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Effects of ocean acidification on salinity tolerance and seawater growth of Atlantic salmon *Salmo salar* smolts

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Human activity has resulted in increasing atmospheric carbon dioxide (CO_2), which will result in reduced pH and higher levels of CO_2 in the ocean, a process known as ocean acidification. Understanding the effects of ocean acidification (OA) on fishes will be important to predicting and mitigating its consequences. Anadromous species such as salmonids may be especially at risk because of their rapid movements between fresh water and seawater, which could minimize their ability to acclimate. In the present study, we examine the effect of future OA on the salinity tolerance and early seawater growth of Atlantic salmon *Salmo salar* smolts. Exposure to 610 and 1010 μatm CO_2 did not alter salinity tolerance but did result in slightly lower plasma chloride levels in smolts exposed to seawater compared with controls (390 μatm). Gill Na^+–K^+-ATPase activity, plasma cortisol, glucose and haematocrit after seawater exposure were not altered by elevated CO_2. Growth rate in the first 2 weeks of seawater exposure was greater at 1010 μatm CO_2 than under control conditions. This study of the effects of OA on *S. salar* during the transition from fresh water to seawater indicates that elevated CO_2 is not likely to affect osmoregulation negatively and may improve early growth in seawater.

KEYWORDS

carbon dioxide, gill Na^+–K^+-ATPase, osmoregulation, pH, salinity, *Salmo salar*

1 | INTRODUCTION

The acidification of the world's oceans due to carbon dioxide (CO_2) released into the atmosphere from the burning of fossil fuels is an area of rapidly emerging global concern. The oceans are a sink for CO_2 and as atmospheric levels have increased as a consequence of industrialization, more CO_2 has been forced into solution. When dissolved in water, CO_2 forms carbonic acid (H_2CO_3), which in turn deprotonates into carbonate (CO_3^{2-}) and hydrogen ions (H^+), lowering pH. Since the Industrial Revolution the average pH of ocean waters has decreased by 0.1 pH units, corresponding to a 30% increase in H^+ concentration (Raven *et al*., 2005). For 300 million years, average ocean pH has been stable, not less than 0.6 units below today's values. However, by current calculations, average pH will decrease by 0.3–0.4 units in the near-term (50 years) and by approximately 0.7 units by the year 2300 (Caldeira & Wickett, 2003). These represent significant changes in both scale and rate from the typically stable ocean environment.

Understanding the physiological response to environmental alterations is one of the fundamental goals of environmental biology. Studies of physiological responses will help in determining the ability of organisms to respond to environmental changes and in defining their potential for acclimatization and adaptation to the new environment. The effect of ocean acidification (OA) on biological organisms has received increasing attention in the last decade. While acidification is expected to present a challenge to an array of species, the majority of recent work employing environmentally relevant levels of CO_2 has generally focused on the implications for calcifying organisms such as coccolithophores, foraminiferans and pteropods, reef-building corals, molluscs and crustaceans (Orr *et al*., 2005). Although researchers have examined the effects of increased dissolved CO_2 (hypercapnia) on fish physiology in relation to aquaculture for several decades, the levels used in these studies are typically much greater and the time scales much shorter than the changes that are expected from ocean acidification (Ishimatsu *et al*., 2008). Only recently have researchers begun

