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The isosmotic point as critical salinity limit for growth and osmoregulation, but not survival, in the wolf eel *Anarrhichthys ocellatus*

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Abstract Members of wolf fish family Anarhichadidae have emerged as potential cold-water marine aquaculture species. This study examined growth performance and osmoregulation in juvenile wolf eel (*Anarrhichthys ocellatus*) held in a series of dilute salinities (30, 14, 9, and 6 ‰) over an 8-week trial. At the conclusion of the growth study, fish were sampled for analysis of gill and intestine enzyme activity, plasma ion content, and muscle moisture. Growth rate remained positive in all salinities throughout the 8-week trial. Specific growth rate was maintained above 3.0% mass day^{-1} at salinities of 30 and 14 ‰, but was significantly reduced at 9 (2.9% mass day^{-1}) and 6 ‰ (2.0% mass day^{-1}). Muscle water content increased with increasing salinity dilution (77.9% water in 30 ‰; 79.8% water in 6 ‰), and plasma osmolality (~320 mOsm kg^{-1}) was maintained in salinities as dilute as 9 ‰ but was significantly lower (~280 mOsm kg^{-1}) in the most dilute salinity of 6 ‰. Segmental linear regression analyses revealed that the calculated isosmotic point for wolf eel of ~10.6 ‰ was a critical limit for maintaining growth performance and osmoregulatory homeostasis. It is an important finding that fish considered to be a typical marine stenohaline organism could maintain ion and water balance as low as the isosmotic point, and exhibit survival and positive growth rates in salinities as dilute as 6 ‰. This work delivers a fundamental step in the empirical examination of this emerging aquaculture species and provides a model for evaluating osmoregulatory performance of marine stenohaline fishes in low-salinity aquaculture.

Keywords Ion regulation · Euryhalinity · Salinity · Growth

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Introduction

Members of the wolffish family, Anarhichadidae, have emerged as potential cold-water aquaculture species (Foss et al. 2004; Moksness and Pavlov 2008; Cross et al. 2017). The largest member of this family, the wolf eel *Anarrhichthys ocellatus* (Ayres, 1855), is a slow-moving, bottom-feeding species found in cold, coastal, northern Pacific waters (Beamish et al. 1999; Mecklenburg et al. 2002; Feeney et al. 2007). Throughout its life, the wolf eel inhabits only a very narrow range of salinities, between 30 and 35 ‰ (900–1050 mOsm kg^{-1}),

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which is typical of coastal marine stenohaline fishes.

In the aquaculture of marine species, the use of cost-efficient, near-shore net pens is decreasing due to growing ecological and environmental concerns (Langan 2009; Angel et al. 2019; Atalah and Sanchez-Jerez 2020; Martin et al. 2021; Wiber et al. 2021; Zajicek et al. 2021). Recent advancements in filtration technology have led to the development of land-based, recirculating aquaculture systems to mitigate these concerns (Ahmed and Turchini 2021). Although recirculating aquaculture systems maximize the biomass produced per volume of water used, the costs of artificial sea salt formulations remain consequential. Thus, there is great interest in investigating the low-salinity aquaculture of marine fish to improve the commercial feasibility of land-based aquaculture of marine species (Riche et al. 2012).

Like most marine fishes, wolf eels in seawater hypo-osmoregulate to maintain an internal osmolality below that of their marine environment (Baldisserotto et al. 2019). To accomplish this, marine fish ingest salt water for its water content (Ferreira and Baldisserotto 2019) and utilize mitochondria- and Na^+/K^+-ATPase (NKA)-rich cells on intestine and gill epithelia to actively absorb salt and water in the intestine and secrete excess salts (mainly Na^+ and Cl^-) from the gills (Grosell 2019; Shaughnessy and Breves 2021). This process of hypo-osmoregulation is only effective in salinities above the isosmotic point, which for most fishes is ~ 290 mOsm kg^{-1} (~ 10 ‰). In salinities more dilute than the isosmotic point, fish must hyper-osmoregulate by stopping drinking and actively absorbing (not secreting) Na^+ and Cl^- across the gill epithelium (Ferreira and Baldisserotto 2019). Most marine fishes are stenohaline—they are adapted to hypo-osmoregulate in salinities above the isosmotic point, but are incapable of adapting to the hyper-osmoregulatory demands of surviving in salinities below the isosmotic point (Schultz and McCormick 2013). Interestingly, it has been suggested that reduced osmoregulatory (and thus energetic) demands in salinities near the isosmotic point may be beneficial for growth (Brett 1979; Jobling 1994; Bœuf and Payan 2001; Sampaio and Bianchini 2002). Thus, for the low-salinity aquaculture of stenohaline marine fishes, the isosmotic point may be the lower limit of salinity in which aquaculture is feasible, but it could also produce the best growth rate due to decreased osmoregulatory demands.

