transfer (Table 4). No significant differences were observed following FW transfer in warm conditions (24 °C) ($\text{pH}=8.13 \pm 0.27$ in SW and 8.19 ± 0.18 (mean $\pm$ SD) in FW).

3.3. Correlations

mRNA levels of transporters that are involved in ion secretion (*cfr* and *nkcc1*) and of key pumps, *nka a1b* and *vha-a*, showed a positive correlation with plasma Cl^- level and negative correlation with Na^+/Cl^- ratio, as shown in Table 3. mRNA levels of transporters involved in ion uptake, *nhe3* and *ncc2a*, showed a negative correlation to plasma Cl^- and Na^+ level. mRNA levels of *nka a1b*, *clc-3* and *vha-a* and *b* are negatively correlated to Na^+/Cl^- ratio. *Rhcg1* was negatively correlated to plasma Na^+ levels. *Nka a1a* and *rhbg* were not correlated to the analyzed blood parameters.

4. Discussion

European sea bass raised in SW were acclimated to two environmental relevant temperatures, 18 °C and 24 °C, in order to investigate the molecular mechanisms underlying acclimation to warm temperature followed by a salinity decrease. The results showed that increased temperature affected the expression of several genes involved in osmoregulation, acid-base balance and ammonia excretion in gills, at both tested salinities.

In this study, we confirm previous results with higher branchial *nka α1a* mRNA levels in FW compared to SW in temperate conditions (Blondeau-Bidet et al., 2016; Jensen et al., 1998). In warm conditions however, *nka α1a* expression was similar between both salinities, which partially explain changes observed previously at the protein activity level (Masroor et al., 2018). NKA activity was increased by 1.72 fold in warm conditions following FW transfer (Table 4) but was overall lower than in temperate conditions (Masroor et al., 2018). Despite *nka α1a* has been identified as a main *nka α1* paralog in sea bass osmoregulatory tissues (Blondeau-Bidet et al., 2016), other paralogous genes encoding for NKA (*nka α2*, β , ...) may also change in warm conditions and contribute to overall branchial ion transport capacities (Vargas-Chacoff et al., 2018), but further analyses remain to be done to support this hypothesis in European sea bass. Branchial *nka α1b* mRNA expression seems to be different only between salinities in warm conditions with higher expression in SW than in FW. This tendency has already been shown in long-term (2.5 years) acclimated European sea bass to FW vs SW controls (Blondeau-Bidet et al., 2016). We did not observe a switch from *nka α1b* to *nka α1a* following FW transfer, a phenomenon previously reported in salmon (McCormick et al., 2009). Recently, Vargas-Chacoff et al. (2018) showed that temperature affect NKA α 1b levels in salmon smolts challenged from 14 °C to 24 °C in SW. In sea bass, it seems that significant differences in *nka*

alb mRNA expression between SW and FW are observed only under specific circumstances as long-term acclimation to extreme salinities (Blondeau-Bidet et al., 2016) or increased temperatures (this study). It is known in the literature that *nka* expression is modulated by temperature change (Michael et al., 2016a; Mitrovic and Perry, 2009; Nilsen et al., 2007) as it seems to be the case in our study in SW notably. Posttranscriptional processes as differential NKA phosphorylation might also be worth investigating in warm vs temperate conditions (Férraille et al., 1999).

4.1. Ion excretion mechanisms

In SW, NKA generates the driving force for ion excretion involving basolateral NKCC1 and apical CFTR (Evans et al., 2005). *Nkcc1* and *cftr* mRNA levels are higher in SW compared to FW when temperature factor is the same, as previously shown in sea bass maintained in temperate conditions (Bodinier et al., 2009; Lorin-Nebel et al., 2006) and numerous other teleost species (Hiroi et al., 2005; Inokuchi et al., 2017; McCormick et al., 2003; Nilsen et al., 2007). Contrary to *nkcc1*, whose expression is strongly affected by salinity only and to a much lesser extent by temperature, there seems to be an additive effect of high salinity and increased temperature on *cftr* expression. Thus, fish exposed to both, high salinity and high temperature (SW, 24° C) have greater *cftr* expression levels than fish exposed to SW without temperature increase. Bodinier et al. (2009) has shown the presence of apical CFTR in gill ionocytes of sea bass maintained in temperate SW. Previous studies have shown no changes in ionocyte density between SW conditions comparing both temperatures (Masroor et al., 2018). The high *cftr* expression observed in warm SW thus suggests an enhanced *cftr* expression in those cells

without necessarily an increased number of ionocytes. CFTR might also be expressed in pavement cells in warm conditions, as it is the case in killifish *F. heteroclitus*, or it could be sub-apically localized as shown in this same species (Marshall et al., 2002). Our results show that CFTR seems to be a key player in contributing to the maintenance of Cl⁻ balance in warm SW-acclimated fish as it has been suggested in temperate conditions (Evans et al., 2005; Bodinier et al., 2009), however further studies are necessary notably by analyzing protein CFTR levels in warm-acclimated sea bass.

4.2. Ion uptake mechanisms

Sea bass gills display remarkable plasticity when it comes to adjusting ion transport in response to salinity changes (Masroor et al., 2018). The switch from hypo- to hyper-osmoregulation is achieved by the activation of ion transporters and channels that are involved in ion uptake (Blondeau-Bidet et al., 2019; Hwang et al., 2011; L'Honoré et al., 2019). Other than NKA, key transporters involved in branchial Cl⁻ and/or Na⁺ uptake, such as *ncc2a* and *nhe3*, are negatively correlated to plasma Na⁺ and Cl⁻ levels, as expected, and higher expressed in temperate FW vs SW conditions. These transporters are apically localized in sea bass FW-type ionocytes and are thus essential for transepithelial Cl⁻ and/or Na⁺ uptake (Blondeau-Bidet et al., 2019; Inokuchi et al., 2017). Few studies have investigated the interactive effects of temperature and salinity on these transporters. In a recent study on stickleback *Gasterosteus aculeatus* from a marine ecotype, the combination of low salinity and low temperature (4 °C and 0.3 ppt) had no interactive effect on the expression of *nhe3*, contrary to *nka*, where an additive effect was observed (Gibbons et al., 2018). In European sea bass, no additive effect was observed for both

of these genes and similar patterns of expression were observed for *nhe3* and *nka a1a* (Fig. 1A, E). Both transporters showed an increased expression in warm SW conditions *vs* temperate SW but no change in expression was observed between salinities in warm conditions (Table 4). No apical NHE3 expression has been observed so far in temperate SW conditions (Blondeau-Bidet et al., 2019) and protein localization should be investigated further, notably in SW warm conditions. *Ncc2a* is upregulated upon warm temperature acclimation, in SW notably. Contrary to *nhe3* and *nka a1a*, there is a significant difference in *ncc2a* expression between salinities in warm conditions and an average mRNA fold change of 1.6 following FW transfer (Table 4). This suggests a differentiation of *ncc2a* expressing cells to take up Cl⁻ and Na⁺. In previous studies we have observed a 883-fold and 10- fold increase of lamellar ionocytes following FW challenge in temperate and warm conditions respectively (Table 4) (Masroor et al., 2018). Lamellar ionocytes are essentially NHE3-type cells whereas NCC2-type cells have been essentially detected on gill filaments (Blondeau-Bidet et al., 2019). This lower density in lamellar ionocytes (NHE3-type) might partially be linked to the lack of *nhe3* increase in warm FW conditions. It is likely that the proportion of NCC- *vs* NHE3-type cells is different in warm FW conditions, but this needs to be investigated further at protein level.