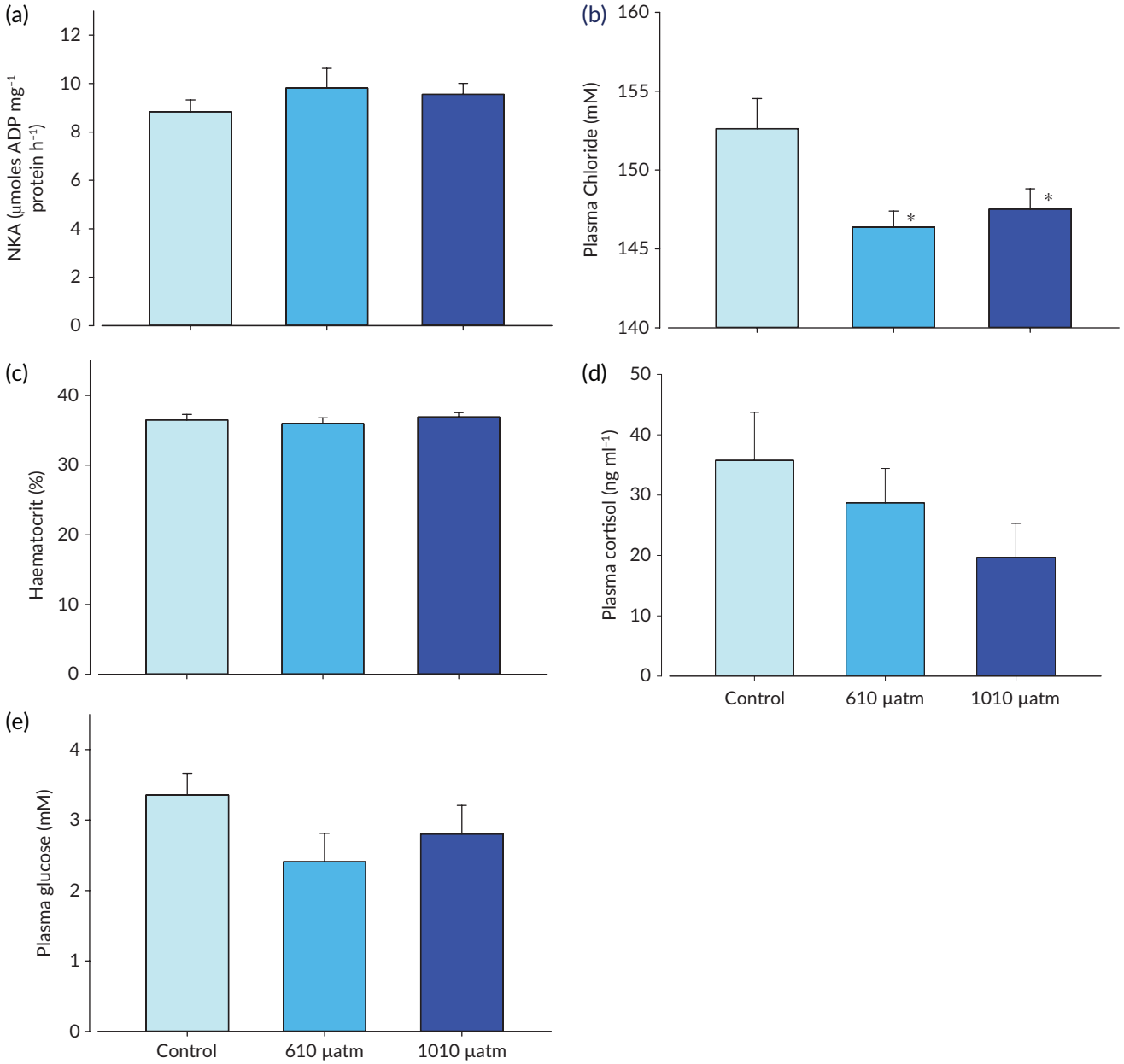

FIGURE 1 Effect (mean + S.E.,) of transferring *Salmo salar* smolts (n = 20–30 fish per group) from fresh water to seawater for 24 h on (a) gill Na^+–K^+-ATPase (NKA) activity CO_2: p = 0.018; Trial: p = 0.13, (b) plasma chloride CO_2: p = 0.30; Trial: p = 0.91, (c) haematocrit CO_2: p = 0.72; Trial: p = 0.92, (d) plasma cortisol CO_2: p = 0.19; Trial: p = 0.16 and (e) plasma glucose CO_2: p = 0.23; Trial: p = 0.17. *Significant difference between samples (p < 0.05); □, control pCO_2 390 μatm

to study the effect of OA conditions on the growth, survival and physiology of fish (Esbaugh, 2017; Heuer & Grosell, 2014).