The goal of this study was to examine the growth performance and osmoregulatory abilities of the wolf eel in dilute salinities, with a particular focus on the isosmotic point as representing a hypothetically critical salinity threshold for both growth and osmoregulation. Growth performance was examined using metrics such as feed conversion ratio (FCR) and specific growth rate (SGR), and osmoregulation was examined using plasma ion concentrations, white muscle water content, and gill and intestine NKA activity. Our investigation in wolf eel aims to deliver an important step forward in the empirical examination of this emerging alternative aquaculture species, and to inform future work seeking to understand the physiological impacts of low-salinity aquaculture on commercially important marine fishes.

Material and methods

Experimental design

Captive-bred, juvenile wolf eels (source: Vancouver Aquarium Marine Science Centre) were reared in an indoor, flow-through seawater system (30 ‰; 250 L tank volume) under natural photoperiod (July–August, ~ 14L:8D) at the Pacific Science Enterprise Centre of the Department of Fisheries and Oceans in West Vancouver, B.C., Canada. Fish were gradually exposed to one of four varyingly dilute salinity regimens (30, 14, 9, or 6 ‰) achieved by mixing locally sourced marine water (30 ‰) with freshwater (well-water). The target salinities of 30, 14, and 9 ‰ were achieved within 1 week, but two extra weeks were taken to acclimate fish to the most dilute salinity of 6 ‰. In all groups, dissolved oxygen was monitored and remained above 8 mg L^{-1} and temperatures were maintained near 8.5 °C. Exposure to target salinity lasted for 8 weeks. Salinity treatments were run in triplicate—3 tanks per salinity regimen, 25 fish per tank. Fish were hand-fed a commercial feed (2 mm "Black Cod Feed," Taplow Feeds, Vancouver, BC) to satiation twice daily in order to carefully monitor the feed intake and ensure satiation was achieved. As provided by the manufacturer, the ingredients for this commercial feed were fish meal, organic

wheat, fish oil, wheat gluten, and calcium propionate. The major dietary components as listed by the manufacturer were 46% protein; 18% fat; 2% fiber; 10% moisture; 10% ash; vitamins A, D, and E; 1% calcium; 1% sodium; and 0.65% phosphorus. Dry feed intake each day was recorded for later feed calculations. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This study was approved by the DFO Pacific Region Animal Care Committee (protocol #: 12–002).

Sampling

Initially and at the end of each week of the 8-week trial, all the fish from each tank were anaesthetized in a non-lethal dose of MS-222 (50 mg L^{-1}, buffered with sodium bicarbonate) blotted dry, weighed to monitor growth, and then returned to the respective experimental tank. After the 8-week trial, a random selection of 3 fish from each tank ($n = 9$ per salinity treatment) were anaesthetized in a bath containing a lethal dose of MS-222 (100 mg L^{-1}) and sampled for tissue and blood. Blood was collected in a heparinized capillary tube from a severed caudal fin, and then centrifuged for hematocrit ratio analysis and plasma extraction. Gill, intestine, and blood plasma samples were quickly sampled and immediately frozen in liquid nitrogen. White muscle tissue (~0.2 g) was collected from the region above the lateral line just posterior to the head, blotted dry, and placed on an aluminum weigh boat for the determination of water content by dehydration at 60 °C in a drying oven.

Blood plasma parameters

Blood plasma parameters were analyzed in duplicate following manufacturer's protocols as previously described (Shaughnessy et al. 2015). Plasma Cl^- was measured using a digital chloridometer (Haake Buchler Instruments Inc., Saddlebrook, NJ). Plasma Na^+ was measured by emission flame photometry (Jenway PFP7, Bibby Scientific Ltd., Staffordshire, UK). Total plasma osmolality was measured using a vapor pressure osmometer (VAPRO 5600, Wescor, Inc., Logan, UT).