In pufferfish *Tetraodon nigroviridis* gills, *clc-3* mRNA expression did not change between FW and SW groups (Tang et al., 2010) as we have also shown in this study in sea bass maintained at 18 °C. This differs from previous results in sea bass, where *clc-3* mRNA expression was lower in FW than in SW whereas protein levels seemed to be higher in FW (Bossus et al., 2013; Tang et al., 2010). In tilapia, *clc-3* mRNA expression was higher in deionized water in comparison to FW and SW (Tang et al., 2010) and several studies suggest an involvement of ClC-3 in basolateral Cl⁻ uptake in FW (Bossus et al., 2013; Tang and Lee, 2011, 2007). In this study, we

showed that *clc-3* expression is strongly affected by temperature but not by salinity. The high *clc-3* expression in warm conditions might be linked to an overall increased ion transport and increased need to regulate cell volume (Duan et al., 1997, 1999; Hermoso et al., 2002). Interestingly, in our previous study, ionocyte cell area was significantly lower in FW warm compared to the three other conditions (Masroor et al., 2018). These same fish also displayed higher plasma chloride levels than in FW temperate conditions (Masroor et al., 2018) suggesting that chloride uptake mechanisms are efficient. No difference in ionocyte cell volume was observed in SW conditions between the two temperature regimes which suggest an efficient ionocyte cell volume regulation in warm SW, maybe partially achieved by the increased *ClC-3* expression as well as other cell volume-regulating proteins as aquaporins (Madsen, 2012) that have not been analyzed in this study.

4.3. Effect of temperature on acid-base regulation

It is well known in marine fish that acid-base homeostasis is mainly regulated at the gill and kidney levels (Heuer and Grosell, 2014) and in larvae at the integument level (Burggren and Bautista, 2019). The link between ionic regulation and acid-base balance with regard to low pH has been investigated in several species (Dymowska et al., 2012; Kwong et al., 2014). Plasma pH levels observed in this study were high compared to the literature. In Shrivastava et al. (2019), sea bass blood pH ranged from around 7.8 at 32 ppt to 7.6 at 2.5 ppt following a three-week salinity challenge. The high levels we measured might be linked to two successive freezing/thawing processes. Absolute values are therefore not compared, however, all samples have been treated similarly and it seems relevant to compare pH changes among treatments. As

374 for the increased plasma Na^+/Cl^- ratio measured previously (Masroor et al., 2018), a significantly
375 higher pH was recorded following FW transfer in temperate conditions clearly indicating a
376 metabolic alkalosis. An increased plasma pH following FW transfer might indicate that sea bass
377 are not fully acclimated to FW after 2 weeks as previously suggested (Jensen et al., 1998).
378 Moreover, L'Honoré et al. (2019) have shown differential FW tolerance in sea bass from the
379 West Mediterranean lineage. In 8-month-old fish, some animals were intolerant to FW and
380 displayed increased plasma Na^+/Cl^- ratios indicating a metabolic alkalosis as shown in this study.
381 It has to be noted that fish from this study are 14 months-old and do not show any other sign of
382 FW intolerance. At the gill level, acid secretion is thought to be coupled to Na^+ uptake either
383 through a Na^+/H^+ exchanger (NHE3 or NHE2) or VHA. In SW, the model with NHEs is more
384 likely given the favorable Na^+ gradient for Na^+/H^+ exchangers. The increased mRNA expression
385 of *nhe3* as well as *vha-a* and *vha-b* in SW warm conditions could be a response to the lower
386 blood Na^+/Cl^- ratio previously shown (Masroor et al., 2018). A low blood Na^+/Cl^- ratio is
387 generally an indicator of metabolic acidosis triggering acid excretion mechanisms (Heuer and
388 Grosell, 2014; Michael et al., 2016b). In 24 °C acclimated sea bass, there is no metabolic
389 acidosis as plasma pH levels are not different from temperate conditions. An overexpression of
390 *nhe3* together with carbonic anhydrase 2 (*ca2*) and Na^+/H^+ exchanger (*nbc1*) has been reported
391 in Osorezan dace *Tribolodon hakonensis* gills when fish were challenged to acidic waters
392 compared to fish maintained in neutral waters (Hirata et al., 2003). In this latter species, apical
393 NHE3 clearly participates to acid excretion as well as in other species studied (Hiroi et al., 2008;
394 Inokuchi et al., 2008; Ivanis et al., 2008). In zebrafish maintained in acidic FW, *vha-a* mRNA
395 expression as well as the density of VHA-enriched ionocyte subtype (called HR cells) were
396 increased following 7 days of acid exposure (Chang et al., 2009). *Vha-a* is negatively correlated

to the Na^+/Cl^- ratio ($r = -0.6471$, $p < 0.0001$) and may be involved in proton excretion in sea bass gills, however the subcellular localization of VHA is not yet clear in this species. Apical (Sullivan et al., 1995; Yan et al., 2007) as well as basolateral (Catches et al., 2006; Malakpour Kolbadinezhad et al., 2018; Uchiyama et al., 2012) localization of VHA has been reported in fish. Basolateral VHA, by pumping protons out of the cell to the blood, would generate a favorable electrochemical gradient for apical $\text{Cl}^-/\text{HCO}_3^-$ exchange and could thus participate to HCO_3^- excretion and chloride uptake (Piermarini and Evans, 2001) whereas apical localization would rather be involved in acid secretion and might be rather functionally linked to a basolateral anion exchanger (AE1) secreting Cl^- in exchange of HCO_3^- (Liu et al., 2016). Interestingly, a high correlation is observed between *vha-a* and blood chloride levels ($r = 0.903$, $p < 0.001$) but further analyses are needed to fully understand if there is a functional link between VHA and chloride levels.

4.4. Effect of temperature on nitrogen excretion

In a previous study on sea bass challenged to FW temperate conditions, *rhcg1* was significantly upregulated compared to SW temperate but *rhbg* did not change between SW and FW conditions (Blondeau-Bidet et al., 2019). This suggests a different handling for nitrogen excretion when comparing different salinity regimes (Frick and Wright, 2002). In our study, we can observe a slightly but not significantly increased *rhcg1* expression in FW compared to SW. More strikingly, a significant upregulation of *rhcg1* was observed in warm temperature-challenged fish at both salinities. In gill ionocytes, RHCG1 might operate in concert with NHE3 and VHA at the apical cell part as shown in other species, but this remains to be analyzed further in sea bass

(Heuer and Grosell, 2014; Nawata et al., 2010). In this study, temperature did not affect *rhbg* expression. In longjaw mudsucker *Gillichthys mirabilis* maintained in SW, warm temperatures (28 °C) seemed to enhance the expression of different transport related genes including branchial *rhbg* and *vha* compared to lower temperature groups (9 °C and 19 °C) and suggested an increased ammonia excretion in warm conditions (Logan and Somero, 2010). Nawata et al. (2010) showed that in seawater maintained pufferfish (*Takifugu rubripes*) exposed to ammonia, gill *rhcg1*, *vha*, *nkcc1*, *nka* and *nhe3* were upregulated, which suggests a tight cooperation between different ion transporters expressed in ionocytes under high ammonia. The involvement of Rhesus proteins (Rh) in ammonia transport processes remains to be clarified in sea bass gills as well as the functional link with other ion transporters, as VHA and NHE3 (Nawata et al., 2007, 2010). However, our data strongly point to a role of *rhcg1* in ammonia excretion in warm temperatures, probably linked to increased metabolism, as shown previously in the same sea bass lineage challenged to increased temperatures (Claireaux and Lagardère, 1999). In another study, Person-le-Ruyet et al. (2004) have shown in the same species a 3 times increased mean daily ammonia excretion rate at 25 °C compared to 13 °C which is in accordance with our data.