In most organisms, CO_2 is an end product of metabolism and is mainly excreted utilizing a partial pressure gradient of high internal fluid levels to low levels in the external environment. With elevated pCO_2 levels expected in the oceans, dissolved CO_2 will equilibrate across biological membranes altering partial pressures essential for CO_2 excretion. In addition, almost all the metabolic processes are tightly regulated by proton gradients and most organisms have some ability to buffer any changes to internal pH levels by active regulation of various ion channels (Pörtner *et al*., 2004). This allows some aquatic organisms to acclimate to a range of external pH and CO_2 levels. However, the metabolic demand associated with acclimation may alter the growth and fitness of individuals and to date relatively few studies have examined elevated CO_2 at levels relevant for OA conditions. In reef fishes, elevated CO_2 caused impaired olfactory responses leading to defects in homing ability in the larvae (Munday *et al*., 2009a). In addition, auditory senses and prey–predator interactions were also affected (Simpson *et al*., 2011). These sensory disturbances are causally linked to inhibition of neurotransmitter function (Nilsson *et al*., 2012).

The effect of OA conditions on growth has been examined in a handful of marine species. Decreased growth in response to elevated CO_2 has been demonstrated in yellowfin tuna *Thunnus albacares* (Bonnaterre 1788) (Frommel *et al*., 2016) and fire clownfish *Amphiprion melanopus* Bleeker 1852 (Miller *et al*., 2012). In pink salmon

Oncorhynchus gorbuscha (Walbaum 1792), aerobic scope and growth rate were lower in fish exposed to relatively high levels of CO_2 (2,000 μatm) (Ou *et al*., 2015). Increased growth has been shown in dolphinfish *Coryphaena hippurus* L. 1758 (Bignami *et al*., 2014), summer flounder *Paralichthys dentatus* (L. 1766) (Chambers *et al*., 2014) and orange clownfish *Amphiprion percula* (Lacépède 1802) (Munday *et al*., 2009b) and no detectable effect on growth found in several other species (Bignami *et al*., 2013; Franke & Clemmesen, 2011; Frommel *et al*., 2013). Thus, there is no clear consensus on the growth response of marine or anadromous species to OA. In addition, most of these studies focused on early larval growth and the effects on juvenile growth have not been widely examined.

Anadromous fishes such as salmonids have complex life cycles that involve migration between fresh water and the marine environment. Spawning occurs in fresh water and juveniles migrate to the sea where they reside until they mature into adults and return to fresh water. In salmon, seawater residency varies between 1 and 4 years depending on the species; this period is usually characterized by rapid growth. Thus, the anadromous life cycle is characterized by ion and osmoregulatory homeostasis in response to extreme changes in environmental salinities (Hoar, 1988; McCormick, 2013). In fresh water, loss of ions by diffusion and water gain are counteracted by active uptake of ions and production of dilute urine, while in seawater diffusive ion gain and water loss is countered by active ion excretion and water consumption. The primary sites of ion and osmoregulation in fish are the gill epithelium, the intestinal epithelium and the kidneys (Edwards & Marshall, 2013). In addition, morphological and behavioural changes also play an important role in this migratory life cycle (Hoar, 1988). There is substantive evidence that environmental factors such as photoperiod and temperature are critical for undergoing physiological transformations necessary for development of high seawater tolerance of smolts (McCormick, 2013). The anadromous life history of salmonids necessitates rapid movement between fresh water and seawater, exposing them as juveniles to simultaneous stressors of high salinity and the potential (and currently unknown) consequences of OA conditions. In spite of their high level of salinity tolerance, movement through estuaries and early ocean entry is a time of high mortality for smolts (Thorstad *et al*., 2012) and additional stressors may have compounding effects on salmonid smolt survival and the sustainability of salmonid populations.

Given the economic and ecological importance of salmonids and their unique life-history strategy, it is imperative to understand the physiological effects of OA on salmonids. In the present study, we focus on the initial phase of seawater entry to examine the influence of elevated CO_2 on salinity tolerance and its associated physiological responses. We also examine its influence on early growth rate in the first 2 weeks of seawater exposure. To our knowledge, this is the first examination of predicted OA conditions on fitness-related characteristics and physiological responses in Atlantic salmon *Salmo salar* L. 1758.