Enzyme activity

Frozen excised gill and intestine samples were prepared on ice in a manual ground glass homogenizer in 750 µL of a homogenizing medium (250 mM sucrose, 10 mM EDTA, 50 mM imidazol, 0.1% sodium deoxycholate; pH 7.5), then centrifuged at 4000 *g* for 2 min. Protein content of the crude homogenates were determined spectrophotometrically (695 nm) using the Bradford protein assay (BioRad Laboratories, Richmond, CA) with a bovine serum albumin standard (Bradford 1976).

NKA activity of the crude gill filament homogenates was determined by closely following previously described methods (Zaugg and McLain 1970; Zaugg 1982; Flik et al. 1983). The assay was performed in duplicate by incubating 10 µL of homogenized tissue in 300 mL of solution designed to either activate (assay solution *A*) or inhibit (assay solution *B*) NKA activity. Assay solution *A* contained (in mM) 156 NaCl, 24 KCl, 3.6 $MgCl_2$, 0.6 EGTA, 50 imidazole, and 0.04 Na_2ATP. Assay solution *B* was identical to *A*, except that 1 mM ouabain was added. After incubation at 20° C for 30 min, the reaction was stopped by adding 500 µL of an ice-cold stopping solution (39.6 mL 0.538 M HCl, 1.29 g ascorbic acid, 2.13 mL 10% ammonium molybdate, and 3.3 mL 20% SDS). Inorganic phosphate (P_i) liberated via hydrolysis of ATP during incubation was measured spectrophotometrically (850 nm) as the phosphomolybdate complex. NKA activity was determined by calculating the difference between P_i liberated in the absence and presence of ouabain and presented as micromoles of P_i per milligram of protein per hour.

Calculations and statistical analysis

Specific growth rate (% body weight day^{-1}) was calculated based on mean initial and final body mass over the 8-week trial using the formula: $SGR = [\ln(mass_{final}) - \ln(mass_{initial})] \div days$. Feed conversion ratio was calculated using the formula: $FCR = feed\ intake \div (mass_{final} - mass_{initial})$. White muscle moisture was calculated as: $Muscle\ moisture = [(mass_{wet} - mass_{dry}) \div mass_{wet}] \times 100$.

Survival was analyzed by chi-square analysis. Mean body mass and relative mean body mass data were analyzed by two-way ANOVA (salinity x time; $n = 3$ tanks per salinity treatment). Physiological data

from terminal sampling after the 8-week trial were analyzed by one-way ANOVA (salinity; $n=9$ fish per salinity treatment). Tukey's post hoc analysis was used to identify differences between salinity groups. The relationships of growth performance (FCR or SGR) or osmoregulation (muscle moisture, plasma ions, NKA activity) with salinity were assessed by regression analysis. Specifically, we sought to test whether growth and osmoregulation were impacted by salinity differently above and below the isosmotic point, which was determined to be 10.6 ‰ based on the plasma osmolality of fish in 30 ‰. Akaike's information criterion (AIC_c) was used to compare two regression models, simple linear regression or segmental (biphasic) linear regression with the break point between the two segments set at the isosmotic point.

All data are presented as mean ± s.e.m. P values are presented in the figures ($\alpha=0.05$). Figure assembly and all statistical analyses were completed using GraphPad Prism 6.0 software (GraphPad Software; La Jolla, CA).

Results

Growth performance

An additional 2 weeks were taken to acclimate wolf eel to the most dilute salinity of 6 ‰. For this reason, wolf eel at this salinity condition had larger starting weights of fish in this salinity (Table 1). All fish across all treatments survived and were in visibly good health except for four individuals in one of the tank replicates for the 6 ‰ treatment at week 7, which were visibly moribund, and thus, the tank replicate had to be removed from the experiment per animal care protocols. Chi-square analyses using either individuals ($n=75$; $X^2_{3,75}=5.119$; $P=0.163$) or tank replicates ($n=3$; $X^2_{3,3}=3.273$; $P=0.352$) as biological replicates determined that survival was not different across salinities in either case. Wolf eels in all salinity treatments gained weight over the 8-week experiment (Fig. 1A), but there were notable differences between salinity treatments. By the end of the trial, wolf eel in 30 ‰ weighed over fourfold their starting weight, whereas wolf eels in 6 ‰ weighed only 2.5-fold their starting weight (Fig. 1B). SGR decreased from 3.22 ± 0.01 in 30 ‰ to 2.02 ± 0.02 in 6 ‰ (Fig. 1C). FCR was lowest in 30 ‰ at 0.78 ± 0.002 and increased with salinity dilution to 1.04 ± 0.008 in 6 ‰ (Fig. 1D).

