

Article

Narrowing the Range of Environmental Salinities Where Juvenile Meagre (*Argyrosomus regius*) Can Be Cultured Based on an Osmoregulatory Pilot Study

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Abstract: Aquaculture in Europe aims to diversify and optimize fish farming. The meagre (*Argyrosomus regius*) arose as a promising species due to its fast growth rates and flesh quality. Thus, it is currently being produced in several Mediterranean countries, mainly in sea-cages and salt-marshes. However, although meagre naturally spend the first years of life in brackish waters, to date it is cultured in seawater. Here, we show that juveniles may not successfully face either freshwater or hyper-osmotic environments as high as 55 ppt salinity. We found that 55 ppt induced catabolism and mobilization of energy metabolites stored in the liver, probably to maintain its osmotic balance. Furthermore, we found that osmoregulatory tissues such as gills managed to maintain plasma osmolality levels without differences in meagre acclimated at 5, 12 and 39 ppt salinity. Our results demonstrate the euryhaline capacity of this species, highlighting that juveniles may be cultured in a wider range of salinities rather than just at seawater. Future studies should focus on optimal environmental salinities for the growth of *A. regius* juveniles, including long-term experiments limited to the range of 5 ppt to full-strength seawater. Minimizing fish energy consumption in osmoregulation could be economically beneficial for the aquaculture industry in Europe.

Keywords: aquaculture; Argyrosomus regius; juveniles; meagre; osmoregulation; salinity

1. Introduction

Most fish and other aquatic animals inhabit waters with markedly different concentrations of solutes compared to the body fluids [1]. Thus, marine species face continuous dehydration processes due to the high concentration of ions in the seawater, while maintaining their internal environment within a narrow range of ion concentrations [2]. This imbalance with the external medium is achieved through the action of the osmoregulatory tissues, such as the gills, intestine, kidney and skin in teleost fish [3–6]. Marine fish drink seawater, absorb water and ions through their intestinal tract, and the excess of monovalent ions (mostly Na⁺ and Cl⁻) are secreted actively through the branchial epithelium [7,8]. This process occurs in the chloride cells (also called ionocytes or mitochondria-rich



cells) placed in the gills, and the efflux of ions is pumped by the Na⁺/K⁺-ATPase (NKA) enzyme [9]. This ATP-demanding process is costly and requires constant energy consumption.

Marine fish devote around 10–50% of the total energy consumption to osmoregulation processes under normal circumstances [10]. Liver, as the major storage tissue of carbohydrates and lipids in teleosts [11], exports energy metabolites such as glucose and triglycerides to the bloodstream to fuel peripheral tissues [12–14]. Liver stores are thus of great importance to face osmoregulatory disturbances [15]. Amino acid consumption is relevant in fish [16], especially after changes in environmental salinity or any other stressful situation [14,17]. Stress responses in teleost fish includes the release of cortisol into the bloodstream [18]. This hormone is involved in the energy management after short- and long-term changes in environmental salinity in teleosts [19,20] including different European aquacultured species like gilthead seabream, Senegalese sole and wedge sole [21–23].

The meagre (*Argyrosomus regius*) arose as a promising species for aquaculture diversification in several Mediterranean countries [24]. There are many studies focused on different aspects of the culture of this species such as dietary requirements [25–27], stocking density [28], handling procedures [29], and even fillet quality [30]. However, as this is a euryhaline species which naturally spends the first two years of life in estuarine-brackish waters [31,32], and is cultured in salt-marshes with great fluctuations on the environmental salinity throughout the year [33], it should also be relevant to study how environmental salinity affects the osmoregulatory and energy management processes of this species.

The aim of this study was to narrow down the environmental salinities where juvenile meagre can be cultured without negative effects on the osmoregulatory system and, hence, on the energy stores. In an attempt to minimize putative stress imbalances due to inadequate environmental salinities, this study was designed as a pilot study. Thus, the experimental time was reduced to 21 days and a minimum number of fish were used.