2 | MATERIALS AND METHODS

Juvenile (0+) *S. salar* were obtained from the Kensington State Hatchery (Kensington, CT, U.S.A.) and brought to the Conte Anadromous Fish Research Center (https://cms.usgs.gov/centers/lsc) in the autumn, 7 months prior to experiments the following spring. Fish used in this study were from the Connecticut River strain and were the F1 progeny of sea-run returns. Until the initiation of the experiment, fish were maintained in 1.7 m diameter tanks supplied with ambient river water at a flow rate of 4 l min^{-1} and provided with continuous aeration. They were maintained under natural photoperiod (42° 36′ N) conditions and fed to satiation using automatic feeders (Zeigler Bros; www.zeiglerfeed.com). The pH of rearing water varied between 6.47 and 7.45 in the month prior to experiments. Fish used in the experiments were large enough to have become smolts [13.7–16.9 cm fork length (L_F), mass (M) 18–40 g), had a silvery appearance and darkened fin margin characteristic of smolts and had elevated gill Na^+–K^+-ATPase (NKA) activity (Figure 1(a)). Experiments were initiated May 21–28 when smolt development is at its peak for the environmental conditions used in prior rearing (McCormick *et al*., 2000). All experiments were carried out under U.S. Geological Survey Institutional Animal Care and Use Committee Guidelines under protocol No.C09070.

We utilized a pH stat system to manipulate seawater CO_2 and pH conditions (Gattuso *et al*., 2011). Seawater was made by mixing artificial sea salt (Crystal Sea Bio-Assay, Marine Enterprises International LLC; www.meisalt.com) with dechlorinated tap water to a salinity of 32. A pH probe was placed in the rearing tank and attached to a meter which controlled a solenoid valve on a pressurized cylinder containing CO_2 gas. Tubing led from the solenoid valve to a gas diffuser at the bottom of the rearing tank. When pH dropped below 0.02 units of its set point, CO_2 would be slowly bubbled into the fish tank. The CO_2 diffuser was placed directly in front of a small submersible pump which circulated water throughout the tank. Independent pH measurements found that pH was uniform (±0.02 units of target). Aeration from pressurized air through a single 10 × 3 × 3 cm air stone was also present in each tank and remained constant. Oxygen levels were monitored twice daily and were always greater than 85% saturation. Two-point calibration was applied to each pH probe just prior to each experiment and in experiment 2 were checked every 5 days.

Partial pressure of CO_2 (pCO_2) was calculated using total alkalinity (TA) and National Bureau of Standards (NBS) scale pH through CO_2calc software (Robbins *et al*., 2010). Total alkalinity was determined using an open-cell titration method modified from Dickson *et al*. (2007). Samples were collected in 250 ml polypropylene flasks and preserved with 0.05 ml saturated $HgCl_2$. Initial pH of the sample was brought to 3.5 with HCl titrant (0.1 M HCl, 0.6 M NaCl). Each sample was stirred for 10 min to allow for CO_2 degassing. The titration continued with pH being measured after every 0.025 ml addition of HCl titrant. As pH approached the endpoint of 3.0, less volume (0.0125 ml) of titrant was added before each measurement. The Gran function was then used for each data point between pH 3.5 and pH 3.0 and plotted against the total mass of HCl titrant added and the equivalence point (x intercept) was then used to calculate TA.

The first series of experiments were designed to examine the effect of OA conditions on salinity tolerance. A series of three trials were conducted at the peak of smolt development on May 21, 25 and 27. In the first trial pH 8.12 (control) and 7.72 were tested and in trials 2 and 3 pH 8.12 (control), 7.92 and 7.72 were tested (390, 610 and

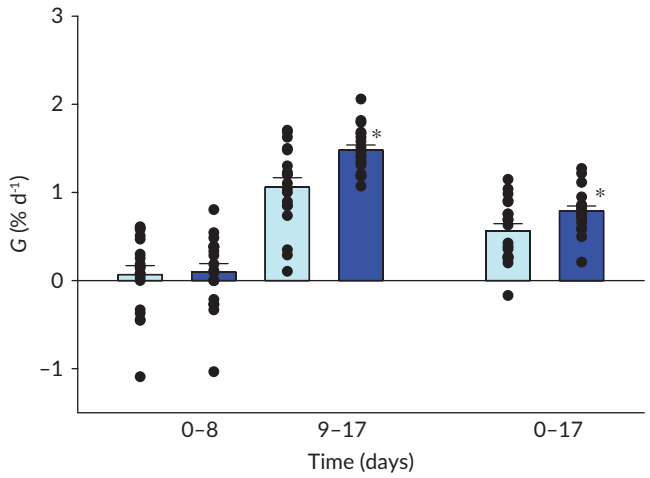

FIGURE 2 Mean (±S.E.) specific growth rate (*G*) of control (□, pCO_2 390 μatm) and CO_2 treated (■, pCO_2 1010 μatm) *Salmo salar* smolts. ●, G of individual fish (18–20 per group); *, significant effect of CO_2 treatment on specific growth rate ($p < 0.05$)