The relationships between salinity and growth performance (SGR and FCR) were better explained (> 99% probability) by segmental linear regression with the isosmotic point as a break point compared to simple linear regression. Below the isosmotic point of 10.6 ‰, SGR sharply decreased and FCR sharply increased.

Osmoregulation

White muscle moisture increased with salinity dilution from 77.9 ± 0.3% water in 30 ‰ to 79.7 ± 0.5% water in 6 ‰ (Fig. 2A). Likewise, plasma ions decreased with salinity dilution (Fig. 2B–D). Plasma osmolality was approximately 320 mOsm kg^{-1} in wolf eels held at 30, 14, and 9 ‰, and no significant differences in plasma osmolality were detected between these groups (Fig. 2B). Fish held at 6 ‰ had significantly lower plasma osmolality (282.6 ± 10.6 mOsm kg^{-1}) (Fig. 2B). Unlike plasma osmolality, plasma Na^+ and Cl^- concentrations decreased more stepwise between 30 ‰ (Na^+: 144.8 ± 2.5 mM; Cl^- 131.6 ± 1.0 mM) and 6 ‰ (Na^+: 125.8 ± 6.9 mM; Cl^-: 111.6 ± 7.4 mM)

Table 1 Growth metrics for juvenile wolf eel held in 30, 14, 9, and 6 ‰ for 8 weeks

Salinity (‰)	Initial body mass (g)	Final body mass (g)	Mass gain (g)	Total dry feed intake (g)
30	7.97 ± 0.03	48.29 ± 0.25	40.31 ± 0.26	31.53 ± 0.17[a]
14	7.82 ± 0.09	45.17 ± 0.58	37.35 ± 0.49	30.10 ± 0.59[b]
9	7.89 ± 0.24	40.27 ± 1.92	32.37 ± 1.8	28.16 ± 0.98[c]
6	12.76 ± 0.15	43.95 ± 0.55	27.04 ± 0.68	28.25 ± 0.49[c]

Data are presented as mean ± s.e.m. ($n=3$). Different letters indicate significant differences (one-way ANOVA with Tukey's post hoc; $P<0.05$)

