Physiological resilience of pink salmon to naturally occurring ocean acidification

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Pacific salmon stocks are in decline with climate change named as a contributing factor. The North Pacific coast of British Columbia is characterized by strong temporal and spatial heterogeneity in ocean conditions with upwelling events elevating CO2 levels up to 10-fold those of pre-industrial global averages. Early life stages of pink salmon have been shown to be affected by these CO2 levels, and juveniles naturally migrate through regions of high CO2 during the energetically costly phase of smoltification. To investigate the physiological response of out-migrating wild juvenile pink salmon to these naturally occurring elevated CO2 levels, we captured fish in Georgia Strait, British Columbia and transported them to a marine lab (Hakai Institute, Quadra Island) where fish were exposed to one of three CO2 levels (850, 1500 and 2000 μatm CO2) for 2 weeks. At 1/2, 1 and 2 weeks of exposure, we measured their weight and length to calculate condition factor (Fulton’s K), as well as haematocrit and plasma [Cl−]. At each of these times, two additional stressors were imposed (hypoxia and temperature) to provide further insight into their physiological condition. Juvenile pink salmon were largely robust to elevated CO2 concentrations up to 2000 μatm CO2, with no mortality or change in condition factor over the 2-week exposure duration. After 1 week of exposure, temperature and hypoxia tolerance were significantly reduced in high CO2, an effect that did not persist to 2 weeks of exposure. Haematocrit was increased by 20% after 2 weeks in the CO2 treatments relative to the initial measurements, while plasma [Cl−] was not significantly different. Taken together, these data indicate that juvenile pink salmon are quite resilient to naturally occurring high CO2 levels during their ocean outmigration.

Key words: CO2, commercial fish, Oncorhynchus, Pacific, upwelling

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Introduction

Climate change is causing the oceans to become warmer, more acidic and more hypoxic, as well as increasing the frequency, magnitude and duration of extreme events (Collins et al., 2019, Gruber, 2011, Hegerl et al., 2011, Jentsch et al., 2007). While sea surface pH in the North Pacific is predicted to reach 7.7 by the end of the century, regional oceanographic conditions cause pH levels to drop below these levels today, such as in the California Current System and the Salish Sea.
(Feely et al., 2008, Jiang et al., 2019), and these extremes are expected to intensify with climate change (McNeil & Sasse, 2016, Pacella et al., 2018). This acidification of the oceans has the potential to impact marine organisms from the individual to the ecosystem level (Fabry et al., 2008).

Numerous studies have examined the effects of ocean acidification (OA) on marine organisms in an array of response variables, from gross measures of survival and growth rates, development and behaviour, to calcification, metabolism and gene expression. Responses to high CO2 in fishes, however, vary widely from strong negative effects, to no effects and even positive effects (Cattano et al., 2018).

One of the main reasons for the diversity of responses is likely local variability of ocean climate on small temporal and spatial scales (Helmuth et al., 2014, Waldbusser et al., 2014), such that pre-exposure to naturally CO2-enriched habitats may provide a buffer to future acidification through local adaptation and phenotypic plasticity (Baumann, 2019). This has been demonstrated in the response to CO2 by marine invertebrates inhabiting areas of graded upwelling regimes of the coast of Chile (Vargas et al., 2017). Studies on fish have shown differences in CO2 vulnerability in two populations of Atlantic cod (Gadus morhua): larvae of the Norwegian coastal population that develop in stable, ambient CO2 conditions were vulnerable to near-future OA (Frommel et al., 2012), whereas larvae of the Baltic population that develop in naturally high CO2 water were resilient to levels up to 4000 μatm CO2 (Frommel et al., 2013). Even within a population, seasonal changes in ocean conditions can affect the vulnerability of individuals to experimental OA (Murray et al., 2014). On the other hand, some studies have found species living in naturally enriched CO2 areas to be robust to current levels of CO2, but highly vulnerable to higher levels (Thomsen et al., 2010). Whether organisms living in areas of naturally high CO2 will be more resilient to future climate change or whether the added acidification represents a tipping point is still unclear and likely to be ecosystem, species or even life-stage specific.