5. Conclusions

In this study focusing on the mechanisms involved in salinity acclimation at two different temperatures, we showed that branchial transcript levels of most analyzed transporters were significantly affected by warm temperatures. We showed a more striking effect of temperature on gene expression patterns in SW warm conditions compared to FW warm. Increased *rhcg1* mRNA expression points to a potential up-regulation of ammonia excretion as a response to enhanced metabolism in warm conditions. Data also support an activation of chloride secretion

in warm sea water contributing to the maintenance of the chloride balance. Moreover, an increased expression in proteins involved in acid excretion (*nhe3* and *vha-a*, and *vha-b*) points to an activation of acid secretion pathways to maintain plasma pH constant. Following FW transfer, a differential response at both temperatures was observed regarding the expression of transporters involved in Na⁺ vs Cl⁻ uptake. These results as well as data obtained on blood parameters (Masroor et al., 2018) indicate physiological as well as molecular modifications at the gill level following a two-week acclimation at 24 °C.

6. Acknowledgements

Sea bass were generously donated by the Ifremer Palavas. We would like to thank Philippe Clair from the qRT-PCR CEMEB platform for his technical guidance. Part of this project was funded by the OSMOVILL project (PRC n°231789, CNRS-MoST).

7. References

- Barnabé, G., 1989. L'élevage du loup et de la daurade, in: Barnabé, G. (Ed.), Aquaculture. Lavoisier, Paris, pp. 675–720.
- Blondeau-Bidet, E., Bossus, M., Maugars, G., Farcy, E., Lignot, J.-H., Lorin-Nebel, C., 2016. Molecular characterization and expression of Na⁺/K⁺-ATPase α 1 isoforms in the European sea bass *Dicentrarchus labrax* osmoregulatory tissues following salinity transfer. Fish Physiol. Biochem. 42, 1647–1664. <https://doi.org/10.1007/s10695-016-0247-x>
- Blondeau-Bidet, E., Hiroi, J., Lorin-Nebel, C., 2019. Ion uptake pathways in European sea bass *Dicentrarchus labrax*. Gene 692, 126–137. <https://doi.org/10.1016/j.gene.2019.01.006>

465 Bodinier, C., Lorin-Nebel, C., Charmantier, G., Boulo, V., 2009. Influence of salinity on the
 466 localization and expression of the CFTR chloride channel in the ionocytes of juvenile
 467 *Dicentrarchus labrax* exposed to seawater and freshwater. *Comp. Biochem. Physiol. A.*
 468 *Mol. Integr. Physiol.* 153, 345–351. <https://doi.org/10.1016/j.cbpa.2009.03.011>

469 Bossus, M., Charmantier, G., Blondeau-Bidet, E., Valletta, B., Boulo, V., Lorin-Nebel, C., 2013.
 470 The ClC-3 chloride channel and osmoregulation in the European Sea Bass, *Dicentrarchus*
 471 *labrax*. *J. Comp. Physiol. B* 183, 641–662. <https://doi.org/10.1007/s00360-012-0737-9>

472 Burggren, W., Bautista, N., 2019. Development of acid-base regulation in vertebrates. *Comp.*
 473 *Biochem. Physiol. A. Mol. Integr. Physiol.* 236, 110518.
 474 <https://doi.org/10.1016/j.cbpa.2019.06.018>

475 Burton, R.F., 1986. Ionic regulation in fish: The influence of acclimation temperature on plasma
 476 composition and apparent set points. *Comp. Biochem. Physiol. A Physiol.* 85, 23–28.
 477 [https://doi.org/10.1016/0300-9629\(86\)90456-1](https://doi.org/10.1016/0300-9629(86)90456-1)

478 Catches, J.S., Burns, J.M., Edwards, S.L., Claiborne, J.B., 2006. Na⁺/H⁺ antiporter, V-H⁺-
 479 ATPase and Na⁺/K⁺-ATPase immunolocalization in a marine teleost (*Myoxocephalus*
 480 *octodecemspinosus*). *J. Exp. Biol.* 209, 3440–3447. <https://doi.org/10.1242/jeb.02384>

481 Chang, W.-J., Horng, J.-L., Yan, J.-J., Hsiao, C.-D., Hwang, P.-P., 2009. The transcription
 482 factor, glial cell missing 2, is involved in differentiation and functional regulation of H⁺-
 483 ATPase-rich cells in zebrafish (*Danio rerio*). *Am. J. Physiol.-Regul. Integr. Comp.*
 484 *Physiol.* 296, 1192–1201. <https://doi.org/10.1152/ajpregu.90973.2008>

485 Chou, M.-Y., Hsiao, C.-D., Chen, S.-C., Chen, I.-W., Liu, S.-T., Hwang, P.-P., 2008. Effects of
 486 hypothermia on gene expression in zebrafish gills: upregulation in differentiation and
 487 function of ionocytes as compensatory responses. *J. Exp. Biol.* 211, 3077–3084.
 488 <https://doi.org/10.1242/jeb.019950>

489 Claireaux, G., Couturier, C., Groison, A.-L., 2006. Effect of temperature on maximum
 490 swimming speed and cost of transport in juvenile European sea bass (*Dicentrarchus*
 491 *labrax*). *J. Exp. Biol.* 209, 3420–3428. <https://doi.org/10.1242/jeb.02346>

492 Claireaux, G., Lagardère, J.-P., 1999. Influence of temperature, oxygen and salinity on the
 493 metabolism of the European sea bass. *J. Sea Res.* 42, 157–168.
 494 [https://doi.org/10.1016/S1385-1101\(99\)00019-2](https://doi.org/10.1016/S1385-1101(99)00019-2)

495 Crockett, E.L., Londraville, R.L., 2006. Temperature, in: Evans, D.H., Claiborne, J.B. (Eds.),
 496 The Physiology of Fishes, Marine Biology Series. CRC, Taylor & Francis, Boca Raton,
 497 FL, pp. 231–269.

498 Dalla Via, J., Villani, P., Gasteiger, E., Niederstätter, H., 1998. Oxygen consumption in sea bass
 499 fingerling *Dicentrarchus labrax* exposed to acute salinity and temperature changes:
 500 metabolic basis for maximum stocking density estimations. *Aquaculture* 169, 303–313.
 501 [https://doi.org/10.1016/S0044-8486\(98\)00375-5](https://doi.org/10.1016/S0044-8486(98)00375-5)

502 Duan, D., Cowley, S., Horowitz, B., Hume, J.R., 1999. A serine residue in ClC-3 links
 503 phosphorylation–dephosphorylation to chloride channel regulation by cell volume. *J.*
 504 *Gen. Physiol.* 113, 57–70. <https://doi.org/10.1085/jgp.113.1.57>

505 Duan, D., Winter, C., Cowley, S., Hume, J.R., Horowitz, B., 1997. Molecular identification of a
 506 volume-regulated chloride channel. *Nature* 390, 417–421.