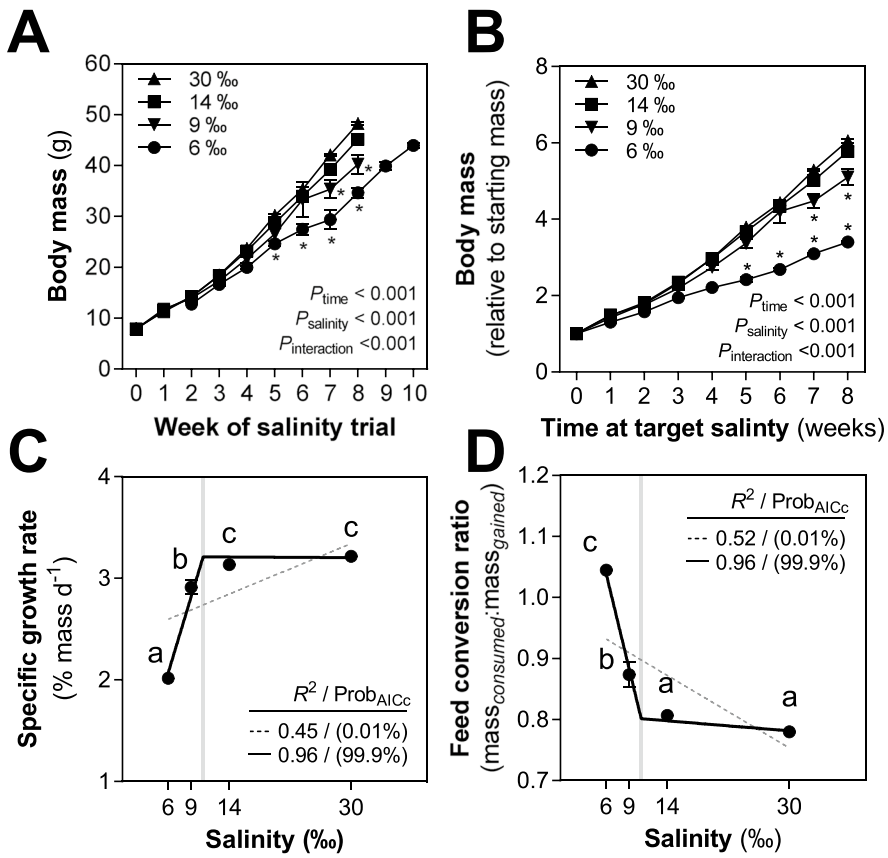

Fig. 1 Growth performance of wolf eel (*Anarrhichthys ocellatus*) reared at different salinities. Mean body mass (**A**) and relative mean body mass (**B**; relative to initial body mass) are shown for juvenile wolf eel held at 30, 14, 9, and 6 ‰ for 8 weeks. Asterisks indicate significant differences from the 30 ‰ treatment (two-way ANOVA with Tukey's post hoc; $n = 3$). Calculated specific growth rate (**C**) and feed conversion ratio (**D**) are shown as a factor of salinity for the 8-week trial. Data in **C** and **D** were fitted by simple (dotted line) and segmental (solid line) linear regression. The calculated isosmotic point for *A. ocellatus* (10.6 ‰) was used as the hypothetical break point for segmental linear regression and is shown as grey vertical bar. The fits were compared using Akaike's information criterion (AIC_c). Coefficient of determination (R^2) and model probabilities ($Prob_{AICc}$) are presented in parentheses (preferred model is denoted by thicker line). Letters on graphs denote statistical differences (one-way ANOVA with Tukey's post hoc; $n = 9$). Data are presented as mean ± s.e.m

(Fig. 2C, D). Interestingly, hematocrit (% red blood cells) was highest at 9 ‰ (26.8 ± 0.6%) and lower at both 30 ‰ (20.4 ± 2.1%) and 6 ‰ (23.0 ± 3.2%) (Fig. 2E).

In every metric of ion and water homeostasis except for plasma Cl^-, the relationships between salinity and ion and water balance were better explained by segmental linear regression with the isosmotic point as a break point compared to simple linear regression. The proportional increase in muscle moisture and decrease in plasma ions with salinity dilution were much greater in salinities below the isosmotic point of 10.6 ‰ than above it.

Gill NKA activity was ~ twofold higher than intestine NKA activity (Fig. 2F). However, unlike ion and water parameters, gill and intestine NKA activities did not change with salinity dilution.

Relationships between salinity, growth, and osmoregulation

Changes in growth performance were tightly correlated ($R^2 > 0.95$) with changes in osmoregulatory homeostasis (Fig. 3A, B). SGR and muscle moisture were strongly negatively correlated ($m = -0.059$; $R^2 = 0.97$; $P = 0.014$) (Fig. 3A). FCR