2. Results

A. regius juveniles did not tolerate freshwater (100% mortality), but at salinities as low as 5 ppt, they managed to survive for at least 21 days. Osmoregulatory parameters revealed that the 55 ppt group could not maintain body fluid homeostasis, as evidenced by increased plasma osmolality levels (Figure 1). In contrast, the 5, 12, and 39 ppt juveniles presented similar plasma osmolality levels amongst them. As the water osmolality increased with environmental salinity (within the range from 5–55 ppt) fitting in a straight regression line ($R^2 = 0.999$, p < 0.05), and no variations were found in plasma osmolality in fish acclimated to the range of experimental salinities between 5–39 ppt, we substituted the average plasma osmolality calculated within this range (346 ± 1 mOsm kg⁻¹) in the regression line calculated for water osmolality, thus resulting in an iso-osmotic salinity for *A. regius* juveniles of 12 ppt (Figure 1).

Furthermore, branchial NKA activity (Figure 2) was significantly higher in the 55 ppt group as compared to the other groups, while the seawater group was also statistically higher than the iso-(12 ppt) and hypo- (5 ppt) osmotic groups, which did not present major differences between them (p > 0.05).



Figure 1. Water and plasma osmolality in *Argyrosomus regius* juveniles acclimated to different environmental salinities (5, 12, 39, and 55 ppt) for 21 days. Results are expressed as the mean \pm standard error of the mean (SEM) (n = 3 water samples per group; and n = 8-10 plasma samples per group). Different letters indicate significantly different groups for both water (lowercase letters) and plasma (capital letters) groups (p < 0.05, one-way analysis of variance or ANOVA followed by Tukey's post-hoc test).



Figure 2. Gill Na⁺/K⁺-ATPase (NKA) activity in *A. regius* juveniles acclimated to different environmental salinities (5, 12, 39, and 55 ppt) for 21 days. Results are expressed as the mean \pm SEM (n = 7–10). Different letters indicate significantly different groups (p < 0.05, one-way ANOVA followed by Tukey's post-hoc test).

The 39 ppt group presented the lowest plasma cortisol values (p < 0.05), with significant differences in respect to 12 ppt. However, there were no statistical differences in plasma cortisol among the juveniles maintained at 5, 12, and 55 ppt salinity (Figure 3).



Figure 3. Plasma cortisol concentration in *A. regius* juveniles acclimated to different environmental salinities (5, 12, 39, and 55 ppt) for 21 days. Results are expressed as the mean \pm SEM (n = 7). Different letters indicate significantly different groups (p < 0.05, one-way ANOVA followed by Tukey's post-hoc test).

Table 1 shows the differentiated plasma energy metabolite availabilities in juveniles acclimated to different environmental salinities (p < 0.05). Maximum glucose concentration was recorded at 39 ppt, minimum concentrations at 5 ppt, and intermediate concentrations were found at 12 and 55 ppt. Both lactate and protein reached the highest values at 55 ppt, being these values statistically different than those observed in the 39 ppt group. Plasma levels of free amino acids increased in the 5 and 12 ppt groups compared to the 39 ppt group. Finally, plasma concentrations of lipid-related metabolites (triglycerides (TAG) and free fatty acids (FFA)) were enhanced at 55 ppt, with significant differences between fish at 5 and 55 ppt salinity (p < 0.05).

Table 1. Plasma metabolites of *A. regius* juveniles acclimated to different environmental salinities (5, 12, 39, and 55 ppt) for 21 days. Data are expressed as the mean \pm standard error of the mean (SEM) (n = 7-10).

