



Effects of an unprecedented summer heatwave on the growth performance, flesh colour and plasma biochemistry of marine cage-farmed Atlantic salmon (*Salmo salar*)

Nicholas M. Wade^{a,*}, Timothy D. Clark^{b,c}, Ben T. Maynard^b, Stuart Atherton^d, Ryan J. Wilkinson^e, Richard P. Smullen^e, Richard S. Taylor^b

^a CSIRO Agriculture and Food, Queensland Biosciences Precinct, St Lucia, QLD 4067, Australia

^b CSIRO Agriculture and Food, Castray Esplanade, Hobart, TAS 7000, Australia

^c Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, TAS 7001, Australia

^d Petuna Aquaculture, 134 Tarleton St, East Devonport, TAS 7310, Australia

^e Ridley Aquafeed, 31 Robart Court, Narangba, QLD 4504, Australia

ARTICLE INFO

Keywords:

Temperature
Relative Condition Index
Predicting performance
Astaxanthin
Thermal stress
Aquaculture

ABSTRACT

Global seawater temperatures are increasing and becoming more variable, with consequences for all marine animals including those in food production systems. In several countries around the world, farming of Atlantic salmon (*Salmo salar*) occurs towards the upper end of the thermal tolerance window for this species, and marked effects on salmon production during summers have been experienced but never empirically investigated. This project tracked the effects of an extreme summer heatwave on two different cohorts of fish stocked into farm cages either during early winter (EW) or late winter (LW). The farm site experienced an unprecedented high water temperature event, with a peak water temperature of 22.9 °C and 117 days above 18 °C. Fish in both EW and LW cohorts experienced a temperature-induced cessation of voluntary feed intake as well as inefficient osmoregulatory, liver and renal function during high temperature periods. Flesh colour declined primarily in the dorsal and ventral regions of the fillet and secondarily along the midline, with over 20% of fish demonstrated a complete loss of flesh colour during the months of March and April. A return to feeding in autumn occurred faster in some fish and caused a marked bimodal size distribution to appear within both the EW and LW cohorts as autumn progressed. However, the LW cohort returned to feeding at seawater temperatures of 20.2 °C, compared with 18.6 °C for the EW cohort. There was a strong positive relationship between fillet colour recovery and residual condition index (RCI). These findings identified alkaline phosphatase as a potential marker to non-destructively track individual fish for signs of recovery after a thermal stress event, and shed light on the physiological consequences of marine heatwaves on fishes. This study also identified that supporting feed intake or promoting a return to feeding may help mitigate the negative impacts of climate warming on cultured Atlantic salmon.

1. Introduction

Fishes have a thermal tolerance range in which their growth performance and health is optimal. Atlantic salmon (*Salmo salar*), for example, typically grow optimally between temperatures of 12 °C and 18 °C (Elliott and Elliott, 2010). However, they have been reported to exhibit a 25 to 50% reduction in feed intake and growth as temperature increased to 19 °C or 20 °C (Hevroy et al., 2012; Oppedal et al., 2011). Growth performance drops further as temperatures approach the upper thermal tolerance limit of 22 to 24 °C (Barton, 1996). Similarly,

dissolved oxygen (DO) levels have a compound effect on fish metabolism and growth, such that performance can be impaired as DO drops to or below critical levels (Claireaux and Chabot, 2016; Remen et al., 2016; Vikesa et al., 2017a). Remen et al. (2016) found that post-smolt feed intake declines below 65% oxygen saturation at 15 °C, but at 19 °C the oxygen threshold is increased to 76.5%. An increase in temperature causes a decrease in water oxygen solubility, while also increasing the metabolism and decreasing the hypoxia tolerance of fishes (McBryan et al., 2013; Portner, 2010; Remen et al., 2016). In temperate environments, the combined effects of temperature and hypoxia during

* Correspondence to: Queensland Biosciences Precinct, 80 Services Rd, St Lucia, QLD 4067, Australia.