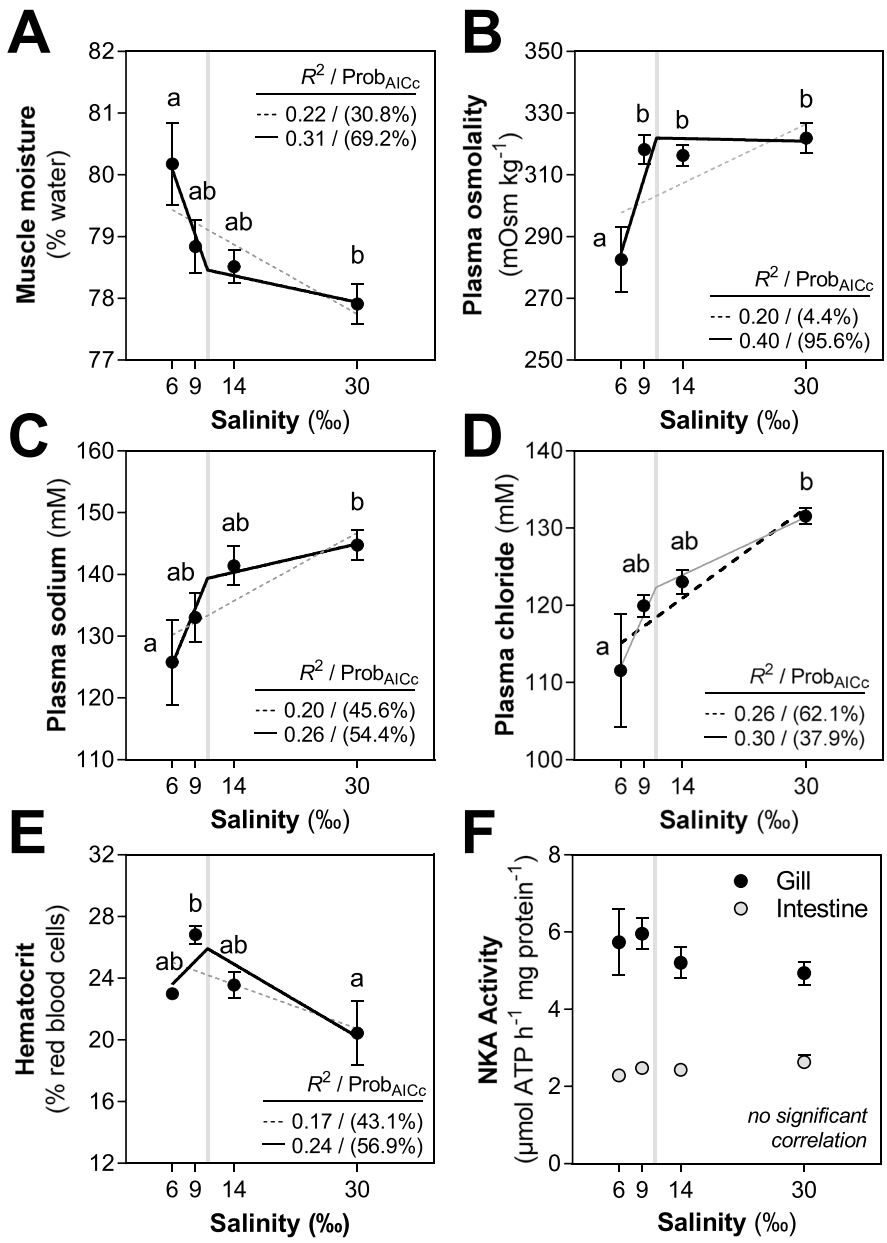

Fig. 2 Osmoregulation in wolf eel (*Anarrhichthys ocellatus*) reared at different salinities. White muscle moisture (**A**), plasma osmolality (**B**), plasma Na^+ (**C**), plasma Cl^- (**D**), hematocrit (**E**), and gill and intestine NKA activity (**F**) in juvenile wolf eel held in 30, 14, 9, or 6 ‰ for 8 weeks are shown as a factor of salinity. See Fig. 1 legend for details on regression analyses. Letters on graphs denote statistical differences (one-way ANOVA with Tukey's post hoc; $n=9$). Data are presented as mean ± s.e.m

and muscle moisture were strongly positively correlated ($m=0.059$; $R^2=0.97$; $P=0.014$) (Fig. 3B).

Discussion

Identifying the impact of dilute salinities on growth in wolf eel delivers an important and necessary step forward in the empirical examination of this emerging aquaculture species. Positive growth rates observed in the present study across all salinity regimens over the 8-week experiment indicate that wolf eel are highly tolerant of dilute salinities. Physiological data demonstrate that suboptimal growth rates in salinity below the isosmotic point correspond with osmoregulatory impairment.

The high ATP usage of the ion-transporting epithelia of the intestine and gills has been cited as evidence that osmoregulating marine fishes commit substantial energetic resources simply to meet the demands