507 Dufour, V., Cantou, M., Lecomte, F., 2009. Identification of sea bass (*Dicentrarchus labrax*)
 508 nursery areas in the north-western Mediterranean Sea. *J. Mar. Biol. Assoc. U. K.* 89,
 509 1367–1374. <https://doi.org/10.1017/S0025315409000368>

510 Dülger, N., Kumlu, M., Türkmen, S., Ölçülü, A., Tufan Eroldoğan, O., Asuman Yılmaz, H.,
 511 Öçal, N., 2012. Thermal tolerance of European sea bass (*Dicentrarchus labrax*) juveniles
 512 acclimated to three temperature levels. *J. Therm. Biol.* 37, 79–82.
 513 <https://doi.org/10.1016/j.jtherbio.2011.11.003>

514 Dymowska, A.K., Hwang, P.-P., Goss, G.G., 2012. Structure and function of ionocytes in the
 515 freshwater fish gill. *Respir. Physiol. Neurobiol.* 184, 282–292.
 516 <https://doi.org/10.1016/j.resp.2012.08.025>

517 Evans, D.H., 2008. Teleost fish osmoregulation: what have we learned since August Krogh,
 518 Homer Smith, and Ancel Keys. *Am. J. Physiol.-Regul. Integr. Comp. Physiol.* 295, 704–
 519 713. <https://doi.org/10.1152/ajpregu.90337.2008>

520 Evans, D.H., Piermarini, P.M., Choe, K.P., 2005. The multifunctional fish gill: Dominant site of
 521 gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste.
 522 *Physiol. Rev.* 85, 97–177. <https://doi.org/10.1152/physrev.00050.2003>

523 Evans, D.H., Piermarini, P.M., Potts, W.T.W., 1999. Ionic transport in the fish gill epithelium. *J.*
 524 *Exp. Zool.* 283, 641–652. [https://doi.org/10.1002/\(SICI\)1097-010X\(19990601\)283:7<641::AID-JEZ3>3.0.CO;2-W](https://doi.org/10.1002/(SICI)1097-010X(19990601)283:7<641::AID-JEZ3>3.0.CO;2-W)

526 Féraïlle, E., Carranza, M.L., Gonin, S., Béguin, P., Pedemonte, C., Rousselot, M., Caverzasio, J.,
 527 Geering, K., Martin, P.-Y., Favre, H., 1999. Insulin-induced stimulation of Na⁺,K⁺-
 528 ATPase activity in kidney proximal tubule cells depends on phosphorylation of the α -
 529 subunit at Tyr-10. *Mol. Biol. Cell* 10, 2847–2859. <https://doi.org/10.1091/mbc.10.9.2847>
 530 Frick, N.T., Wright, P.A., 2002. Nitrogen metabolism and excretion in the mangrove killifish
 531 *Rivulus marmoratus* I. The influence of environmental salinity and external ammonia. *J.*
 532 *Exp. Biol.* 205, 79–89.
 533 Gibbons, T.C., McBryan, T.L., Schulte, P.M., 2018. Interactive effects of salinity and
 534 temperature acclimation on gill morphology and gene expression in threespine
 535 stickleback. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 221, 55–62.
 536 <https://doi.org/10.1016/j.cbpa.2018.03.013>
 537 Giorgi, F., 2006. Climate change hot-spots. *Geophys. Res. Lett.* 33, L08707.
 538 <https://doi.org/10.1029/2006GL025734>
 539 Goss, G.G., Perry, S.F., Fryer, J.N., Laurent, P., 1998. Gill morphology and acid-base regulation
 540 in freshwater fishes. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 119, 107–115.
 541 [https://doi.org/10.1016/S1095-6433\(97\)00401-7](https://doi.org/10.1016/S1095-6433(97)00401-7)
 542 Hermoso, M., Satterwhite, C.M., Andrade, Y.N., Hidalgo, J., Wilson, S.M., Horowitz, B., Hume,
 543 J.R., 2002. ClC-3 is a fundamental molecular component of volume-sensitive outwardly
 544 rectifying Cl⁻ channels and volume regulation in HeLa cells and *Xenopus laevis* Oocytes.
 545 *J. Biol. Chem.* 277, 40066–40074. <https://doi.org/10.1074/jbc.M205132200>
 546 Heuer, R.M., Grosell, M., 2014. Physiological impacts of elevated carbon dioxide and ocean
 547 acidification on fish. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* 307, 1061–1084.
 548 <https://doi.org/10.1152/ajpregu.00064.2014>
 549 Hickman, C.P., Trump, B.F., 1969. Excretion, ion regulation and metabolism, in: Hoar, W.S.,
 550 Randall, D.J. (Eds.), *Fish Physiology*, Vol. I, The Kidney,. Academic Press, New York,
 551 pp. 91–239.
 552 Hirata, T., Kaneko, T., Ono, T., Nakazato, T., Furukawa, N., Hasegawa, S., Wakabayashi, S.,
 553 Shigekawa, M., Chang, M.-H., Romero, M.F., Hirose, S., 2003. Mechanism of acid
 554 adaptation of a fish living in a pH 3.5 lake. *Am. J. Physiol.-Regul. Integr. Comp. Physiol.*
 555 284, 1199–1212. <https://doi.org/10.1152/ajpregu.00267.2002>

556 Hiroi, J., McCormick, S.D., Ohtani-Kaneko, R., Kaneko, T., 2005. Functional classification of
 557 mitochondrion-rich cells in euryhaline Mozambique tilapia (*Oreochromis mossambicus*)
 558 embryos, by means of triple immunofluorescence staining for Na⁺/K⁺-ATPase,
 559 Na⁺/K⁺/2Cl⁻ cotransporter and CFTR anion channel. J. Exp. Biol. 208, 2023–2036.
 560 <https://doi.org/10.1242/jeb.01611>
 561 Hiroi, J., Yasumasu, S., McCormick, S.D., Hwang, P.-P., Kaneko, T., 2008. Evidence for an
 562 apical Na–Cl cotransporter involved in ion uptake in a teleost fish. J. Exp. Biol. 211,
 563 2584–2599. <https://doi.org/10.1242/jeb.018663>
 564 Hsu, H.-H., Lin, L.-Y., Tseng, Y.-C., Horng, J.-L., Hwang, P.-P., 2014. A new model for fish ion
 565 regulation: identification of ionocytes in freshwater- and seawater-acclimated medaka
 566 (*Oryzias latipes*). Cell Tissue Res. 357, 225–243. [https://doi.org/10.1007/s00441-014-](https://doi.org/10.1007/s00441-014-1883-z)
 567 1883-z
 568 Hu, M.Y., Michael, K., Kreiss, C.M., Stumpp, M., Dupont, S., Tseng, Y.-C., Lucassen, M.,
 569 2016. Temperature modulates the effects of ocean acidification on intestinal ion transport
 570 in Atlantic cod, *Gadus morhua*. Front. Physiol. 7.
 571 <https://doi.org/10.3389/fphys.2016.00198>
 572 Hutchison, V.H., Maness, J.D., 1979. The role of behavior in temperature acclimation and
 573 tolerance in ectotherms. Am. Zool. 19, 367–384. <https://doi.org/10.1093/icb/19.1.367>
 574 Hwang, P.-P., 2009. Ion uptake and acid secretion in zebrafish (*Danio rerio*). J. Exp. Biol. 212,
 575 1745–1752. <https://doi.org/10.1242/jeb.026054>
 576 Hwang, P.-P., Lee, T.-H., 2007. New insights into fish ion regulation and mitochondrion-rich
 577 cells. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 148, 479–497.
 578 <https://doi.org/10.1016/j.cbpa.2007.06.416>
 579 Hwang, P.-P., Lee, T.-H., Lin, L.-Y., 2011. Ion regulation in fish gills: recent progress in the
 580 cellular and molecular mechanisms. Am. J. Physiol. Regul. Integr. Comp. Physiol. 301,
 581 28–47. <https://doi.org/10.1152/ajpregu.00047.2011>
 582 Hwang, P.-P., Lin, L.-Yih., 2014. Gill ionic transport, acid-base regulation, and nitrogen
 583 excretion, in: Evans, D.H., Claiborne, J.B., Currie, S. (Eds.), The Physiology of Fishes.
 584 pp. 205–233.
 585 Inokuchi, M., Hiroi, J., Watanabe, S., Lee, K.M., Kaneko, T., 2008. Gene expression and
 586 morphological localization of NHE3, NCC and NKCC1a in branchial mitochondria-rich