E-mail address: nick.wade@csiro.au (N.M. Wade).

<https://doi.org/10.1016/j.jtherbio.2018.12.021>

Received 21 May 2018; Received in revised form 1 November 2018; Accepted 24 December 2018

Available online 27 December 2018

0306-4565/ © 2018 Published by Elsevier Ltd.

severe summer heatwaves may challenge the physiological capacity of fish species such as Atlantic salmon and induce stress responses that incur a substantial physiological cost (Oppedal et al., 2011; Vikesa et al., 2017a).

Farmed Atlantic salmon have been shown to modify their depth in cages to select seawater temperatures of between 16 °C and 18 °C, and preferentially avoid low DO waters (Johansson et al., 2006; Stehfest et al., 2017). Atlantic salmon grown throughout Tasmania may be exposed water temperatures that can exceed 20 °C for prolonged periods during summer. At these times, feed intake may decline and the fish can exhibit classical symptoms of heat stress such as reduced growth rate, decreased condition, variable size distribution due to ‘failing’ sub-populations, increased disease susceptibility, and disrupted gonadal development (Nakano et al., 2014; Pankhurst and King, 2010). Heat stress in salmon has been studied under controlled conditions, and has been found to affect heat shock proteins and antioxidant pathways (Lund et al., 2002; Olsvik et al., 2013; Pankhurst and King, 2010) as well as the production of reactive oxygen species (ROS) (Lushchak, 2011). Size has also been established as a key determinant of the response to heat stress, in that larger salmon show reduced blood oxygen levels and slower metabolic recovery (Clark et al., 2012, 2008), indicating that they may be more prone to the effects of chronic warm water exposure.

The red colouration of salmon flesh is an important product quality criterion (Skrede and Storebakken, 1986) and the concentration of the carotenoid pigments astaxanthin (Axn) and/or canthaxanthin (Cxn) retained in the flesh are the key determinant of overall flesh colour (Buttle et al., 2001). Carotenoid pigments such as Axn are important to salmonid health and welfare, affecting growth (Storebakken and Goswami, 1996), immune function (Amar et al., 2012) and disease resistance (Amar et al., 2012). Carotenoids also function as antioxidant molecules that are thought to protect animals against the effects of oxidative stress (Britton, 1995; Cheng et al., 2017; Lushchak, 2011). Therefore, a potential link exists between the increased production of harmful free radicals during times of stress and the demand for protective anti-oxidants such as vitamin A (Vit A) and carotenoids. In pufferfish (*Takifugu rubripes*), dietary levels of 80–320 mg/kg Axn have been shown to improve resistance to high temperature stress. The effects of short-term thermal stress in Atlantic salmon can lead to a visible decline in flesh colouration (Grunenwald et al., 2016). However, increased dietary Axn, Cxn or Vit A was unable to prevent pigment loss during heat stress (Grunenwald et al., 2016).

Although the growth of cultured Atlantic salmon may be depressed during summer, fish typically exhibit compensatory growth in autumn when animals rapidly increase weight, condition and fat deposits. Compensatory autumn growth is thought to be linked to increased and differential muscle fibre hypertrophy and hyperplasia (Johnston et al., 2000, 2014), with hyperplasia being the dominant process during periods of rapid growth (Higgins and Thorpe, 1990). Nevertheless, it appears that there may be long-term effects on flesh colouration that can seasonally impact product quality. Despite some anecdotal reports from the aquaculture industry, the prolonged effects of thermal stress on growth performance, flesh colour or plasma biochemistry have not been investigated within production cages.