Variable	5 ppt	12 ppt	39 ppt	55 ppt
Glucose (mM)	$2.8\pm0.1~\mathrm{c}$	3.6 ± 0.2 bc	5.1 ± 0.4 a	$3.8\pm0.2b$
Lactate (mM)	$1.4\pm0.2~\mathrm{ab}$	$1.5\pm0.1~\mathrm{a}$	$0.9\pm0.1~\mathrm{b}$	1.5 ± 0.2 a
Proteins (g dL ⁻¹)	$30.1\pm1.2~\mathrm{b}$	$31.5\pm0.6~\mathrm{ab}$	$30.4\pm0.8b$	$33.5\pm0.7~\mathrm{a}$
Amino acid (mM)	$16.0\pm0.8~\mathrm{a}$	$15.7\pm0.4~\mathrm{a}$	$13.1\pm0.5b$	$13.9\pm0.5~\mathrm{ab}$
TAG (mM)	$0.9\pm0.1~\mathrm{b}$	$1.1\pm0.1~\mathrm{ab}$	$1.0\pm0.1~\mathrm{ab}$	$1.4\pm0.1~\mathrm{a}$
FFA (mM)	1.7 ± 0.2 b	$2.6\pm0.2b$	$2.6\pm0.2b$	5.4 ± 0.4 a

Different letters indicate significantly different groups (p < 0.05, one-way ANOVA followed by Tukey's post-hoc test). TAG means triglycerides, and FFA, free fatty acids.

The hepatic parameters of juvenile *A. regius* are shown in Table 2. The hepatosomatic index (HSI) values of the 55 ppt group were significantly lower than those of the 39 ppt group (p < 0.05). Glycogen stores as well as amino acids decreased while free glucose levels increased significantly in the 55 ppt group compared to the other groups (p < 0.05). No major changes in hepatic TAG or FFA were observed for the distinct groups assessed.

Table 2. Hepatic parameters (the hepatosomatic index (HSI) and metabolites) of *A. regius* juveniles acclimated to different environmental salinities (5, 12, 39, and 55 ppt) for 21 days. Data are expressed as the mean \pm SEM (n = 7-10) and refer to the liver as an individual unit of storage.

Variable	5 ppt	12 ppt	39 ppt	55 ppt
HSI (%)	$2.0\pm0.2~\mathrm{ab}$	$1.8\pm0.2~\mathrm{ab}$	2.2 ± 0.2 a	$1.5\pm0.1~{ m b}$
Glycogen (mg liver $^{-1}$)	$36.8\pm2.2~\mathrm{ab}$	$34.1\pm2.2~\mathrm{b}$	$36.6\pm2.8~\mathrm{a}$	$26.6\pm4.0~\mathrm{c}$
Glucose (mg liver $^{-1}$)	$5.1\pm0.3~\mathrm{ab}$	$4.6\pm0.3~\mathrm{b}$	$4.3\pm0.4~\mathrm{b}$	$6.1\pm0.6~\mathrm{a}$
TAG (mg liver $^{-1}$)	4.5 ± 0.3	3.7 ± 0.2	4.9 ± 0.5	4.3 ± 0.2
Amino acid (μ mol liver ⁻¹)	$258\pm16~\text{a}$	$267\pm9~a$	$289\pm18~\mathrm{a}$	$172\pm14\mathrm{b}$

Different letters indicate significantly different groups (p < 0.05, one-way ANOVA followed by Tukey's post-hoc test). TAG means triglycerides.

Changes in weight gain (WG) and condition factor (K) for juveniles acclimated to 5, 15, 39, and 55 ppt salinity over the experimental period (21 days) are shown in Table 3. In all cases, the 55 ppt group showed statistically lower values compared to the other groups (p < 0.05).

Table 3. Changes in weight gain (WG) and condition factor (K) values in *A. regius* juveniles acclimated to different environmental salinities (5, 12, 39, and 55 ppt) for 21 days. Data are expressed as the mean \pm SEM (n = 10). Different letters indicate significant differences among groups (p < 0.05, one-way ANOVA followed by Tukey's post-hoc test).