The eastern North Pacific is a hotspot for OA, and CO2 levels >1000 μatm are seasonally measured on the continental shelf in association with upwelling, in inland coastal areas, and estuarine habitats (Evans et al., 2019, Feely et al., 2010). The Salish Sea, at the southern end of British Columbia, is a semi-enclosed estuary due to narrow connections with the open Pacific in the north (Queen Charlotte Strait) and south (Juan de Fuca Strait) and a large freshwater discharge, mainly from the Fraser River. Water circulation is driven by tides and winds and is strongly affected by the bathymetry, as well as seasonal cycles with interannual variability in spring and summer pH and CO2 levels from river discharge (Janson et al., 2016, Moore-Maley et al., 2016). Modelling simulations have shown that corrosive (P_{aragonite} < 1) conditions in the Northern Salish Sea are driven by anthropogenic CO2 emissions, were likely absent before 1900 and will likely be exacerbated below biological thresholds for vulnerable species during most of the year (Evans et al., 2019). With the Fraser River watershed, British Columbia has one of the world’s largest salmon-producing river systems. However, since the 1990s, stocks of Pacific salmon have been in decline, with climate change named as one of the main contributing factors (Cohen, 2012, Riddell et al., 2013). Salmon are particularly vulnerable during their early life history, and high mortalities during this time may be responsible for interannual variability and long-term declines in salmon recruitment (Friedland et al., 2000, Mueter et al., 2002). Anadromous salmon migrate out of freshwater into seawater as juveniles, a process that requires complete remodelling of their physiology for osmoregulation, acid-base status, respiration, circulation and metabolism, termed smoltification (Hoar, 1988), to prepare them for life in the ocean (Hoar, 1988, Høgåsen, 1998). High mortality at this life stage has been linked to a combination of suboptimal ocean conditions and poor food availability during an energetically costly life stage (Beamish et al., 2001, McKinnell et al., 2014, Mueter et al., 2005).

Pink salmon (Oncorhynchus gorbuscha) migrate to the ocean at the earliest developmental stage and the smallest size of all Pacific salmon. Their small size creates challenges for osmotic and ion regulation, as they have a high surface area to volume ratio. Additionally, they migrate into seawater prior to completing smoltification and their gill Na⁺/K⁺-ATPase (NKA) activity continues to increase for weeks following seawater entry (Gallagher et al., 2012, Grant et al., 2009). Gill ion- and osmoregulation may be further challenged by elevated environmental CO2 conditions associated with additional acid-base regulatory challenges that may impact subsequent condition and performance. Juvenile pink salmon exhibit up to a 30% reduction in maximum oxygen consumption rate after 2 weeks in high CO2 seawater (1600 μatm CO2) independent of previous freshwater CO2 exposure (10 weeks in either 450 or 2000 μatm CO2), indicating no carry-over effects from the freshwater to seawater phase (Ou et al., 2015). From this study, it was hypothesized that pink salmon may be particularly vulnerable to OA based on their unique life-history strategy.

This is especially relevant for Fraser River salmon, as they experience high CO2 conditions, high temperature and low O2 during their seaward migration. After entering the ocean in early May, a key migration route leads Pacific salmon from the northern Strait of Georgia through Johnstone Strait and Queen Charlotte Strait before entering the Gulf of Alaska (Welch et al., 2011), exposing them to highly varied hydrographical conditions along the way (Fig. 1). The Strait of Georgia is a complex estuarine habitat, with the southern region strongly influenced by the Fraser River plume. This freshwater input lowers the total alkalinity of the water, weakening the buffering capacity for CO2 acidification (Evans et al., 2019). In the central and northern Strait of Georgia, there is a distinct seasonal cycling in pCO2 with warm, stratified and productive waters in summer with CO2 levels around 300 μatm and highly mixed in winter with pCO2 levels around 700 μatm (Evans et al., 2019). However, this
system is also characterized by periodic upwelling events due to strong northwesterly winds, when $pCO_2$ levels above 1000 μatm can be measured at the sea surface in winter and spring during peak salmon migration (Tortell et al., 2012). After leaving the Strait of Georgia, the salmon pass a sill into the Discovery Islands, where the water is persistently deeply mixed. Cold, dense and CO2-rich deep water is mixed through the surface and this region has been termed the tidal mixing zone (TMZ). The TMZ is particularly challenging both in terms of oceanographic and ecological conditions where persistent winds and tidal mixing elevate the $pCO_2$ of the entire water column (Evans et al., 2019), as well as create inadequate food supply (Stormer et al., 2016, Thomson et al., 2012). Field data shows that juvenile salmon have low to no foraging success in the TMZ (Discovery Islands and Johnstone Strait), due to unfavourably small prey (James et al., 2020) leading to mean gut fullness index (GFI, to % body weight) of 0.41% in pink salmon across the TMZ with up to 40% empty guts (Fladmark, unpublished). Based on tagging studies with sockeye salmon (Rechisky et al., 2017) and swim speeds of juvenile pink salmon (Nendick et al., 2011), travel time from the entrance to the TMZ to the exit of the Johnstone Strait is estimated to be a minimum of 2 weeks for juvenile pink salmon, exposing them to these unfavourable conditions for the duration of our experiment.