587 cells of Mozambique tilapia (*Oreochromis mossambicus*) acclimated to a wide range of
 588 salinities. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 151, 151–158.
 589 <https://doi.org/10.1016/j.cbpa.2008.06.012>
 590 Inokuchi, M., Nakamura, M., Miyanishi, H., Hiroi, J., Kaneko, T., 2017. Functional
 591 classification of gill ionocytes and spatiotemporal changes in their distribution after
 592 transfer from seawater to freshwater in Japanese seabass. *J. Exp. Biol.* 220, 4720–4732.
 593 <https://doi.org/10.1242/jeb.167320>
 594 Ivanis, G., Esbaugh, A.J., Perry, S.F., 2008. Branchial expression and localization of SLC9A2
 595 and SLC9A3 sodium/hydrogen exchangers and their possible role in acid-base regulation
 596 in freshwater rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* 211, 2467–2477.
 597 <https://doi.org/10.1242/jeb.017491>
 598 Jensen, M.K., Madsen, S.S., Kristiansen, K., 1998. Osmoregulation and salinity effects on the
 599 expression and activity of Na⁺,K⁺-ATPase in the gills of European sea bass,
 600 *Dicentrarchus labrax* (L.). *J. Exp. Zool.* 282, 290–300.
 601 [https://doi.org/10.1002/\(SICI\)1097-010X\(19981015\)282:3<290::AID-JEZ2>3.0.CO;2-H](https://doi.org/10.1002/(SICI)1097-010X(19981015)282:3<290::AID-JEZ2>3.0.CO;2-H)
 602 Kreiss, C.M., Michael, K., Lucassen, M., Jutfelt, F., Motyka, R., Dupont, S., Pörtner, H.-O.,
 603 2015. Ocean warming and acidification modulate energy budget and gill ion regulatory
 604 mechanisms in Atlantic cod (*Gadus morhua*). *J. Comp. Physiol. B* 185, 767–781.
 605 <https://doi.org/10.1007/s00360-015-0923-7>
 606 Kumai, Y., Perry, S.F., 2012. Mechanisms and regulation of Na⁺ uptake by freshwater fish.
 607 *Respir. Physiol. Neurobiol.* 184, 249–256. <https://doi.org/10.1016/j.resp.2012.06.009>
 608 Kwong, R.W.M., Kumai, Y., Perry, S.F., 2014. The physiology of fish at low pH: the zebrafish
 609 as a model system. *J. Exp. Biol.* 217, 651–662. <https://doi.org/10.1242/jeb.091603>
 610 Kyprianou, T.-D., Pörtner, H.O., Anestis, A., Kostoglou, B., Feidantsis, K., Michaelidis, B.,
 611 2010. Metabolic and molecular stress responses of gilthead sea bream *Sparus aurata*
 612 during exposure to low ambient temperature: an analysis of mechanisms underlying the
 613 winter syndrome. *J. Comp. Physiol. B* 180, 1005–1018. [https://doi.org/10.1007/s00360-](https://doi.org/10.1007/s00360-010-0481-y)
 614 [010-0481-y](https://doi.org/10.1007/s00360-010-0481-y)
 615 L'Honoré, T., Farcy, E., Chatain, B., Gros, R., Ruelle, F., Hermet, S., Blondeau-Bidet, E.,
 616 Naudet, J., Lorin-Nebel, C., 2019. Are European sea bass as euryhaline as expected?

617 Intraspecific variation in freshwater tolerance. Mar. Biol. 166, 102.
618 <https://doi.org/10.1007/s00227-019-3551-z>

619 Liu, S.-T., Horng, J.-L., Chen, P.-Y., Hwang, P.-P., Lin, L.-Y., 2016. Salt secretion is linked to
620 acid-base regulation of ionocytes in seawater-acclimated medaka: new insights into the
621 salt-secreting mechanism. Sci. Rep. 6, 31433.

622 Logan, C.A., Buckley, B.A., 2015. Transcriptomic responses to environmental temperature in
623 eurythermal and stenothermal fishes. J. Exp. Biol. 218, 1915–1924.
624 <https://doi.org/10.1242/jeb.114397>

625 Logan, C.A., Somero, G.N., 2011. Effects of thermal acclimation on transcriptional responses to
626 acute heat stress in the eurythermal fish *Gillichthys mirabilis* (Cooper). Am. J. Physiol.
627 Regul. Integr. Comp. Physiol. 300, 1373–1383.
628 <https://doi.org/10.1152/ajpregu.00689.2010>

629 Logan, C.A., Somero, G.N., 2010. Transcriptional responses to thermal acclimation in the
630 eurythermal fish *Gillichthys mirabilis* (Cooper 1864). Am. J. Physiol.-Regul. Integr.
631 Comp. Physiol. 299, 843–852. <https://doi.org/10.1152/ajpregu.00306.2010>

632 Lorin-Nebel, C., Boulo, V., Bodinier, C., Charmantier, G., 2006. The Na⁺/K⁺/2Cl⁻ cotransporter
633 in the sea bass *Dicentrarchus labrax* during ontogeny: involvement in osmoregulation. J.
634 Exp. Biol. 209, 4908–4922. <https://doi.org/10.1242/jeb.02591>

635 Madeira, D., Narciso, L., Cabral, H.N., Vinagre, C., Diniz, M.S., 2013. Influence of temperature
636 in thermal and oxidative stress responses in estuarine fish. Comp. Biochem. Physiol. A.
637 Mol. Integr. Physiol. 166, 237–243. <https://doi.org/10.1016/j.cbpa.2013.06.008>

638 Madsen, S.S., 2012. Aquaporins in fishes—expression, localization, and functional dynamics.
639 Front. Physiol. 3. <https://doi.org/10.3389/fphys.2012.00434>

640 Malakpour Kolbadinezhad, S., Coimbra, J., Wilson, J.M., 2018. Effect of dendritic organ ligation
641 on striped eel catfish *Plotosus lineatus* osmoregulation. PLOS ONE 13, e0206206.
642 <https://doi.org/10.1371/journal.pone.0206206>

643 Marshall, W.S., Lynch, E.M., Cozzi, R.R.F., 2002. Redistribution of immunofluorescence of
644 CFTR anion channel and NKCC cotransporter in chloride cells during adaptation of the
645 killifish *Fundulus heteroclitus* to sea water. J. Exp. Biol. 205, 1265–1273.