Variable	5 ppt	12 ppt	39 ppt	55 ppt
WG (%) K (no units)	25.4 ± 10.7 a 0.99 ± 0.01 a	16.9 ± 6.4 a 0.96 ± 0.01 a	20.0 ± 9.9 a 0.99 ± 0.01 a	$-12.6 \pm 7.0 \text{ b}$ $0.89 \pm 0.01 \text{ b}$

3. Discussion

The present study is the first to test and propose a range of appropriate environmental salinities for culture of *A. regius* juveniles. Based on osmoregulatory responses and energy management, future approaches should be limited to the range from 5 ppt salinity to full-strength seawater, as freshwater and hyper-osmotic environments above seawater have detrimental consequences on this life stage. Aquaculture of this species may be relevantly optimized by modifying environmental salinity. For this purpose, long-term experiments should be conducted to better characterize the best environmental salinity for the culture of *A. regius* juveniles and other life stages.

The results of this study show that *A. regius* juveniles managed to maintain their plasma osmolality levels constant within the range from 5–39 ppt salinity. This euryhaline response coincides with what was described in the sciaenid shi drum, *Umbrina cirrosa* [34], the European seabass, *Dicentrarchus labrax* [35] and the sub-Antarctic *Eleginops maclovinus* [36]. However, other euryhaline species cultured in Europe such as *Sparus aurata*, *Dicologoglossa cuneata* and *Scophthalmus maximus* manage to maintain constant plasma osmolality levels within a narrower range of salinities, from brackish water close to the iso-osmotic point of these species to seawater [23,37,38], while *Solea senegalensis*, *Scophthalmus rhombus* and *Pagrus pagrus* show increasing plasma osmolality as environmental salinity increased [39–41].

Plasma osmolality levels are controlled by the osmoregulatory tissues, with branchial NKA activity as an enzyme of paramount relevance [1,9]. This enzyme is placed in the basolateral membrane of specialized branchial cells called chloride-cells, or mitochondrial-rich cells, or ionocytes. These cells actively secrete ions (mainly Na⁺ and Cl⁻) through the branchial epithelium energized mainly by

NKA in hyperosmotic environments, while actively absorb these ions in hypo-osmotic environments in collaboration with other basal/apical ion transporters/channels [41]. In this sense, extreme salinities induce two different NKA profiles depending on the species. Our results evidenced higher branchial NKA activity at higher salinities, as was described for *S. senegalensis* and *S. rhombus* [40,41]. The other NKA activity profile in fish was described in *S. aurata, Mugil liza* and *D. labrax*, showing the lowest branchial NKA activity in salinities close to the iso-osmotic point of these species and increased activities in the extreme salinities [35,37,42]. However, it was described for the sciaenid *U. cirrosa* that juveniles maintained for 84 days within a range of environmental salinities from 4 to 40 ppt, did not show differences in their branchial NKA activity [34]. Changes in the NKA activity in gills are related to the quantity and quality of the NKA subunits in the branchial ionocytes [43,44]. Moreover, several ionocyte populations were described depending on the environmental salinity, with differentiated ion-transport processes aiming at a sharp control of ion fluxes between the blood and the external media [45,46]. All these osmoregulatory changes are under endocrine control, with cortisol as a hormone involved in both hypo- and hyperosmotic acclimation [47].