Here, we report the performance of fish in farm cages as they experienced and recovered from an unprecedented marine heatwave that swept Tasmania in the austral summer of 2015/2016 (Oliver et al., 2017). This study brings together farm data on environmental conditions, feeding rates and growth to examine the impact of thermal stress over a 7-month period. Moreover, we characterise the effect of extreme summer conditions on biometric condition indices, flesh colour and blood biochemistry, and compare the responses of fish stocked as early- and late-winter smolt. Our ultimate objective was to elucidate the physiological processes associated with growth performance and changes in flesh pigmentation during and following thermal stress. These physiological changes were investigated to define potential non-

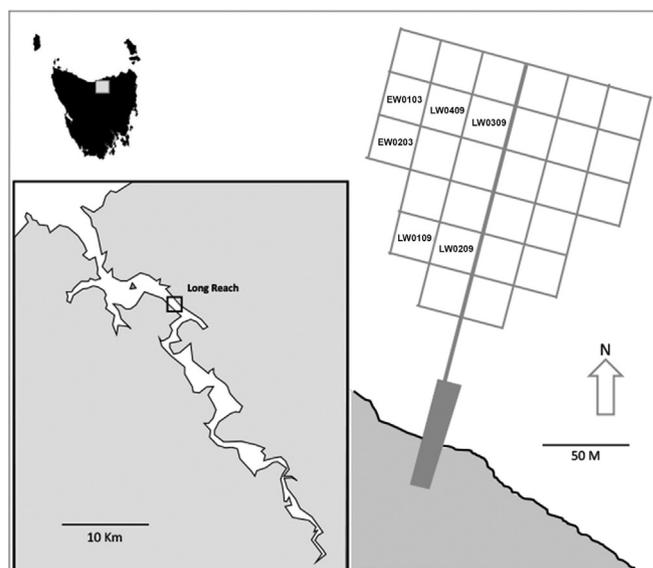


Fig. 1. Study location along the Tamar estuary in northern Tasmania (decimal degrees – 41.165019, 146.914241) and cage layout showing the location of the four main study cohorts (EW103, EW203, LW109 and LW209). The arrow indicates north.

destructive markers of individual fish performance, and inform strategies to mitigate the impacts of summer heatwaves on salmon production.

2. Methods

2.1. Farm details and cage locations

This study was based at Van Diemen Aquaculture, Tasmania (Fig. 1). Sampling occurred between January and August 2016. All procedures were approved under the Tasmanian Government Department of Primary Industries, Parks, Water and Environment (DPIPWE) Animal Ethics Committee (AEC Project number 17/2015-16).

The farm is located in the ‘Long Reach’ section of the Tamar estuary approximately 15 km inland from Low Head. The estuary is a drowned river valley extending 70 km north from Launceston to Low Head. The farm consists of a system of 28 square steel cages (25 m × 25 m) with direct shore access. The lease ranges in depth from 14 m close to the shore to 26 m on the northern boundary. The South Esk and North Esk rivers are the main source of freshwater into the estuary causing extreme salinity drops during flood events and a frequent brackish water influence in the surface layers. The river experiences semidiurnal tides with a high tidal range (3 m) occurring approximately every 12 h (BOM, 2016). This section of the estuary experiences strong flows of 1–2 m/s (DPIPWE, 2000) that ensures good water mixing to depth and generally good oxygen availability. In order to cope with these strong flows the farm employs a unique system of heavy brass fish nets which maintain their shape, repel fouling organisms and prevent predation by seals. Daily measurements of dissolved oxygen (DO), salinity and temperature were recorded at an ‘out of cage’ position on the north-eastern corner of the cage flotilla using Model 420 DO Sensor (5 m depth) and Oxyguard salinity and DO61 temperature probes at 5 m and 10 m depth. Fish were offered feed to satiation throughout the study by centralised feeding system, with feeding activity and uneaten pellets monitored by in-cage camera. Feed amounts offered to each cage were recorded daily.