646 Masroor, W., Farcy, E., Gros, R., Lorin-Nebel, C., 2018. Effect of combined stress (salinity and
647 temperature) in European sea bass *Dicentrarchus labrax* osmoregulatory processes.

648 Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 215, 45–54.
649 <https://doi.org/10.1016/j.cbpa.2017.10.019>

650 McCormick, S.D., Regish, A.M., Christensen, A.K., 2009. Distinct freshwater and seawater
651 isoforms of Na⁺/K⁺-ATPase in gill chloride cells of Atlantic salmon. J. Exp. Biol. 212,
652 3994–4001. <https://doi.org/10.1242/jeb.037275>

653 McCormick, S.D., Sundell, K., Björnsson, B.T., Brown, C.L., Hiroi, J., 2003. Influence of
654 salinity on the localization of Na⁺/K⁺-ATPase, Na⁺/K⁺/2Cl⁻ cotransporter (NKCC) and
655 CFTR anion channel in chloride cells of the Hawaiian goby (*Stenogobius hawaiiensis*). J.
656 Exp. Biol. 206, 4575–4583. <https://doi.org/10.1242/jeb.00711>

657 Metz, J.R., Burg, E.H. van den, Bonga, S.E.W., Flik, G., 2003. Regulation of branchial Na⁺/K⁺-
658 ATPase in common carp *Cyprinus carpio* L. acclimated to different temperatures. J. Exp.
659 Biol. 206, 2273–2280. <https://doi.org/10.1242/jeb.00421>

660 Michael, K., Koschnick, N., Pörtner, H.-O., Lucassen, M., 2016a. Response of branchial Na⁺/K⁺
661 ATPase to changes in ambient temperature in Atlantic cod (*Gadus morhua*) and whiting
662 (*Merlangius merlangus*). J. Comp. Physiol. B 186, 461–470.
663 <https://doi.org/10.1007/s00360-016-0970-8>

664 Michael, K., Kreiss, C.M., Hu, M.Y., Koschnick, N., Bickmeyer, U., Dupont, S., Pörtner, H.-O.,
665 Lucassen, M., 2016b. Adjustments of molecular key components of branchial ion and pH
666 regulation in Atlantic cod (*Gadus morhua*) in response to ocean acidification and
667 warming. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 193, 33–46.
668 <https://doi.org/10.1016/j.cbpb.2015.12.006>

669 Mitrovic, D., Perry, S.F., 2009. The effects of thermally induced gill remodeling on ionocyte
670 distribution and branchial chloride fluxes in goldfish (*Carassius auratus*). J. Exp. Biol.
671 212, 843–852. <https://doi.org/10.1242/jeb.025999>

672 Mitter, K., Kotoulas, G., Magoulas, A., Mulero, V., Sepulcre, P., Figueras, A., Novoa, B.,
673 Sarropoulou, E., 2009. Evaluation of candidate reference genes for QPCR during
674 ontogenesis and of immune-relevant tissues of European seabass (*Dicentrarchus labrax*).
675 Comp. Biochem. Physiol. B Biochem. Mol. Biol. 153, 340–347.
676 <https://doi.org/10.1016/j.cbpb.2009.04.009>

677 Morrison, J.F., Guynn, S.R., Scofield, M.A., Dowd, F.J., Petzel, D.H., 2006. Warm acclimation
678 changes the expression of the Na⁺,K⁺-ATPase α subunit isoforms in Antarctic fish gills. J.
679 Exp. Mar. Biol. Ecol. 333, 129–139. <https://doi.org/10.1016/j.jembe.2005.12.048>

680 Moyes, C.D., Ballantyne, J.S., 2011. Membranes and temperature: Homeoviscous adaptation, in:
681 Farrell, A.P., Stevens, E.D., Cech, J.J., Richards, J.G. (Eds.), Encyclopedia of Fish
682 Physiology: From Genome to Environment. Academic Press, an imprint of Elsevier,
683 London ; Waltham, MA, pp. 1725–1731.

684 Nawata, C.M., Hirose, S., Nakada, T., Wood, C.M., Kato, A., 2010. Rh glycoprotein expression
685 is modulated in pufferfish (*Takifugu rubripes*) during high environmental ammonia
686 exposure. J. Exp. Biol. 213, 3150–3160. <https://doi.org/10.1242/jeb.044719>

687 Nawata, C.M., Hung, C.C.Y., Tsui, T.K.N., Wilson, J.M., Wright, P.A., Wood, C.M., 2007.
688 Ammonia excretion in rainbow trout (*Oncorhynchus mykiss*): evidence for Rh
689 glycoprotein and H⁺-ATPase involvement. Physiol. Genomics 31, 463–474.
690 <https://doi.org/10.1152/physiolgenomics.00061.2007>

691 Newton, A., Mudge, S.M., 2003. Temperature and salinity regimes in a shallow, mesotidal
692 lagoon, the Ria Formosa, Portugal. Estuar. Coast. Shelf Sci. 57, 73–85.
693 [https://doi.org/10.1016/S0272-7714\(02\)00332-3](https://doi.org/10.1016/S0272-7714(02)00332-3)

694 Nilsen, T.O., Ebbesson, L.O.E., Madsen, S.S., McCormick, S.D., Andersson, E., Bjornsson,
695 B.Th., Prunet, P., Stefansson, S.O., 2007. Differential expression of gill Na⁺,K⁺-ATPase -
696 and -subunits, Na⁺,K⁺,2Cl⁻ cotransporter and CFTR anion channel in juvenile
697 anadromous and landlocked Atlantic salmon *Salmo salar*. J. Exp. Biol. 210, 2885–2896.
698 <https://doi.org/10.1242/jeb.002873>

699 Person-Le Ruyet, J., Mahé, K., Le Bayon, N., Le Delliou, H., 2004. Effects of temperature on
700 growth and metabolism in a Mediterranean population of European sea bass,
701 *Dicentrarchus labrax*. Aquaculture 237, 269–280.
702 <https://doi.org/10.1016/j.aquaculture.2004.04.021>

703 Piermarini, P.M., Evans, D.H., 2001. Immunochemical analysis of the vacuolar proton-ATPase
704 B-subunit in the gills of a euryhaline stingray (*Dasyatis sabina*): effects of salinity and
705 relation to Na⁺/K⁺-ATPase. J. Exp. Biol. 204, 3251–3259.

706 Schulte, P.M., 2011. Effects of temperature: An introduction, in: Farrell, A.P., Stevens, E.D.,
707 Cech, J.J., Richards, J.G. (Eds.), Encyclopedia of Fish Physiology: From Genome to

Environment. Academic Press, an imprint of Elsevier, London ; Waltham, MA, pp. 1688–1694.

Shrivastava, J., Ndugwa, M., Caneos, W., De Boeck, G., 2019. Physiological trade-offs, acid-base balance and ion-osmoregulatory plasticity in European sea bass (*Dicentrarchus labrax*) juveniles under complex scenarios of salinity variation, ocean acidification and high ammonia challenge. *Aquat. Toxicol.* 212, 54–69. <https://doi.org/10.1016/j.aquatox.2019.04.024>

Sullivan, G., Fryer, J., Perry, S., 1995. Immunolocalization of proton pumps (H⁺-ATPase) in pavement cells of rainbow trout gill. *J. Exp. Biol.* 198, 2619–2629.