In this study, plasma cortisol levels were maximum at the highest environmental salinity (55 ppt). Under such an extreme condition, the described high plasma cortisol concentrations in A. regius juveniles may be related to the high NKA activity in the gills. Altogether with the mineralocorticoid functioning of cortisol, that allow teleost fish to modify the osmoregulatory processes and acclimate to different environmental salinities [48], this hormone is also related to chronic stress responses [18]. In this sense, cortisol stimulates several aspects of intermediary metabolism and sustained high levels are interpreted as a signal of distress [49], regulating lipid metabolism and possibly promoting gluconeogenesis [50]. In this line, it was described that chronic stress situations, such as inadequate stocking densities, induced consumption of hepatic stores, increased glycolytic and glycogenolytic pathways, mobilized lipids and increased amino acid consumption in S. rhombus, D. cuneata, Rhamdia quelen, P. pagrus and E. maclovinus [51–55]. These cortisol actions are also reflected in the groups maintained at 5 ppt, but especially in those maintained at 12 ppt salinity. In these groups, plasma cortisol concentration was higher (but not significantly higher) than at 39 ppt (but lower than at 55 ppt), coinciding with lowered HSI and stored liver glycogen. Thus, other groups rather than the seawater-acclimated (39 ppt) group are exposed to long-term energy-consuming conditions. Thus, growing at salinities different than 39 ppt can result in a slight decrease in growth if maintained.

Energy mobilization, as seen by the differentiated plasma free fatty acids, TAG and free protein levels in the 55 ppt salinity group, are in accordance with the previous idea of cortisol acting as an anabolic hormone. Moreover, *A. regius* acclimated to 55 ppt salinity for 21 days showed the lowest glycogen levels and HSI of all groups tested. Glucose is thus mobilized from liver to the plasma at higher salinities, while amino acids and lipids may be consumed from other tissues. According to our results, the idea of energy demand for osmoregulation being minimized in water salinity close to the iso-osmotic condition in fish [10,42,56] should be better studied in the future for *A. regius* juveniles.

The present study, although designed as a pilot study without replicated tanks, show that *A. regius* juveniles maintained at 55 ppt salinity for 21 days decreased their body weights and condition factor. These results are supported by the low glycogen stores in the liver at this salinity, as well as the high concentrations of plasma proteins and lipids, mobilized to fuel energy demanding tissues. Thus, *A. regius* growth seems to be compromised at hyper-osmotic environments such as 55 ppt salinity, while failing to survive in freshwater. Scarce literature has evaluated growth rates of sciaenids cultured at different environmental salinities. It was published that *U. cirrosa* and *Argyrosomus japonicus* juveniles showed better growth rates at full-strength seawater (SW), equal or lower growth at iso-osmotic salinities (10–12 ppt), and displayed the lowest performances at hypo-osmotic environments (4–5 ppt salinity) [34,57]. Moreover, it has been demonstrated that *A. regius* juveniles acclimated to environmental salinities close to the iso-osmotic point for this species (12 ppt) enhanced *igf1* mRNA expression, thus suggesting better growth rates [58]. Altogether, this study

7 of 13

highlights the differentiated responses in the osmoregulatory processes between closely-related fish species, which may have important consequences on their growth at different environmental salinities.

4. Materials and Methods

4.1. Experimental Design

Juvenile *A. regius* were provided by the El Toruño Center for Investigation and Formation in Fishery and Aquaculture (IFAPA, El Puerto de Santa María, Cádiz, Spain) in April 2009. All experimental procedures complied with the guidelines of the University of Cadiz (Spain) and the European Union (86/609/EU) for the use of animals in research, and the Commission of Ethics and Animal Research of the University of Cadiz approved the experiment.

One-year-old seawater-acclimated *A. regius* juveniles (144.67 \pm 5.50 g body weight, mean \pm standard error of the mean, SEM) were transferred to the wet laboratories of the Faculty of Marine and Environmental Sciences (University of Cadiz, Puerto Real, Cádiz, Spain), where they were acclimated to seawater (39 ppt) in circular 1000-L tanks until the start of the experiment. Preliminary experiments indicated that after an abrupt change and five-days acclimation period from 39 to 12 ppt, the minimum salinity at which *A. regius* juveniles could be held with no mortality was 5 ppt. Fish could not tolerate 0 ppt (100% mortality within a few hours) and thus 5 ppt was the lowest salinity investigated in this study.