1010 μatm CO_2, respectively). In each trial fish were directly transferred from fresh water to 32 ppt seawater at 14.5–15.6°C, followed by measurement of plasma ion and osmolality levels after 24 hours. This seawater challenge protocol has been widely used to examine developmental changes in salinity tolerance in salmonids (Clarke *et al*., 1996). Ten fish of 13.7 to 16.2 cm L_F were utilized in each pH condition and after 24 h they were sampled as outlined below.

The second experiment was designed to examine the effect of OA conditions on growth rate in the first 17 days after seawater exposure. On May 28, *S. salar* in fresh water (14.1–16.9 cm L_F) were anesthetized using 100 mg l^{-1} MS-222 buffered with $NaHCO_3$ to pH 7.0. Body mass to the nearest 0.1 g, L_F and total length (L_T) to the nearest mm were recorded for each individual and they received a paint mark between the fin rays which allowed for identification of all individuals in each tank. Fish were then transferred to one of four tanks at a salinity of 32, 2 at pH 8.12 (control, 390 μatm) and two at pH 7.72 (1,010 μatm). Temperature was recorded daily and was maintained at 14.8–16.2°C throughout the experiment. After 8 and 17 days fish were anesthetized as above and length and mass measured. Fish were fed twice daily ad libitum throughout the experiment, except for the day prior to inventory. The tanks were isolated from the rest of the laboratory so that the daily feeding was the only disturbance that the fish experienced. At each feeding, fish were carefully observed so that food was offered only until the point where it was no longer being consumed (when one or two pellets would drop to the bottom of the tank). The amount of feed consumed at each feeding was measured to the nearest 0.1 g. Feed was withheld from the fish for 24 h prior to the start of the experiment and at any other time that length and mass were measured.

At the end of each experiment fish were anesthetized with 200 mg l^{-1} MS-222 and *M* and L_F were measured. Blood was drawn from the caudal vessels into a 1 ml ammonium heparinized syringe then placed in a microcentrifuge tube. Blood for haematocrit measurement was collected from this pool into heparanized micro-haematocrit capillary tubes and centrifuged at 13,500*g* for 5 min in a micro-haematocrit centrifuge and read on a micro-capillary reader (Damon/IEC Division, Needham, MA, U.S.A.). The remaining blood was centrifuged at 3,200*g* for 5 min at 4°C, then plasma was aliquoted and stored at −80°C. Gill biopsies of four to six primary gill filaments were placed into 100 μl of ice-cold SEI buffer (250 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3) and frozen at −80°C for measurement of gill NKA activity.

Plasma chloride was analyzed by the silver titration method using a Buchler-Cotlove digital chloridometer (Labconoco; www.labconco.com) and external standards. Plasma glucose was measured by enzymatic coupling with hexokinase and glucose 6-phosphate dehydrogenase (Carey & McCormick, 1998). Plasma cortisol levels were measured by a validated direct competitive enzyme immunoassay as previously outlined (Carey & McCormick, 1998). Sensitivity as defined by the standard curve was 1–160 ng ml^{-1}. The lower detection limit was 0.3 ng ml^{-1}. Using a pooled plasma sample, the average intra-assay variation was 7.2% and the average inter-assay variation was 11.8%.

Gill NKA activity was determined with a kinetic assay run in 96-well microplates at 25°C and read at a wavelength of 340 nm for 10 min as described by McCormick (1993). Gill tissue was homogenized in 150 μl of SEID (SEI buffer and 0.1% deoxycholic acid) and centrifuged at 5,000*g* for 30 s. Two sets of duplicate 10 μl samples were run, one set containing assay mixture and the other assay mixture and 0.5 mM ouabain. The resulting ouabain-sensitive ATPase activity is expressed as μmoles ADP mg^{-1} protein h^{-1}. Protein concentrations were determined using BCA (bicinchoninic acid) protein assay (Pierce; www.piercenet.com). Both assays were run on a THERMOmax microplate reader using SOFTmax software (Molecular Devices; www.moleculardevices.com).