2.2. Sampling points, data recorded and tissues collected

The study was designed to sample twenty fish each from the same four commercial cage populations each month over the summer and

through the subsequent autumn and winter. These populations of all-female fish were derived from two batches of S1 smolt that were put to sea two months apart as ‘early winter’ (EW Cohort, June 2015, aged 13 months) and ‘late winter’ (LW Cohort, August 2015, aged 15 months). The input populations (EW003 and LW009) were split immediately pre-summer by passing fish through in-line electronic counters (Aquascan CSE 3150, accuracy \pm 2%), to produce cages EW103, EW203 and LW109, LW209, respectively. Each cage held approximately 34,000 fish. Following sampling observations that indicated a bimodal size structure had developed in the LW cohort, the two cages were graded and merged to form cages LW309 (biggs) and LW409 (smalls) in early June 2016. The LW309 population was sampled in early July.

In order to minimise handling stress effects upon blood parameters, fish were sampled rapidly according to the methods of Clark et al. (2011). Briefly, fish were captured using a 5 m \times 5 m \times 4 m ‘box net’ which was set at depth and lifted through the water column, enabling a sub-sample of the population to be rapidly crowded. Fish were sequentially processed in runs of four fish and the box net was lowered between each run to allow remaining fish to swim back to the holding cage. Individual fish were dip-netted from the box net, captive bolt stunned (MT-5 Percussive Stunner, Seafood Innovations, Australia) and blood sampled within 2 min of initial capture. Mass and fork length (FL) were recorded then each fish was tagged with a waterproof label cable tied through the mouth and operculum. Blood was collected from the caudal vein using a 3–5 mL lithium-heparinised syringe and an 18 G needle. Each blood sample was transferred to a Vacutainer™ tube and stored on ice. After severing of the isthmus anterior to the heart, the fish was then bled out for two minutes in a tank of ambient water. The fish was then gutted, liver and gonad weighed and the carcass stored on ice until filleting. Immediately after cage-side processing of each cage sample (20 fish), blood sample tubes were centrifuged at 1000 \times g for 5 min and plasma was transferred to a new Eppendorf™ tube and snap frozen in liquid nitrogen. Sampling of all four cages of fish (80 fish total) was conducted over 2 consecutive days and the order in which cages were sampled was randomised at each sample point.

2.3. Feed types

A range of diets were fed to the study cohorts due to commercial strategies independent of this study. All cages remained on a constant 45:25 protein to lipid ratio (apart from a few weeks in late April when the LW cohort were fed a higher lipid diet). The EW cohort were fed a low fish meal diet over summer, and a similar diet was used on the LW in late April. Vitamin C (500 mg/kg) and E (1500 mg/kg) levels were kept high relative to normal winter grower diets. Feed pigment levels were elevated at 40–60 mg/kg (astaxanthin) and 25–40 mg/kg (canthaxanthin). The ratio, and the levels of astaxanthin to canthaxanthin was varied but generally remained at around 1.2–1 (data not shown).

2.4. Farm average weight and biomass data for calculation of feed rates

The population and average weight of fish in each cage was estimated from a combination of farm weight checks (40–60 fish, 19 measures across the 4 cages late Dec (W) to late June) and monthly experimental sample data (20 fish S1–S6). Daily population numbers were adjusted each day from the initial pre-summer counted populations. Any population discrepancies identified when cages were later counted for grading or harvest were subsequently back-calculated linearly to the previous count. Therefore, the daily specific feed rate (SFR) was calculated as feed offered/biomass, calculated as population \times average weight for each day. SFR data was calculated for the period of 1/12/2015 until 31/5/2016.

2.5. Condition index and residual condition index

Condition index (CI) was calculated using Fulton's CI equation: CI

= mass \times 100,000/FL³ (Fulton, 1904). Since the fish spanned a large size range, a positive relationship between CI and FL was evident (Supplementary Fig. 1A), and indicated that CI was not independent of FL. As such, the residual condition index (RCI) was also calculated. RCI was determined by plotting an exponential regression through the mass vs. FL relationship and the resulting formula was used to calculate how much each fish deviated from the regression (Supplementary Fig. 1B). Values were divided by 1000 to enhance clarity. This RCI was used as a means for comparison across individuals without being influenced by fish size, as fish growth over such long periods of time is more allometric than isometric, to which a range of different types of normalisations can be applied (Bolger and Connolly, 1989). These types of residual indices, indexed by residuals from the least-squares regressions of CI, are useful for testing the relationship between physiological and morphometric condition indices (Sutton et al., 2000). Individuals that fell above the RCI vs. FL regression relationship were considered to have above-expected condition, while those that fell below the regression relationship were considered to have below-expected condition (Supplementary Fig. 1C).