To investigate the effects of ocean acidification on juvenile salmon physiology during their migration through naturally CO2 enriched waters, we caught wild juvenile pink salmon at the entrance to the TMZ and exposed them to three levels of CO2 (850, 1500 and 2000 μatm CO2) for 2 weeks at the Hakai Institute’s Quadra Island Field Station experimental facility. Fish were sampled at regular intervals to determine condition, haematocrit and plasma [Cl−] to gain insight into osmoregulatory and acid-base status. At each sampling time, fish were subjected to additional stressors associated with climate change—namely, thermal and hypoxia challenge, to provide a more comprehensive picture of the overall physiological condition of the fish exposed to these CO2 treatments.

Methods

Fish capture and husbandry

Juvenile pink salmon (500 fish, 67–87 mm fork length, 2.75–4.3 g body weight) were caught with a juvenile fish seine in Granite Bay (50°15’27.3”N 125°20’50.1”W) off the west coast of Quadra Island in the TMZ of the Discovery Passage between the Strait of Georgia and the Johnstone Strait. Fish
were held in aerated water in a large holding tank and immediately transported back to the Marna Laboratory at the Hakai Institute's Quadra Island Field Station. Here, 40 fish were placed in each of 9 large (90 L) holding tanks, each provided with flow-through (300 ml min\(^{-1}\)) filtered and UV sterilized seawater taken directly from the bay (at 20 m depth) in front of the lab. Three of the 90 L large holding tanks were held within each of three large water baths (5000 L) maintained at 13°C using industrial chillers. The salinity remained between 29 and 30 for the duration of the experiment in all 90 L holding tanks. Before the start of the experiment, fish were allowed to acclimate to the 90 L holding tanks for 24 h after which a baseline sample was taken from 29 fish for wet weight, length, blood, haematocrit and plasma (see below). After 24 h, each of the 9–90 L tanks was randomly assigned one of three CO\(_2\) treatments: control CO\(_2\) (compressed air only), medium CO\(_2\) (1500 \(\mu\)atm CO\(_2\)) and high CO\(_2\) (2000 \(\mu\)atm CO\(_2\)), each replicated three times. Target CO\(_2\) levels were achieved using mass-flow controllers bubbling premixed air/CO\(_2\) at 0.8 L min\(^{-1}\) into each tank. The fish were held for 2 weeks under these conditions with measurements of thermal and hypoxia tolerance conducted at \(1/2\), 1 and 2 weeks of CO\(_2\) exposure (see below). The fish were not fed during this 2-week exposure to mimic poor natural ocean feeding conditions (Fassbender, unpublished), as well as to prevent confounding effects of potential CO\(_2\)-induced differences in feeding success. No mortalities (outside of scheduled sampling-induced mortalities) occurred during the course of the experiment. Water was measured twice a day (morning and evening) for temperature, salinity, pH and O\(_2\) using a handheld multimeter (VWR H30PCD), and oxygen saturation remained above 92% in all tanks (mean 96% ± 4.3% O\(_2\)). Ammonia levels were measured three times a week with a water test kit and remained below 0.1 mg/L in all tanks. Water samples were taken at the beginning, middle and end of the experiment and analyzed for carbonate chemistry using a Burke-o-Lator \(pCO_2/TCO_2\) analyzer (BoL, Dakunalytics, LLC) following the approach described in Evans et al., 2019. Briefly, \textit{in situ} \(pCO_2\) and pH\(_{Total}\) were calculated from directly measured \(pCO_2\), TCO\(_2\), temperature and salinity using CO2SYS with the carbonic acid dissociation constants (K1 and K2) from Lueckert et al., 2000; the KHSO4 dissociation constant from Dickson et al., 1990; the boron/chlorinity ratio from Uppstrom, 1974; and the aragonite solubility constant from Mucci, 1983. Control water was higher than the global average of 400 \(\mu\)atm due to the water being taken out of the Bay in the TMZ that is persistently CO\(_2\)-enriched. For a summary of the carbonate parameters, see Table 1.