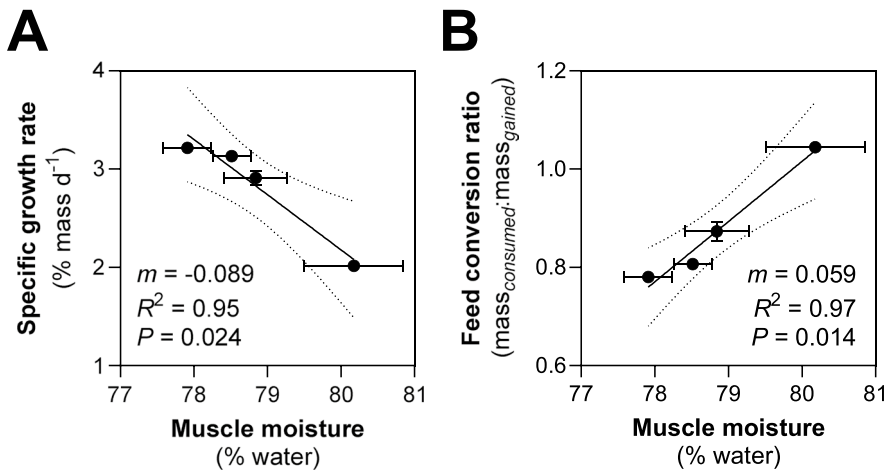

Fig. 3 Relationship between growth performance and osmoregulation in wolf eel (*Anarrhichthys ocellatus*) reared at different salinities. Calculated specific growth rate (**A**) and feed conversion ratio (**B**) are shown as a factor of white muscle moisture. Data are analyzed by simple linear regression analysis (thick line = fit; dotted line = 95% confidence interval). The slope (*m*), coefficient of determination (R^2), and significance (*P*) of each correlation are given. Data are presented as mean ± s.e.m

of osmoregulation (Brett 1979; Jobling 1994; Bœuf and Payan 2001; Sampaio and Bianchini 2002). This high energetic cost of osmoregulation may limit the energy resources available to other physiological processes. Thus, it has been hypothesized that growth and osmoregulation may be related in this way—that fishes spending more energy resources to meet osmoregulatory requirements would have fewer such resources available for growth (Brett 1979; Jobling 1994; Bœuf and Payan 2001). It is predicted then that osmoregulating teleost fishes would have the lowest cost of osmoregulation in an isosmotic environment, and thus exhibit better growth performance compared to fishes in salinities further from isosmotic.

Based on data from our study, the apparent homeostatic set point for plasma osmolality in wolf eel was approximately 320 mOsm kg^{-1}, as this osmolality was maintained at 30, 14, and 9 ‰. With full-strength seawater being approximately 1050 mOsm kg^{-1}, we estimated an isosmotic salinity for wolf eel was approximately 10.6 ‰. In the present study, neither a decrease in gill and intestinal NKA activity nor an increase in growth rate at 14 or 9 ‰ compared to 30 ‰ was observed. Thus, the hypothesis that growth rates are greater nearer to the isosmotic point due to reduced osmoregulatory demands was not supported by our data in wolf eel. Indeed, a universal effect of salinity on growth in fishes may not exist, with optimal salinities for growth varying from hyper-osmotic to hypo-osmotic depending on the species (Bœuf and Payan 2001). Of the 22 species reviewed by Brett (1979), only 4 showed an optimal salinity range for growth within an isosmotic range between 7 and 12 ‰. However, it is interesting that wolf eel growth performance in the 50% seawater dilution (14 ‰ treatment) was equal to that in their native salinity range of > 30 ‰. This result reflects a similar result in a closely related species to wolf eel, the spotted wolffish (*Anarhichas minor*), which exhibited no differences in growth across salinities ranging from 12 to 34 ‰ (Foss et al. 2001). Considering the salinity costs of recirculating marine aquaculture, the feasibility of a 50% seawater dilution in the aquaculture of Anarhichadidae could have considerable cost benefits.

The influence of salinity on feeding behavior and FCR, like growth performance, can vary substantially between fish species. In a comparison between 6 stenohaline and euryhaline fishes, Altinok and Grizzle (2001) found that feed conversion efficiency at the isosmotic salinity of 9 ‰ was slightly improved in 3 species and dramatically worse in 2 species. Kang'ombe and Brown (2008) found higher feed conversion efficiency at 10 ‰ than 5 or 15 ‰ in redbreast tilapia (*Coptodon rendalli*), and Imsland et al. (2001) found no effect of salinity on FCR in turbot (*Scophthalmus maximus*). In the present study, wolf

eel exhibited higher dry feed intake at 30 ‰ compared to 9 ‰, which alone might support the hypothesis that there is a greater need to mediate the energetic cost of osmoregulating in salinities further from the isosmotic point. However, the lack of differences in intestine and gill NKA activity levels across salinities prohibits this interpretation. Additionally, FCR increased with salinity dilution, indicating that wolf eel raised nearer to the isosmotic point and below were converting even less food to body weight than wolf eel at higher salinities. It is important to note that the FCR in wolf eel reared in 30 ‰ and 14 ‰ (FCR was ~ 0.8) was quite productive with respect to the FCRs in other fishes, which are approximately 1–2 (Naylor et al. 2009). This is a good indication that wolf eel would be well-suited for aquaculture even when reared at a 50% seawater dilution.