2.6. Digital photography and quantification of fillet colour

Fish were filleted pre-rigor within four hours of harvest. Each fillet was photographed (Supplementary Fig. 2) with the fish identification number clearly displayed, the image contained a MacBeth ColorChecker for validation and a lineal Salmofan™. Images were taken under standardised light conditions on a black Visy Board background at a distance of 40 cm using a digital SLR camera (Canon D-450) fitted with an 18 mm lens, with fixed settings of ISO1600, aperture F22 and 1/125th sec shutter speed. Fillets were photographed in a 38 \times 50 cm light box illuminated with 4 \times 10 W 50 cm White LED lights (Animates Aqua One) set at 45° along the top edges in order to minimise glare on the fillet surface. Where necessary, image intensity was adjusted and validated between photographs using known RGB values for the MacBeth ColorChecker and Salmofan values measured from each photograph (data not shown).

Average fillet colour for all sampled fish was tracked over time on a cage by cage basis. This was calculated from the average RGB values across a 3600 pixel square from three boxes at each measurement site A–G (Supplementary Fig. 2) using ImageJ software (Schneider et al., 2012). The RGB colour space is an additive colour space based on varying levels of the colours red (R), green (G) and blue (B). The RGB values can be averaged to produce a single colour that is representative of the average colour of each of the 20 fish sampled from that cage, or a subset of animals as required through further analysis. A threshold to define colour loss was developed using the RGB values quantified from digital images and the ratio between the average R (Rav) and average B (Bav) was particularly informative. This was further utilised to define ‘unaffected’ (Rav/Bav \geq 2.5), ‘mildly affected’ (Rav/Bav \geq 1.5 \leq 2.5) and ‘severely affected’ (Rav/Bav \leq 1.5) sites, and these criteria were applied for each fillet measurement site for each fish over the 7 months of sampling.

2.7. Blood plasma analysis

Plasma samples were analysed for electrolytes and clinical chemistry markers of physiological stress at QML Vets (Murarrie, Qld, Australia) using a Cobas Integra 800 automated chemistry analyser (Roche Diagnostics GmbH). Each of the assays used was a standard kit developed for the auto-analyser (Roche Diagnostics GmbH). Measured plasma metabolite values were compared with normal haematological and clinical parameters in healthy adult Atlantic salmon fed a well balanced commercial diet in sea cages at 2.0–7.7 °C (Sandnes et al., 1988). The measurement of plasma sodium and chloride levels is used to indicate osmoregulation efficacy. Anion gap represents the difference between serum cations (sodium Na⁺ and potassium K⁺) and anions

(chloride Cl^- and bicarbonate HCO_3^-). Plasma-borne AST and ALT is indicative of the liver “leaking” enzymes into the bloodstream, as AST is found in muscle and liver tissue while ALT is exclusively found in the liver. The major functions of AP are assistance with protein breakdown and transportation across cell membranes.