Analysis of Variance (ANOVA) with trial as a nested covariate was used to determine if OA conditions affected physiological parameters after seawater challenge. If significant ($p < 0.05$), Dunnett's test was then used to determine if 7.92 and 7.72 treatments were significantly different from the control (8.12) treatment. Specific growth rate (*G*) in mass was calculated as: $G = 100(\ln M_F - M_0)t^{-1}$, where M_0 is initial mass, M_F is final mass and t is time in days. Analysis of Variance (ANOVA) with tank as a nested covariate was then used to determine if OA conditions affected individual growth rates.

3 | RESULTS

Plasma chloride after 24 h seawater exposure in the control group was < 160 mM, indicative of fish that have completed smolting (Figure 1(b)). CO_2 treated fish had reduced plasma chloride levels compared with controls (CO_2 $p < 0.05$, trial $p > 0.05$, interaction $p > 0.05$) and were significantly lower in both the 610 and 1010 μatm groups. Gill NKA activity in the control group was > 8 μmoles ADP mg^{-1} protein h^{-1}, also indicative that fish had completed smolting (Figure 1(a)). There was no significant effect of CO_2 treatment on gill NKA activity (CO_2 $p > 0.05$, trial $p > 0.05$, interaction $p > 0.05$). Haematocrit also did not differ as a function of CO_2 treatment or trial (CO_2 $p > 0.05$, trial $p > 0.05$, interaction $p < 0.05$) (Figure 1(c)). Plasma cortisol was highest in the control group in two of the three trials, but there was no significant effect of CO_2 treatment (CO_2 $p > 0.05$, trial $p > 0.05$, interaction $p > 0.05$) (Figure 1(d)). Plasma glucose was not

TABLE 1 Effect of 17 days of ocean acidification conditions on plasma chloride, gill NKA activity, haematocrit (Hct), plasma cortisol and glucose of *Salmo salar* smolts (mean ± s.e. n = 18–20 per group)

Treatment (pH/μatm)	Plasma chloride (mM)	Gill NKA activity (μmoles ADP mg^{-1} protein h^{-1})	Hct (%)	Plasma cortisol (ng ml^{-1})	Plasma glucose (mM)
8.12 / 390	145 ± 0.6	18.5 ± 0.8	35 ± 0.8	4.4 ± 1.8	6.1 ± 0.3
7.72 / 1010	141 ± 0.6	19.5 ± 0.7	34 ± 1.0	3.1 ± 0.7	6.8 ± 0.2

significantly affected by CO_2 treatment (CO_2 > 0.05, trial p > 0.05, interaction p > 0.05) (Figure 1(e)).

Experiment 2 examined the effect of CO_2 treatment on growth rate. In the first treatment interval (day 0–8) the mean specific growth rate in the control group was not significantly different from 0, but 3 of 16 fish lost weight (Figure 2). Similar results occurred for the 1010 μatm CO_2 treated fish and there was no significant effect on growth rate in this interval (CO_2 p > 0.05, tank p > 0.05). In the second growth interval (day 8–17) the mean growth rate was positive for both groups and was 40% greater in the 1010 μatm CO_2 treated fish relative to controls (CO_2 p < 0.01, tank p > 0.05). All fish in this interval had positive growth rates. Growth rate of the entire experiment (day 0–17) were also significantly higher for the CO_2 treated fish (40% higher, CO_2 p > 0.05, tank p > 0.05). Food consumption for the 17 day period was significantly greater in the CO_2 treated tanks than in controls (p < 0.01, 0.917 ± 0.002 and 0.834 ± 0.004% body mass d^{-1}, respectively), but food conversion efficiency did not differ (p > 0.05, 76.5 ± 2.0 and 59.6 ± 9.7 g food g^{-1} wet mass, respectively).