Tang, C.-H., Hwang, L.-Y., Lee, T.-H., 2010. Chloride channel ClC-3 in gills of the euryhaline teleost, *Tetraodon nigroviridis*: expression, localization and the possible role of chloride absorption. *J. Exp. Biol.* 213, 683–693. <https://doi.org/10.1242/jeb.040212>

Tang, C.-H., Lee, T.-H., 2011. Ion-deficient environment induces the expression of basolateral chloride channel, ClC-3-like protein, in gill mitochondrion-rich cells for chloride uptake of the *Tilapia Oreochromis mossambicus*. *Physiol. Biochem. Zool.* 84, 54–67. <https://doi.org/10.1086/657161>

Tang, C.H., Lee, T.H., 2007. The effect of environmental salinity on the protein expression of Na⁺/K⁺-ATPase, Na⁺/K⁺/2Cl⁻ cotransporter, cystic fibrosis transmembrane conductance regulator, anion exchanger 1, and chloride channel 3 in gills of a euryhaline teleost, *Tetraodon nigroviridis*. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 147, 521–528. <https://doi.org/10.1016/j.cbpa.2007.01.679>

Trancart, T., Feunteun, E., Lefrançois, C., Acou, A., Boinet, C., Carpentier, A., 2016. Difference in responses of two coastal species to fluctuating salinities and temperatures: Potential modification of specific distribution areas in the context of global change. *Estuar. Coast. Shelf Sci.* 173, 9–15. <https://doi.org/10.1016/j.ecss.2016.02.012>

Tzaneva, V., Perry, S.F., 2010. The control of breathing in goldfish (*Carassius auratus*) experiencing thermally induced gill remodelling. *J. Exp. Biol.* 213, 3666–3675. <https://doi.org/10.1242/jeb.047431>

Uchiyama, M., Komiyama, M., Yoshizawa, H., Shimizu, N., Konno, N., Matsuda, K., 2012. Structures and immunolocalization of Na⁺, K⁺-ATPase, Na⁺/H⁺ exchanger 3 and vacuolar-type H⁺-ATPase in the gills of blennies (Teleostei: Blenniidae) inhabiting rocky

- intertidal areas. J. Fish Biol. 80, 2236–2252. <https://doi.org/10.1111/j.1095-8649.2012.03277.x>
- Vargas-Chacoff, L., Regish, A.M., Weinstock, A., McCormick, S.D., 2018. Effects of elevated temperature on osmoregulation and stress responses in Atlantic salmon *Salmo salar* smolts in freshwater and seawater. J. Fish Biol. 93, 550–559. <https://doi.org/10.1111/jfb.13683>
- Vasić, V., Momić, T., Petković, M., Krstić, D., 2008. Na⁺,K⁺-ATPase as the target enzyme for organic and inorganic compounds. Sensors 8, 8321–8360. <https://doi.org/10.3390/s8128321>
- Wang, L., Chen, L., Jacob, T.J.C., 2000. The role of ClC-3 in volume-activated chloride currents and volume regulation in bovine epithelial cells demonstrated by antisense inhibition. J. Physiol. 524, 63–75. <https://doi.org/10.1111/j.1469-7793.2000.t01-1-00063.x>
- Yan, J.-J., Chou, M.-Y., Kaneko, T., Hwang, P.-P., 2007. Gene expression of Na⁺/H⁺ exchanger in zebrafish H⁺-ATPase-rich cells during acclimation to low-Na⁺ and acidic environments. Am. J. Physiol.-Cell Physiol. 293, 1814–1823. <https://doi.org/10.1152/ajpcell.00358.2007>

Figure legend

Fig. 1. Box and whisker plot showing the median, minimum and maximum mRNA expression level of *nka α1a* (*atp1a1a*) (A), *nka α1b* (*atp1a1b*) (B), *cfr* (C), *nkcc1* (*slc12a2*) (D), *nhe3* (*slc9a3*) (E), *ncc2a* (*slc12a3-like*) (F), *clc-3* (*clcn3*) (G), *vha-a* (*atp6v1a*) (H), *vha-b* (*atp6v1a*) (I), *rhhg* (J) and *rhcgl* (K) in gills of sea bass exposed to FW and SW at 18 °C (temperate) and 24 °C (warm). mRNA levels were normalized to *ef1α*. Different letters indicate significant differences between conditions (two-way ANOVA followed by a Fisher Least Significant Difference (LSD) post hoc test $p < 0.05$, $N = 8-13$). FW: fresh water; SW: seawater.

Fig. 1S. Mean plasma pH in sea bass exposed to FW and SW at 18 °C (temperate) and 24 °C (warm). Different letters indicate significant differences between conditions (two-way ANOVA followed by a Fisher Least Significant Difference (LSD) post hoc test $p < 0.05$, $N = 8-13$).

Table 1: Sequences and efficiencies of the primers used for qRT-PCR. F: forward primer; R: reverse primer; Sequence ID: identification number from sea bass genome or GenBank identification number.

Sequence ID	Target gene	Primer name	Sequence (from 5' to 3')	Amplicon size	Efficiency	Reference
KP400258	<i>atp1a1a</i>	NKA α 1a F NKA α 1a R	CCTCAGATGGCAAGGAGAAG CCCTGCTGAGATCGGTTCC	146	1.89	(Blondeau-Bidet et al., 2016)
KP400259	<i>atp1a1b</i>	NKA α 1b F NKA α 1b R	AGCAGGGCATGAAGAACAAG CCTGGGCTGCGTCTGAGG	204	1.99	(Blondeau-Bidet et al., 2016)
DQ501276	<i>Cftr</i>	CFTR F CFTR R	GACTGATGCGTTCGGTAG CCTCAATGACATCTCCTTC	215	1.917	(Bodinier et al., 2009)
DLAgn_00080120	<i>slc12a2</i>	NKCC1 F NKCC1 R	TCAGCTCACAGTTCAAGGCC TTGTGGAGTCCATAGCGGC	102	2.08	(Lorin-Nebel et al., 2006)
JN998891	<i>clcn3</i>	CIC-3 F CIC-3 R	CAAGTACAGCAAGAACGAGGC ACAGCGTCTTGAGAGGGAAG	146	2.069	(Bossus et al., 2013)
DLAgn_00204050	<i>slc9a3</i>	NHE3 F NHE3 R	GGATACCTCGCCTACCTGAC AAGAGGAGGGTGAGGAGGAT	251	1.98	(Blondeau-Bidet et al., 2019)
DLAgn_00038210	<i>slc12a3-like</i>	NCC2a F NCC2a R	ATGATGAGCCTCTTCGAGCC ACAGAAGGTGATGAGAGCAGC	278	1.94	
DLAgn_00076370	<i>atp6v1a</i>	VHA-A F VHA-A R	GGCAGTCACATCACAGGAGG CCAGCTCCATCACCACATCG	154	1.98	
DLAgn_00018050	<i>atp6v1b2</i>	VHA-B F VHA-B R	TTGCCATAGTCTTCGAGCC CTTCTCGCACTGGTAGGC	194	1.90	
DLAgn_00222650	<i>Rhbg</i>	RHBG F RHBG R	CCTCATGGTGACCCGAATCC TATGTGGACAGAGTGCAGGC	218	1.97	
DLAgn_00166370	<i>rhcg1</i>	RHCG1 F RHCG1 R	TCAGGGAATTGTGTGACCGC CCCAGCGTGGACTTGATTCT	118	2.01	
AJ866727	<i>ef1a</i>	EF1-F EF1-R	GGCTGGTATCTCTAAGAACG CCTCCAGCATGTTGTCTCC	239	2.09	(Lorin-Nebel et al., 2006)

Table 2: Two-way ANOVA results of gill gene expression data and plasma pH with salinity and temperature as the main factors. ns: not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. N= 8-13 per condition.