**Condition (Fulton’s K)**

Wet weight and fork length were measured in all fish sampled. The Fulton’s condition factor (\(K\)) was determined by dividing the weight by the cubed length.

**Haematocrit and plasma [Cl\(^{-}\)]**

A subset of five fish per tank were randomly sampled at 1, 4, 8 and 14 days of CO\(_2\) exposure (referred to as baseline, \(1/2\), 1 and 2 weeks from this point forward) and euthanized with an overdose of buffered MS222 (500 mg/L). The caudal peduncle was severed, and blood was collected from the caudal vein directly into 75-mm heparinized haematocrit tubes in triplicate per fish. Tubes were spun down with a haematocrit centrifuge and callipers were used to measure the height of packed red blood cells (RBCs) which was divided by the total height of RBCs and plasma combined to yield haematocrit. The haematocrit tube was scored at the interface of the RBCs and plasma, and plasma was expelled into 0.5 ml of microcaps and frozen in liquid nitrogen for later measurement of plasma [Cl\(^{-}\)]. Immediately prior to analysis, plasma samples were thawed and centrifuged. Plasma [Cl\(^{-}\)] was measured in duplicate (provided there was sufficient plasma) with coulometric titration (ChloroChek\textsuperscript{®} Chloridometer\textsuperscript{®}).

**Acute upper thermal tolerance**

Acute upper thermal tolerance was measured by the critical thermal maximum (CT\(_{\text{max}}\)) using the standard methodology for fish (Beitinger et al., 2000). On Days 1, 3, 7 and 13 of CO\(_2\) exposure (referred to as baseline, \(1/2\), 1 and 2 weeks from this point forward), a subsample of five fish per tank (sampled in random order) were transferred to 3 × 20 L plastic aquaria filled with water from their respective CO\(_2\) treatment and covered with black foil to reduce stress. An air-stone supplied from the respective mass-flow controllers was used to keep \(pCO_2\) relatively constant as the temperature was increased, as well as to help mix the water to create a homogeneous water temperature throughout the tank. After a 1-h acclimation period, the previously inserted heat stick was turned on, and the water was heated at a constant rate of 0.3°C min\(^{-1}\) until loss of equilibrium (LOE) (Jung, 2018). The temperature was measured using high-accuracy digital thermometers (Hanna Checktemp1) with values manually recorded every 5 min to

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**Table 1**: Mean +/− SD carbonate chemistry parameters: temperature, salinity and pH\(_{Total}\) from bi-daily measurements with a hand-held multimeter (VWR) TCO\(_2\) and \(pCO_2\) measured at distinct sampling intervals with the BoL; TA calculated with CO2SYS

| Treatment | Temperature (°C) | Salinity | pH\(_{Total}\) | \(pCO_2\) (μatm) | TCO\(_2\) (μmol/kg) | TA (μmol/kg) |
|-----------|-----------------|----------|---------------|------------------|------------------|--------------|
| Control   | 13.07 ± 0.61    | 30.70 ± 0.11 | 7.72 ± 0.02   | 856.70 ± 73.85   | 1996.16 ± 10.39  | 2050.73 ± 9.77 |
| Medium    | 13.14 ± 0.54    | 30.70 ± 0.06 | 7.53 ± 0.02   | 1520.47 ± 133.22 | 2080.29 ± 17.33  | 2074.53 ± 7.41 |
| High      | 12.94 ± 0.50    | 30.72 ± 0.15 | 7.41 ± 0.03   | 2042.33 ± 235.99 | 2106.13 ± 24.48  | 2064.94 ± 11.06 |
track the rate of heating. The end-point LOE was determined when the individual fish was not able to right itself when gently prodded with forceps, at which point the temperature (CTmax) and time were noted, and the fish was quickly removed and euthanized with an overdose of buffered MS222 (500 mg/L).