2.8. Statistical analysis

All values are means unless otherwise specified. Effects of different parameters were examined using one-way or two-way ANOVA, with significance limits set at $P < 0.05$, and levels of significance determined using a Fishers Least Significant Difference (LSD) test. Regression models (linear or 2nd, 3rd or 4th order polynomials) were sequentially tested until additional factors did not significantly improve model statistics. Linear regression analysis of cessation of feeding was performed using temperature and SFR data between 10/12/2015 and 21/2/2016, while refeeding used data between 19/3/2016 and 31/5/2016. Linear regression analysis of colour was performed using colour (Rav/Bav) and RCI as the dependent variables, respectively, applying an F-test to attribute overall significance. Comparison of colour of selected fish was performed using 3-way ANOVA, with Rav/Bav set as the dependent variable, with cohort, month and size as dependent variables, and levels of significance determined using Fishers LSD test. No statistical comparison of colour groups (severely affected, mildly affected, unaffected) was performed as the cutoff values were arbitrarily set to visually demonstrate the effect on fish colour. Comparison of EW and LW cohort performance was assessed by 2-way ANOVA using RCI as the dependent variable, with month and cohort as dependent variables. Polynomial regression analysis applied to selected fish used RCI as the dependent variable, and month as the independent variable, applying an F-test to attribute overall significance. Comparison of EW and LW cohort plasma biochemistry was assessed by 2-way ANOVA using AST, ALT or AP as the dependent variable, with month and cohort as dependent variables. Regression analysis applied to plasma biochemistry used AST, ALT or AP enzyme levels as the dependent variable, and month as the independent variable, applying an F-test to attribute overall significance. Statistical analyses were performed using StatPlus: Mac 2009 (AnalystSoft Inc).

3. Results

3.1. Farm data

Temperatures at the Van Diemen lease rose rapidly from mid-December (17.8 °C) to peak at 22.9 °C on 13th February (Fig. 2) with little difference at depth (22.2 °C at 10 m). The farm experienced 117 days over 18 °C and 83 days over 20 °C at 5 m depth (Fig. 2). There was record rain in the north and northeast of Tasmania at the end of January (BOM, 2016). Heavy rainfall (145 mm between 28th and 30th January) caused a rapid drop in salinity to 27 ppt at 5 m through most of February and a subsequent drop in water temperature to 20.5 °C for the latter half of the month. Temperatures began to climb again to 20.5 °C in early March before declining during the onset of autumn. Temperatures did not drop below 15 °C until early May. Heavy rainfall in early June caused an extreme salinity drop (6.3 ppt at 5 m), with salinity remaining consistently between 15 and 25 ppt through to the end of the study. Daily oxygen records indicated that out of cage oxygen levels averaged 89.8% saturation throughout the study period.

3.2. Feed input and growth performance

After removal of the LW cages that were size graded in July, a two-way ANOVA showed that weight was significantly affected by month for both the EW cohort ($F = 51.3$, $P < 0.0001$) and LW cohort ($F = 11.4$, $P < 0.0001$). There was no effect of cage on fish weight or RCI, and all cage weight data was best described by 3rd order or 2nd order

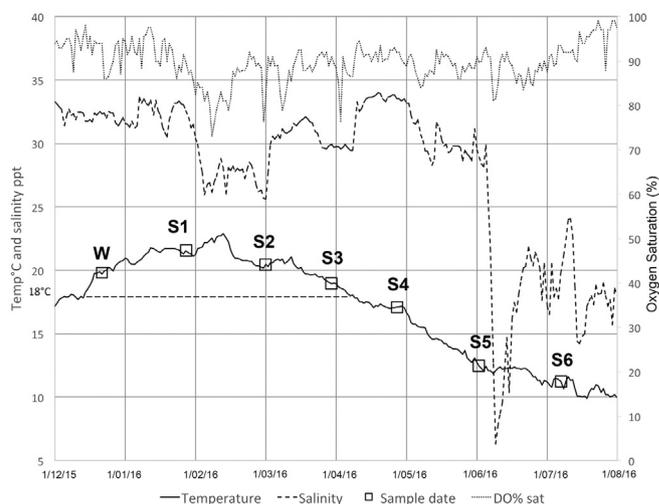


Fig. 2. Environmental conditions recorded at 5 m depth for oxygen saturation (%), salinity (ppt) and temperature (°C) over the duration of the sampling period. The period above 18 °C is indicated by the dashed line. One weight only event (W) and six sampling events (S1–S6) are denoted by □ superimposed on the temperature trend.