Genes	Interaction	Salinity	Temperature
<i>nka α1a (atp1a1a)</i>	**	**	**
<i>nka α1b (atp1a1b)</i>	Ns	**	ns
<i>cfr</i>	Ns	****	****
<i>nkcc1 (slc12a2)</i>	Ns	***	*
<i>nhe3 (slc9a3)</i>	Ns	**	**
<i>ncc2a (slc12a3-like)</i>	Ns	****	**
<i>clc-3 (clcn3)</i>	Ns	ns	****
<i>vha-a (atp6v1a)</i>	Ns	**	****
<i>vha-b (atp6v1b2)</i>	Ns	ns	*
<i>Rhbg</i>	Ns	ns	ns
<i>rhcg1</i>	Ns	*	****
pH	***	*	****

Table 3: Spearman correlation for gill gene expression vs plasma Cl^- , Na^+ and Na^+/Cl^- ratio (these data have been obtained from the same fish obtained in Masroor et al. (2018)). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. N= 8-13 per condition.

Genes	Cl^- level (mmol.L ⁻¹)	Na^+ level (mmol.L ⁻¹)	Na^+/Cl^- ratio
<i>nka a1a (atp1a1.a)</i>	-0.2798	-0.2858	-0.02371
<i>nka a1b (atp1a1.b)</i>	0.3303*	-0.01329	-0.3031*
<i>Cftr</i>	0.5442***	0.07930	-0.3796*
<i>nkcc1 (slc12a2)</i>	0.4615**	-0.05339	-0.3365*
<i>nhe3 (slc9a3)</i>	-0.3059*	-0.3437*	0.005587
<i>ncc2a (slc12a3-like)</i>	-0.3913**	-0.4037**	-0.02552
<i>clc-3 (clcn3)</i>	0.1989	-0.2980	-0.4097**
<i>vha-a (atp6v1a)</i>	0.4903***	-0.2927	-0.6471****
<i>vha-b (atp6v1b2)</i>	0.2822	-0.2027	-0.3201*
<i>rhbg</i>	0.1040	-0.09353	-0.07771
<i>rhcg1</i>	-0.1198	-0.3655*	-0.1780

Table 4 : Fold changes for mean relative mRNA levels regarding genes involved in ion uptake (*nka α1a*, *ncc2a* and *nhe3*), Na⁺/K⁺-ATPase activity, ionocyte density at filament and lamellar level (data have been obtained from the same fish obtained in Masroor et al. (2018)) and plasma pH changes (indicated in % of change of mean pH values). Comparisons are done in sea bass following freshwater (FW) transfer at temperate (18 °C) and warm (24 °C) conditions.

	Gene name	Relative mRNA levels	NKA activity (μmol Pi. mg ⁻¹ protein. h ⁻¹)	Filament ionocytes (number/300 μm)	Lamellar ionocytes (number/300 μm)	Plasma pH
FW transfer at 18 °C	<i>nka α1a (atpl1a1a)</i>	2.23	1.70	1.70	883.62	+5.00
	<i>nhe3</i>	3.19				
	<i>ncc2a</i>	5.20				
FW transfer at 24 °C	<i>nka α1a (atpl1a1a)</i>	0.98	1.68	1.29	9.70	-0.83
	<i>nhe3</i>	1.26				
	<i>ncc2a</i>	1.61				

Fig. 1

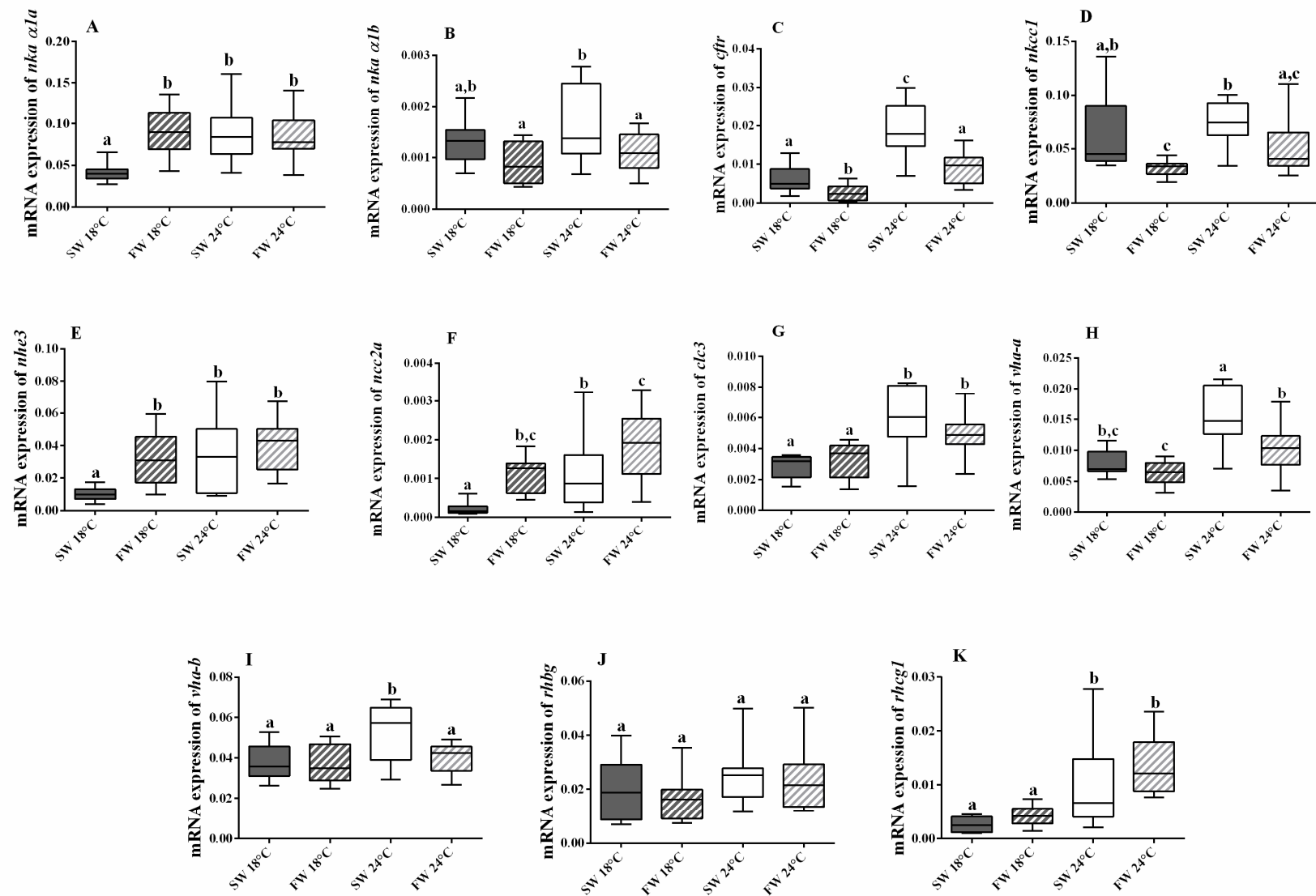


Fig. 1S