**Hypoxia tolerance**

Hypoxia tolerance was determined from the incipient lethal oxygen saturation (ILOS) (Claireaux and Chabot 2016). A subset of five fish per CO2 treatment tank were sampled on Days 1, 3, 7 and 13 of CO2 exposure (referred to as baseline, 1/2, 1 and 2 weeks from this point forward) and transferred to a 20 L covered plastic aquaria with water from the respective CO2 treatment tank. After a 1-h acclimation, medical grade nitrogen was bubbled into the tank via an air-stone to reduce the water oxygen saturation by ~0.3% min⁻¹ (Jung, 2018). Oxygen levels were measured with a calibrated portable O₂ meter (YSI) and recorded every 5 min to monitor the rate of O₂ decrease. The incipient lethal oxygen saturation (ILOS) was determined as percent oxygen saturated air (100% O₂ refers to air saturation) at which the fish lost equilibrium and was not able to right itself in response to gentle prodding with forceps. At this point, % O₂ and the time were noted, and the fish was quickly removed and euthanized with an overdose of buffered MS222 (500 mg/L).

**Statistics**

All statistics were performed in R (R Core Team, 2018), and graphics were created using ggplot. P-values were generated using MANOVAs and Pillai’s trace test to account for type 1 error with small sample sizes. Post hoc comparisons were run on separate sampling days. Beginning (baseline) and final values (after 2-week CO2 exposure) were compared for each treatment with an effect size analysis, using the natural logarithm of the response ratio (LnRR) and calculating 95% confidence intervals (Hedges & Olkin, 1985).

**Animal care**

This study was carried out under strict accordance with the animal care protocol approved by the University of British Columbia Animal Care Committee (certificate # A15-0266). Fish were collected under the Fisheries and Oceans permit number XR 63 2019.

**Results**

**Mortality**

No mortality occurred in any of the treatments, and thus, 100% of the fish survived all of the CO2 treatments for the entire 2-week duration of the experiment.

**Condition**

Fulton’s index of condition (Fulton’s K) was significantly affected by both treatment ($F_1 = 15.093; P < 0.001$) and date ($F_1 = 116.836; P < 0.001$). A post hoc test revealed that this was due to the decrease in condition relative to the baseline (Tukey HSD, $p_{adj} < 0.001$ for all treatments). After 2 weeks, K was reduced by 10% in all treatments relative to the baseline (Fig. 2). Excluding the baseline from the ANOVA showed no significant difference in treatment over the course of the
Table 2: Mean ± SD of wet weights for initial sample (start) and after 1/2, 1 and 2 weeks of experiment for three different CO2 treatments

| Treatment | Start     | 1/2 week  | 1 week   | 2 weeks  |
|-----------|-----------|-----------|----------|----------|
| Baseline  | 3.49 ± 0.43|           |          |          |
| Control   | 3.57 ± 0.44| 3.54 ± 0.56| 3.32 ± 0.34|          |
| Medium    | 3.89 ± 0.59| 3.60 ± 0.46| 3.12 ± 0.69|          |
| High      | 3.42 ± 0.58| 3.72 ± 0.53| 3.34 ± 0.45|          |

experiment ($F_2 = 1.58; P = 0.207$). Table 2 summarizes the wet weights for fish throughout the experiment.

**Upper thermal maximum (CTmax)**

Overall, there was a significant effect of treatment ($F_3 = 4.758; P = 0.00388$), days of exposure ($F_1 = 12.921; P > 0.001$) and the interaction (Fig. 3). There was a significant difference between treatments only after 1 week of exposure, with fish in the high CO2 treatment losing equilibrium at a significantly lower temperature than the control and medium CO2 treatments ($F_2 = 14.21, P > 0.001$), indicating a lower thermal tolerance. The weight of the fish was not significant ($F_1 = 0.536, P = 0.466$) when included in the model.

**Hypoxia tolerance (ILOS)**

Overall ILOS increased by an average of 25% from the start to 2 weeks, with a significant difference in treatment ($F_2 = 14.21, P > 0.001$) and the interaction of both ($F_2 = 12.921, P > 0.001$) with the medium CO2 treatment exhibiting a 45% increase in ILOS and the high CO2 treatment a 25% increase. After 1/2 and 2 weeks, the treatment levels were not significantly different to the control group.

**Haematocrit**

Haematocrit was not different between the treatments after 1/2 and 1 week, but mean haematocrit significantly increased by 16% in the high CO2 treatment relative to the control CO2 after 2 weeks of exposure ($F_2 = 4.38; P = 0.0187$).

**Plasma [Cl–]**

There was no significant difference in plasma [Cl–] between the treatments ($F_3 = 1.636; P = 0.186$) or day ($F_1 = 0.444; P = 0.507$).