At the end of 17 days treatment, 1010 μatm CO_2 treated fish had reduced plasma chloride levels compared with controls (CO_2 p < 0.001, tank p > 0.05) (Table 1). Gill NKA activity in the control group was > 18 μmoles ADP mg^{-1} protein h^{-1}, which was substantially higher (two-fold) than in the seawater challenge trials indicating that the smolts had responded to salinity exposure. There was no significant effect of CO_2 treatment on gill NKA activity (CO_2 p > 0.05, tank p > 0.05). Haematocrit also did not differ as a function of CO_2 treatment (CO_2 p > 0.05, tank p > 0.05). Plasma cortisol was low in all groups and there was no significant effect of CO_2 treatment (CO_2 p > 0.05, tank p < 0.05) (Table 1). Plasma glucose was not significantly affected by CO_2 treatment (CO_2 p > 0.05, tank p > 0.05).

4 | DISCUSSION

In the present study, the effect of future OA conditions on the short term osmoregulatory and growth responses of *S. salar* smolts immediately after exposure to seawater was investigated for the first time. Under the conditions used in this study, results indicate that OA will not negatively affect the survival and ion regulatory ability of *S. salar* smolts. Furthermore, rather than have a detrimental effect, OA conditions may actually improve the growth of *S. salar* in the first 2 weeks after seawater exposure.

The change in plasma ions levels 24 h after direct transfer to seawater has been widely used as an indicator of the salinity tolerance of *S. salar* smolts (Clarke, 1982). We used slightly lower total salinity (32 rather than the typical 35) so that the same salinity could be used in both acute (24 h) and short-term (17 days) studies without incurring mortalities that might occur at higher salinity. The levels of plasma chloride of the control group were low and gill NKA activity was high, indicating that all fish used in the study had achieved the high level of salinity tolerance that is typical of *S. salar* smolts. Neither of the OA conditions tested had negative effects on survival after seawater exposure and both the acute and short term physiological sampling resulted in plasma chloride levels that are typical of fully developed smolts. Thus, our results indicate that under the conditions used in this study, elevated CO_2 that may occur in future OA conditions did not compromise salinity tolerance.

Significantly lower levels of plasma chloride were seen after acute (24 h) and short-term (17 days) OA exposure, though the magnitude of these changes was relatively small. If OA conditions were affecting salinity tolerance itself, we would have expected larger changes in plasma chloride especially during the first 24 h. Previous studies have determined that increased plasma HCO_3^- levels are a common response of teleosts to OA conditions (Esbaugh, 2017) and there is an associated decrease in plasma chloride with increased plasma HCO_3^- in both fresh water and seawater. Thus, we suggest that the lower plasma chloride levels we observed were not an effect of OA on salinity tolerance per se, but rather the effect of OA on a new set point for plasma chloride in seawater.

Since most of the work to date has focused on strictly marine species, to our knowledge previous studies have not examined the simultaneous change in salinity and realistic OA conditions faced by anadromous fish. Several studies have examined the effect of seawater exposure at hypercarbic conditions of a magnitude seen in certain aquaculture settings. In white sturgeon *Acipenser transmontanus* Richardson 1837 exposure to seawater at 6 kPa CO_2 (200 fold higher than current equilibrium CO_2 levels) resulted in higher perturbations in plasma osmolality in the first several days of seawater exposure than at normocarbic conditions (Shaughnessy *et al*., 2015). Interestingly, plasma osmolality and sodium returned to baseline levels after 10 days of seawater exposure, but plasma chloride remained at levels 20 mM below those seen in fresh water or after seawater acclimation. Thus, lower plasma chloride may be a common feature of the simultaneous exposure of euryhaline species to seawater and both moderate and severe hypercarbic conditions. In cod *Gadus morhua* L. 1758 plasma chloride was significantly lower after 4 weeks exposure to both 1200 and 2200 μatm CO_2 (Kreiss *et al*., 2015), whereas in red drum *Sciaenops ocellatus* (L. 1766) there was no significant effect on plasma chloride after 2 weeks exposure to 1000 μatm CO_2 (Esbaugh *et al*., 2016), while plasma Na^+ levels did not differ in either species.