After 60 days of acclimation, the juveniles (n = 40) were anaesthetized with 2-phenoxyethanol (Sigma, St. Louis, MO, USA; P1126, 0.5 mL L⁻¹, 0.05% v/v), had total length and body weight measured, and were randomly divided into four 400-L tanks (n = 10 juveniles per tank). Three tanks contained SW (39 ppt, 1090 mOsm kg⁻¹ H₂O), and one was kept at a salinity of 12 ppt (364 mOsm kg^{-1} H₂O, presumably close to the iso-osmotic point of this species). After five days, the juveniles from the 39 ppt tanks were transferred to similar tanks with salinities of either 12 ppt (364 mOsm kg^{-1} H₂O; one tank), 39 ppt (1090 mOsm kg^{-1} H₂O; one tank, control), or 55 ppt (1546 mOsm kg^{-1} H₂O; one tank). The group challenged to 55 ppt salinity was included as in the South of Spain, fish are cultured in saltmarshes that could be extremely hypersaline in summer months. Previous studies conducted in the same geographical area, with locally-important species such as gilthead seabream (Sparus aurata), Senegalese sole (Solea senegalensis) and wedge sole (Dicologoglossa cuneata), included an experimental group challenged to 55 ppt salinity, establishing a common ground to compare the results obtained in this study [21,23,59]. In turn, the juveniles first acclimated in the 12 ppt tank were transferred to a tank with a salinity of 5 ppt (140 mOsm kg⁻¹ H₂O). The experimental salinities were assessed in single tanks as this study was designed as a pilot experiment aiming at reducing the number of animals employed. We refused to include more tanks with less fish in each (and therefore augment the statistical independence of the data) as recent studies has shown this species is less stressed in high stocking densities [28] due to its gregarious natural behavior [60]. Each tank had its own water recirculation system equipped with an external filter (Hydor Prime 30; Hydor, Sacramento, CA, USA) to ensure optimal water conditions. Every other day, approximately 20% of the water in each tank was replaced. The experimental salinities were obtained by mixing SW with dechlorinated tap water or by mixing SW with natural marine salt (Salina de La Tapa, Puerto de Santa María, Cádiz, Spain). Juveniles were maintained for 21 days under natural photoperiod (July; latitude 36°31′34′′ N) and water temperature (21–22 °C) conditions. Temperature, salinity, oxygen and ammonia were monitored daily inside the tanks, and no major changes were observed during the experimental period (temperature, 18.7–19.3 °C; oxygen, >5 mg O₂ L⁻¹; and ammonia, 0.0–0.2 mg NH₄ L⁻¹). Juveniles were fed commercial dry pellets once daily in proportion to 1% body weight (Dibaq Diproteg SA, Segovia, Spain). No mortalities were recorded during the experiment.

4.2. Blood and Tissue Sampling

Blood and tissue samples were taken at day 21 post-transfer. Animals were fasted for 24 h before sampling. Juveniles were deeply anaesthetized with 2-phenoxyethanol (1 mL L⁻¹, 0.1% v/v), and were then weighed and sampled. Blood was collected from the caudal peduncle into previously cooled 1 mL ammonia-heparinized syringes. Euthanization was confirmed by severing the spinal cord with a sharp knife. Plasma for each fish was separated from whole blood by centrifugation (3 min, 4 °C at 10,000 × *g*), immediately frozen in liquid nitrogen, and stored at -80 °C until analysis. From each fish, the second gill arch on the left side was excised, dried with absorbent paper, and a small portion was cut using fine-point scissors. These small portions were placed in 100 µL of ice-cold sucrose-EDTA-imidazole buffer (150 mM sucrose, 10 mM EDTA, and 50 mM imidazole, pH 7.3) and frozen at -80 °C until analyses of NKA activity. The liver was excised, weighed, and snap frozen at -80 °C.