polynomial regressions for the EW or LW cohorts, respectively (Fig. 3). A polynomial regression through the average sample weight of the two EW cages (Fig. 3A) and two LW cages (Fig. 3C) showed that weight declined from the start of the trial in December. Therefore cage data was combined for further analysis. This also resulted in a marked decline in condition factor (data not shown). The weight and condition of the LW stocks began to recover noticeably from late March onwards (Fig. 3C). Improved condition and positive growth did not begin in the EW cohort until late April (Fig. 3A). Two-way ANOVA showed that cohort had the most significant effect on RCI ($F = 23.4$, $P < 0.0001$), followed by the interaction of month and cohort ($F = 3.3$, $P = 0.004$), then month ($F = 2.8$, $P = 0.01$). A widening size distribution and development of bimodality became apparent (particularly in the LW cages) as autumn progressed, and this bimodal response was investigated in further analyses using selected the most extreme fish from each cage as detailed below (Section 3.4). This indicated that the increased feed intake was generated more by a subpopulation of early performers rather than as a general uplift across the entire population. As conditions improved, more fish were promoted into the feeding/performing portion of each cohort. This view was supported by the increasing ratio of fish with feed in the gut noted at monthly samplings and the associated return to feeding.

Despite being offered, feeding behaviour and therefore recorded feed intake declined rapidly as temperatures increased (Fig. 3B and D). There was a significant linear correlation between feed input (% daily intake) and temperature as temperature increased (Supplementary Fig. 3), for both the EW cohort ($y = -511x + 21.5$, $R^2 = 0.77$, $P < 0.0001$) and LW cohort ($y = -272x + 22.6$, $R^2 = 0.65$, $P < 0.0001$). The y-intercept (temperature at which feeding stopped) was 21.5 °C in the EW cohort, consistent with the observed complete cessation of feeding from the last week of January as the temperature increased above 21 °C. Meanwhile, the LW cohort had a reduced slope and increased y-intercept of 22.6 °C, and this cohort maintained a low feed intake levels through to early February. The significant correlation between feed input and temperature was maintained as temperature decreased, for both the EW cohort ($y = -581x + 18.6$, $R^2 = 0.87$, $P < 0.0001$) and LW cohort ($y = -436x + 20.2$, $R^2 = 0.86$, $P < 0.0001$). The y-intercept (temperature at which feeding returned) of the EW cohort was 18.6 °C, consistent with a return to feeding behaviour as temperatures began to drop in early April (up arrow on Fig. 3A and B). Meanwhile, the y-intercept of the smaller LW cohort was