In the present study gill NKA activity was higher in the fish sampled after 17 days in seawater compared with 24 h in seawater, a response that has been seen in previous studies on *S. salar* (McCormick *et al*., 2013). OA conditions, however, did not have a significant effect on this ion-transport enzyme. These results are

consistent with previous studies that have found that transcription and abundance of ion transporters in the gill, including NKA, are not affected by OA conditions (Allmon & Esbaugh, 2017; Esbaugh *et al*., 2016; Michael *et al*., 2016). Esbaugh (2017) has suggested that ion transporters are sufficiently abundant in ionocytes to respond to elevated CO_2 without a transcriptional response. Nonetheless, gill NKA activity in red drum was significantly upregulated after 14 days exposure to 1000 μatm CO_2 (Esbaugh *et al*., 2016), indicating that at least in some species there is plasticity of gill-ion transporters in response to OA conditions.

As noted in the introduction, effects ranging from negative effects, no effect and positive effects of OA on growth have been found in other studies on marine teleosts and to date there are no published studies on anadromous fish. We found that growth rate was higher in *S. salar* smolts exposed to OA conditions (1010 μatm CO_2) in the first 2 weeks of seawater exposure. The mechanism for this increase in growth rate is unclear. Although we did not find a significant effect of OA on food consumption, our power to detect this effect was low since it only occurred at the tank level rather than the individual level that we measured for growth rate. The mean values of food consumption were higher for the OA group and of a similar magnitude of change as that of growth rate, so we cannot rule out the possibility that OA conditions resulted in higher food consumption which in turn resulted in higher growth rate. If this is the case it would be of value for future studies to examine the effect of OA conditions on appetitive behaviour of *S. salar* and other fish whose growth is affected by OA.

Our results contrast with those of Ou *et al*. (2015) who found that OA had negative effects on growth of *O. gorbuscha* smolts. This difference could be explained by difference in pCO_2 levels used in the two studies, which were two-fold higher in the latter study. It should be noted that Ou *et al*. (2015) also found reduced growth in smolts that were exposed to pCO2 that fluctuated daily between 450 and 2,000 μatm. *Oncorhynchus gorbuscha* smolts are much smaller than *S. salar* smolts and this size difference may have also played a role in the different growth responses seen in the two species. Clearly more work will be necessary before we can come to a fuller understanding of OA on growth and physiological responses in salmonids.

Plasma cortisol and glucose were higher in the 24 h seawater exposure groups compared with the 17 day fish, consistent with previous studies that have found short-term increases in these stress variables in the first several days after seawater exposure followed by return to baseline levels (McCormick *et al*., 2013). Plasma cortisol and glucose were not significantly affected after both 24 h and 17 days exposure to OA conditions, indicating that these are not perceived as stressful to *S. salar* smolts. Plasma cortisol has been shown to have negative effects on growth rate in fishes (Small, 2004), therefore it is possible that the elevated cortisol seen in the first 24 h after seawater exposure is related to the low growth rate seen in the first week after seawater exposure. By the same token, the low levels of cortisol seen at the end of the 17 days growth study are consistent with the higher growth seen in this period. Since there was no difference in cortisol between the OA and control groups, cortisol is unlikely to be involved in the higher growth seen in the 1010 μatm CO_2 exposed fish. Haematocrit of *S. salar* smolts was also unaffected by OA conditions, consistent with previous studies on marine teleosts (Esbaugh, 2017) and indicative that at least this variable of respiratory physiology is consistently unaltered by OA conditions. It would be of value to measure other metabolic parameters associated with the transition to seawater under OA conditions. Ou *et al*. (2015) found that decreased aerobic scope was associated with lower growth rate of *O. gorbuscha* exposed to 2000 μatm pCO_2. It would also be valuable to measure hormones associated with growth such as growth hormone and insulin-like factor I to determine if they are associated with and possibly causal to growth changes after exposure to OA conditions.

Our results indicate that under the experimental conditions tested here, OA is not likely to have detrimental effects on salinity tolerance and may improve early seawater growth of *S. salar* smolts. Under natural and aquaculture scenarios environmental conditions may vary, including salinity, temperature and food availability. It will be important to examine the effects of these and other variables to determine their interaction with elevated CO_2. It will also be of interest to determine if the absence of effect on salinity tolerance and possible benefits to growth are common to other anadromous fish species.

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