**Overall effect of the 2-week exposure to elevated CO2 levels**

An effect size analysis of the different CO2 treatments relative to the baseline after 2 weeks of exposure shows the overall effect in the different factors examined (Fig. 7). After 2 weeks, the condition of the juvenile salmon was reduced by a mean of 10% in all treatments relative to the baseline. CTmax was increased by a mean of 1% in all treatments relative to the baseline, while ILOS was significantly increased by a mean ($F_3 = 4.758; P = 0.00388$), day ($F_1 = 47.049; P < 0.001$) and the interaction of both ($F_2 = 12.921; P < 0.001$). A post hoc test showed that, after 1 week of exposure, ILOS in the medium and high CO2 treatments were significantly higher relative to the control ($F_2 = 78.75; P > 0.001$) with the medium CO2 treatment exhibiting a 45% increase in ILOS and the high CO2 treatment a 25% increase. After 1/2 and 2 weeks, the treatment levels were not significantly different to the control group.

![Figure 3: Upper thermal maximum (CTmax) of juvenile pink salmon as measured by the temperature at which the fish lost equilibrium (LOE) at the start of the experiment (baseline, yellow) and after a 1/2, 1 and 2 weeks of exposure to control (C, light green), medium (M, aquamarine) and high (H, dark cyan) CO2 treatments. n = 15. Significance code: 0****0.001.](#)
30, 23 and 18% in the control, medium and high treatment, respectively. Haematocrit was significantly higher in both the medium and high treatments relative to the baseline (mean 17 and 22%, respectively), while the control was not significantly different. Plasma [Cl\(^-\)] levels were not significantly different between the baseline and any treatment after 2 weeks but had a large individual variation.

**Discussion**

Despite our predictions that juvenile pink salmon would be vulnerable to high levels of CO\(_2\) during their energetically costly phase of seaward migration, our data indicate that juvenile pink salmon appear to be quite robust to the levels of CO\(_2\) exposure used in this study. Although the fish were exposed to CO\(_2\) concentrations up to 2000 μatm for 2 weeks in this experiment and not fed, there was no mortality. Their condition factor (Fulton’s K) decreased an average of 10% by the end of the experiment; however, this was not affected by CO\(_2\) treatment (Figure 7) and likely the result of food deprivation. Significant reductions in tolerance to additional acute stressors of high temperature or low oxygen were only seen after 1 week of exposure, but not after 1/2 or 2 weeks. Haematocrit was slightly increased in the high CO\(_2\) treatment relative to the control after 2 weeks of exposure, possibly indicating an enhanced capacity for oxygen transport that may be associated with stress. Elevated haematocrit due to an increase in red blood cells aids oxygen delivery to the tissues. Therefore, higher oxygen delivery to the brain and nervous system may have postponed loss of equilibrium in the ILOS and CTmax trials after 2 weeks in the medium and high CO\(_2\) treatments, leading no significant differences between control and high CO\(_2\) treatments. Plasma [Cl\(^-\)], however, remained unchanged over the experimental period indicating a stable osmoregulatory status with CO\(_2\) exposure. Together, these findings indicate that levels of CO\(_2\) up to 2000 μatm for 2 weeks at this life stage of pink salmon are not associated with physiological impairment as measured in this study and have only a small effect on simultaneous acute stressors.

While many studies find no effects of OA on fish (Cattano et al., 2018), negative effects of elevated CO\(_2\) have been demonstrated for salmon in both fresh- and saltwater for growth and survival, behaviour and olfaction in larval and juvenile stages (Ou et al., 2015, Williams et al., 2019). Ou et al. (2015) showed a decrease in maximal metabolic rate and aerobic scope in smolting pink salmon exposed to 2000 μatm CO\(_2\), irrespective of previous freshwater CO\(_2\) exposure. Since aerobic scope and tolerance to low oxygen and high temperature have been correlated in salmon, with a proposed functional link through oxygen supply (Zhang et al., 2018), we predicted that hypoxia and thermal tolerance may be reduced in salmon at higher CO\(_2\) concentrations.