4.3. Plasma Measurements

Plasma and water osmolality were measured with a Fiske One-Ten vapor pressure osmometer (Fiske Associates, Advanced Instruments, Norwood, MA, USA). The isosmotic point was estimated according to previous studies [61] as the intersect of the iso-osmotic line and the regression lines between plasma and water osmolality. Plasma glucose, lactate, and TAG levels were measured on 96-well microplates using commercial kits (Spinreact, St. Esteve de Bas, Spain; Glucose-HK Ref. 1001200; Lactate Ref. 1001330; TAG Ref. 100131101). Total plasma proteins were determined using the Bicinchoninic Acid Protein Assay Kit #23225 (Pierce, Rockford, IL, USA) with bovine serum albumin as the standard. Total α -amino acid levels were assessed through colorimetric analysis using the ninhydrin method [62] adapted to microplates and using L-alanine as the standard. Free fatty acids were analyzed with a commercial kit (Wako Chemicals GmbH, (Neuss, Germany) using oleic acid as the standard. All assays were carried out on a microplate reader (Bio-Tek Instruments, Winooski, VT, USA) using the KCjunior Gen5 Data Analysis Software for Microsoft Windows XP. Plasma cortisol levels were measured using an enzyme-linked immunosorbent assay performed in microtiter plates (MaxiSorp, Nunc, Roskilde, Denmark), as previously described for other teleost species [63].

4.4. Gill Na⁺/K⁺-ATPase Activity

Gill NKA activity was determined as previously described [64], with modifications [65].

4.5. Liver Metabolite Levels

Each excised and frozen liver was homogenized as described previously [66]. The homogenate was centrifuged (30 min, $13,000 \times g$, 4 °C), and the supernatants were stored in different aliquots at -80 °C until use in the metabolite assays. Tissue glycogen concentrations were assessed using described methodologies [67]. The levels of glucose after glycogen breakdown (i.e., after subtracting free glucose levels) were determined with a commercial kit (Spinreact, St. Esteve de Bas, Spain). Total α -amino acid and TAG levels were determined as described above for the plasma samples.

4.6. Growth Parameters

The evaluated parameters were weight gain (WG) and Fulton's condition factor (K). At the end of the experimental period (day 21), the hepatosomatic index (HSI) was also calculated. The above parameters were determined as follows:

WG (%) =
$$100 \times ((Wf-Wi) * Wi^{-1})$$

K = $100 \times (Wt Lt^{-3})$
HSI = $100 \times (Wl Wt^{-1})$

where Wt = total wet body weight (g); Wf = final wet body weight (g); Wi = initial wet body weight (g); Wl = wet liver weight (g); and Lt = total body length (cm).

4.7. Statistics

Differences between groups were tested using a one-way analysis of variance (ANOVA) with environmental salinity as the factor of variance. When necessary, data were logarithmically transformed to fulfil the requirements for parametric ANOVA. Normality and homogeneity of variances were analyzed using the Kolmogorov–Smirnov's and the Levene's test, respectively. When ANOVA yielded significant differences, Tukey's post-hoc test was used to identify significantly different groups. Statistical significance was accepted at p < 0.05. All the results are given as mean \pm SEM.

5. Conclusions

The present study, designed as a pilot study, indicates that *A. regius* could be considered as a partially euryhaline species. In this sense, freshwater resulted too much of a challenge, while a hyper-osmotic environment of 55 ppt salinity induced consumption of energy stores and fish failed to osmoregulate properly. However, culture of this species within a range covering 5 ppt to seawater salinity may be further evaluated. Growth rates in the appropriate salinity can be favored, with the consequent economic benefits for the Aquaculture sector in Europe.

Author Contributions: S.C. and J.M.M. designed the experiment. I.R.-J., P.M., L.V.-C. and J.A.M.-S. conducted the experiments and analysis. I.R.-J., L.V.-C. and J.A.M.-S. interpreted the results. I.R.-J. and J.M.M. wrote the draft of the manuscript. All authors critically revised the manuscript.

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