Importantly, we observed significant decreases in growth performance in salinities below the isosmotic point. Segmental linear regression analysis identified the isosmotic point as a critical salinity threshold in which the relationship between growth performance and salinity changes (Fig. 1C, D). This is likely due to the inability of wolf eel to physiologically adapt to salinities more dilute than the isosmotic point, despite a clear ability to survive in and tolerate these dilute salinities. Our analyses of wolf eel osmoregulation across the salinity treatments largely support this interpretation.

The estimated isosmotic point of 10.6 ‰ appeared to be the limit of osmoregulatory control in wolf eel. For nearly all osmoregulatory metrics measured (i.e., muscle moisture, plasma osmolality, plasma Na^+, and hematocrit), the isosmotic point was a critical salinity value at which the relationship between osmoregulation and salinity changed. In salinities above the isosmotic point, osmoregulatory homeostasis was relatively well-maintained, but osmoregulatory control seemed to be lost in salinities below the isosmotic point. Perhaps the most telling metric of this osmoregulatory limit was the changes in hematocrit, which appeared to increase gradually with salinity dilution from 30 to 14 ‰, then sharply increased from 14 to 9 ‰, then sharply decreased from 9 to 6 ‰ (Fig. 2E). This may be due to unregulated red blood cell swelling below the isosmotic point and partial osmotic lysis in the most dilute salinity of 6 ‰. Additionally, it is interesting that white muscle moisture was affected differently by salinity than plasma osmolality. Whereas plasma osmolality remained stable down to 9 ‰, muscle moisture increased stepwise with each salinity dilution. A possible explanation is that protecting plasma osmolality, and thus red blood cell volume, is prioritized over white muscle water content. In this view, the white muscle tissue may be acting as a buffer by incorporating proportionally more water from the dilute environment than the plasma compartment to confer the impressive salinity tolerance observed.

Historically, marine stenohaline fishes have been defined by an inability to maintain ion and water homeostasis outside of a narrow range of salinity. Schultz and McCormick (2013) expanded this view of marine stenohaline fishes by offering an analysis showing that most SW fishes studied to date, many of which are considered marine stenohaline, are tolerant of salinities down to and below the isosmotic point, despite having a narrow halohabitat of full-strength seawater. The present study, demonstrating that wolf eel can survive, feed, and grow in a salinity as dilute as 6 ‰, supports this hypothesis that the salinity tolerance of marine fishes is far broader than their halohabitat would suggest. The close correlation between growth performance and osmoregulation we observed (Fig. 3) clearly suggests the two are related in some way. More detailed mechanistic studies are needed to determine how marine fish can tolerate such low salinities, despite apparent disturbances to osmotic homeostasis.

In conclusion, the present study demonstrates that the stenohaline marine fish, the wolf eel, survives in and tolerates dilute salinities down to and below the isosmotic point, despite a clear shift in growth and osmoregulation at the isosmotic point. Importantly, our work here shows that wolf eel exhibit impressive feed conversion and growth rates in dilute salinities, fulfilling a necessary step as the first empirical examination of this emerging cold-water aquaculture species. We hope this work can serve as a model for future investigations into low-salinity aquaculture of marine species.

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Author contribution CS performed the experiments, analyzed the data, and wrote the original draft of the manuscript.

SB conceived and performed the experiments, facilitated the research, and contributed to the final draft of the manuscript. JB performed the experiments, facilitated the research, and contributed to the final draft of the manuscript. All authors approved the final draft of this manuscript.

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Availability of data and material All data is presented in this manuscript.

Code availability Not applicable.

Declarations

Ethics approval This study was approved by and performed in accordance with the DFO Pacific Region Animal Care Committee (Protocol No: 12–002).

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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