CTmax was only significantly reduced in the high CO\(_2\) treatment after 1 week of exposure (Figure 3). However, this difference appears to be largely driven by an increased thermal tolerance in the control and medium CO\(_2\) treatment compared to the start and 1/2 week of exposure and thus may signal a lack of adjustment in the high CO\(_2\) treatment compared to the control and medium treatment. Interestingly, this significant difference in thermal tolerance following 1 week of exposure to high CO\(_2\) was also associated with a reduction of hypoxia tolerance. After 1 week of CO\(_2\) exposure, the medium and high CO\(_2\) levels showed a 25 and 45% increase in ILOS, respectively (Figure 4). However, this significant difference was largely driven by a lower ILOS in the control treatment, relative to the medium and high CO\(_2\) exposure. If thermal tolerance and hypoxia tolerance are functionally related, a reduction in thermal performance at high temperatures may be the result of oxygen limitation (Anttila et al., 2013) as is the case for hypoxia tolerance. Thus, while it is interesting that there are significant reductions in both thermal and hypoxia tolerance at 1 week of CO\(_2\) exposure, the basis is unclear and further studies should be conducted to clarify this.

To compensate for a CO\(_2\)-induced acidosis, fish generally elevate plasma [HCO\(_3^-\)] in exchange for [Cl\(^-\)], which is associated with net proton excretion (Brauner et al., 2019). The increase in plasma [HCO\(_3^-\)] during CO\(_2\) exposure is matched with an equimolar reduction in plasma [Cl\(^-\)]. In our study, no differences in plasma [Cl\(^-\)] were detected between CO\(_2\) treatments at any sampling day (Figure 6). As plasma [Cl\(^-\)] was very variable within each treatment and the expected changes in [Cl\(^-\)] at 2000 μatm pCO\(_2\) relative to our control treatment would be <2 mmol/L (Brauner et al., 2019, Heuer et al., 2016), which may be below our ability to detect this change. Plasma [Cl\(^-\)] could also be affected by osmoregulatory status; however, given that no significant changes were observed, it does not appear that this CO\(_2\) level resulted in any negative osmoregulatory effects.

Following a 2-week exposure to high CO\(_2\), there was a statistically significant increase in haematocrit (Figure 5), which may have been due to either an elevation in red blood cell numbers or associated with adrenergic stimulation of red blood cells (Nikinmaa, 1990). The elevated haematocrit could have been the compensatory mechanism improving ILOS and CTmax in the CO\(_2\) treatments after 2 weeks of exposure through enhancing O\(_2\) unloading (Brauner & Wang, 1997, Rimmer et al., 2013). While there was a decrease in hypoxia tolerance by an average of 25% after 2 weeks, compared with the baseline, there was no difference between the treatment levels (Figure 7). This could indicate accumulation of physiological effects of stress in the fish due to confinement, as well as a starvation effect. While loss of equilibrium during CTmax trials seem to be controlled by neural failure (Jutfelt et al., 2019), the end-point of ILOS generally occurs when adenosine triphosphate (ATP) levels in the brain are depleted (Speers-Roesch et al., 2013). As ATP production is highly dependent on glycogen stores, a fish that has not fed for 2 weeks will likely have lower glycogen stores, leading to LOE sooner at higher oxygen concentrations. This was reflected in
Figure 4: Hypoxia tolerance (ILOS) of juvenile pink salmon as measured by the % oxygen saturation (100% O₂ refers to air saturation) at which the fish loses equilibrium (LOE) at the start (baseline, yellow) of the experiment and after a 1/2, 1 and 2 weeks of exposure to control (C, light green), medium (M, aquamarine) and high (H, dark cyan) CO₂ treatments. n = 15. Significance code: 0 "***" 0.001.

Figure 5: Haematocrit in juvenile pink salmon at the start (baseline, yellow) and after 1/2, 1 and 2 weeks of exposure to control (C, light green), medium (M, aquamarine) and high (H, dark cyan) CO₂ treatments. n = 15. Significance code: 0.01 "*" 0.05.

the condition factor that showed an average 10% decrease in all treatments after 2 weeks relative to the baseline (Figure 7). Therefore, the reduction in glycogen stores after 2 weeks without food may be masking more subtle effects of CO₂ exposure. Future studies should include fed fish during CO₂ exposure to investigate the effects of food deprivation and water chemistry on tolerance assays in juvenile salmon.

However, studies looking at the gut contents have shown that like juvenile sockeye (James, 2019), pink and chum feed poorly during their migration through the TMZ, with a large proportion (up to 40%) having empty stomachs throughout most of this region (Fladmark, unpublished data). This has been linked to depressed growth of fish sampled in the Johnstone and Queen Charlotte Strait, indicating a
lower condition compared to salmon caught in the Strait of Georgia (Ferriss et al., 2014, Journey et al., 2018). Suboptimal ocean conditions, an altered Redfield ratio, and low plankton abundance and diversity may all be contributing factors to low salmon foraging success in this area (Mackas et al., 2007, Preikshot et al., 2013, Schweigert et al., 2013). After exiting the TMZ, feeding commences and growth and condition improve (Ferriss et al., 2014). The travel time of juvenile salmon through this region is estimated to be around 2 weeks (the duration of our study), based on telemetry data available for sockeye salmon (Rechisky et al. 2017). Therefore, 2 weeks of elevated CO2 conditions and food deprivation are naturally encountered by juvenile salmon during this section of their migration and may have primed them to compensate these conditions for the duration they experience them in the wild.

Salmonids are well adapted to periods of fasting and are able to fully remobilize metabolic reserves when food becomes available again (Navarro & Gutiérrez 1995). From several studies, we know juvenile salmonids have a high resistance to starvation, with survival up to 13 weeks of
starvation in juvenile chum (Akiyama & Nose, 1980) and actively swimming rainbow trout, (Simpkins et al. 2003), with hormonal and weight responses generally setting in after 2-weeks of food deprivation (Pierce et al. 2001). In a study on the stress response of fasted adult Atlantic salmon, it was found that plasma cortisol levels, a primary stress response marker, were moderately increased after 1 week of fasting but returned to initial values after 2 weeks of fasting (Waagbø et al. 2017). This could explain our significant findings after 1 week, but not 2 weeks. Juvenile chinook and chum salmon have also shown to be resistant to food deprivation, where a 2-week fasting period did not induce mortality or reduce condition factor (Ban et al. 1996, Snyder, 1980). In rainbow trout, 1 week of fasting significantly decreased their O2 consumption and CO2 excretion, while increasing ammonia excretion, however, after 2 weeks of fasting levels were not significantly different to control levels (Lauff and Wood 1996). This may have been similar in our study and may have caused the significant effects seen after 1 week of fasting and CO2 exposure, but not after 2 weeks.

These stocks experience a natural high CO2 zone in the Discovery Islands and Johnstone Strait every year, and they have been using this migration route for years on evolutionary time scales. As the water is so deeply mixed, there is a high degree of inertia on the environment experienced by fish in the TMZs, and the changes that they may experience with climate change is likely mostly in the long-term. Conversely, in the Strait of Georgia to the south and Queen Charlotte Strait north of the TMZ, fish are exposed to fluctuations in ocean conditions on seasonal and inter-annual scales.

It has been shown that fish experiencing naturally elevated CO2 conditions may have adapted to tolerate future global levels of OA (Frommel et al., 2013, Lonthair et al., 2017), and pre-exposure of the adults to high CO2 can have positive effects on the offspring (Allan et al., 2014, Parker et al., 2012). Therefore, pink salmon populations that experience high CO2 levels in their yearly migration may be pre-adapted to high CO2 levels. On a shorter time scale, tolerance to acute stressors is often dependent on pre-exposure to those stressors, and prior rearing in high temperature and low oxygen has been found to protect against acute increases in temperature and hypoxia in salmon early life stages (Del Rio et al., 2019). Studies using hatchery-spawned and reared salmon (Del Rio et al., 2019, Ou et al. 2015, Williams et al. 2019) may lose this phenotypic plasticity and therefore exhibit greater effects to climate stressors in relation to our study with wild-caught fish (Zhang et al., 2016).

Pink salmon are one of the few species of Pacific salmon. Alternatively, their life history may make them more robust to OA. It is also possible that the individuals we captured at Quadra Island are the survivors that have made it through the costly phase of smoltification and therefore are the strongest individuals.

Conclusion

Contrary to our hypothesis, that pink salmon would be vulnerable to suboptimal ocean conditions, due to their small size, high surface to volume ratio and previously documented vulnerability to OA, juvenile pink salmon were not greatly affected by high CO2 conditions in this study. Previous exposure to high CO2 in the Strait of Georgia may have primed them for the CO2 conditions in our experiment, or the fish may have been able to rapidly acclimate to the CO2 levels used in this study that are similar to those already occurring in the TMZ. This study indicates that wild juvenile pink salmon may be robust to current and future OA. Whether this trait is unique for pink salmon, or all wild salmon populations undertaking the migration through high CO2 water, remains to be investigated.

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