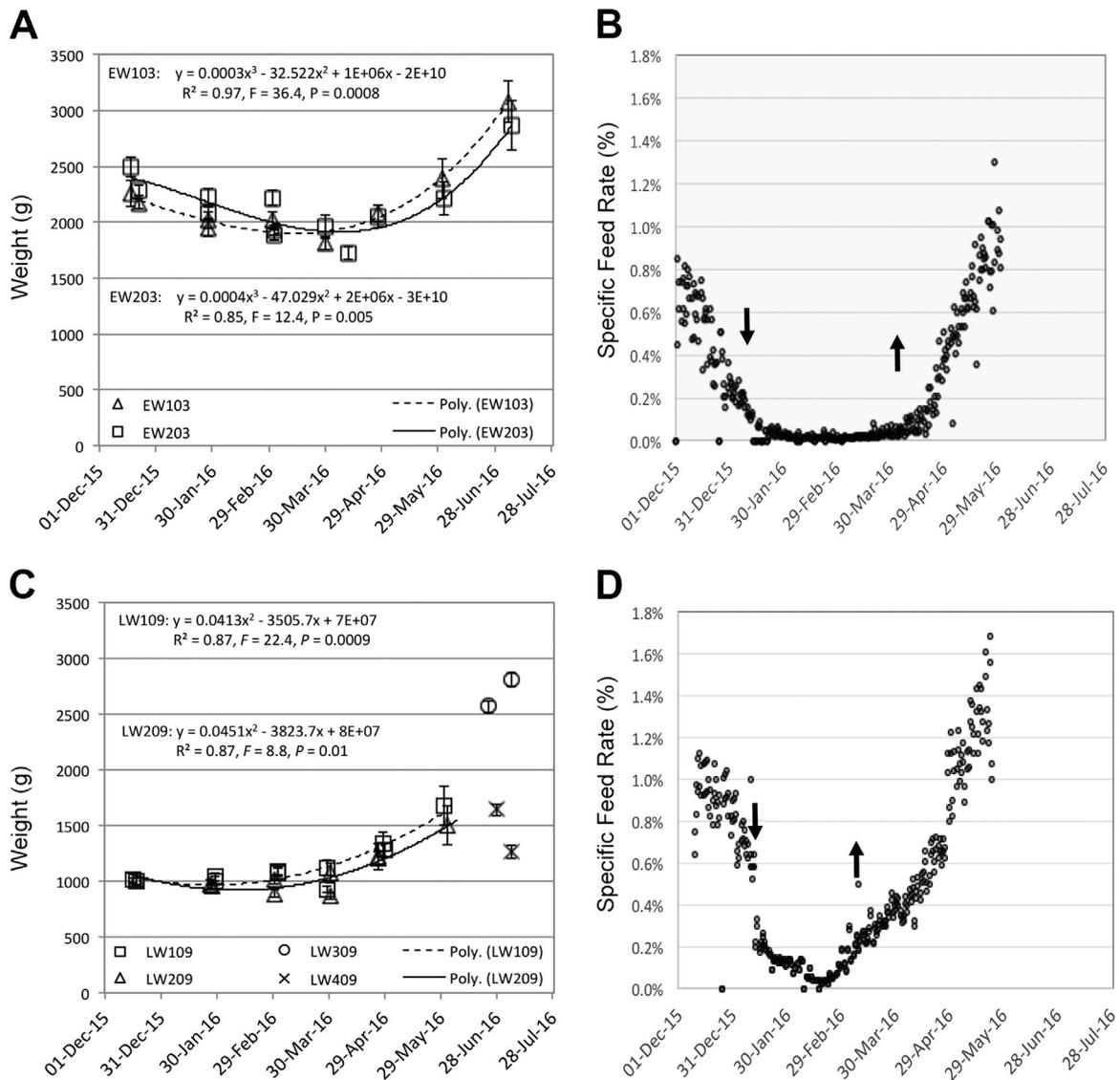


Fig. 3. Average weight and daily feed inputs (% bodyweight/day) recorded for each cohort (EW – A; LW – B) during the sampling period. Dashed lines indicate a polynomial regression trend of weight over time for each cage. Regression equations and corresponding significance values are shown.

20.2 °C, consistent with an earlier and more rapid recovery of feed input from early March onwards in the LW cohort (up arrow on Fig. 3C and D).

3.3. *Flesh colour*

The ratio between the average R and B values (Rav/Bav) for all fillet sites (A–G, Supplementary Fig. 2) was used as a measure of average fillet colour for each fish, and this was plotted against RCI for all sampled fish each month (Fig. 4). Initially there was no relationship between Rav/Bav and RCI, however as fish returned to condition, a significant positive relationship developed during the recovery period of April and May (significant regression statistics shown on graphs).

The ratio of Rav/Bav was also used to visually determine the proportion of fish that were severely affected, mildly affected or unaffected at each site (Fig. 5). During the months of March, April and May, in excess of 90% of fish showed signs of mild or severe colour loss, with approximately 20–30% of fillets largely devoid of colour. Results over the entire sampling period demonstrated the progressive colour loss from dorsal (sites A and D) and belly flap (sites C and F) regions of the fillet, prior to the loss of colour through the midline (sites B and E). These data define the potential progressive colour loss or potential

colour variability across the sampling period. However, without the ability to non-destructively track the colour of each individual fish over time, the true progression of colour loss cannot be reliably determined as these measures are independent of one another. By selecting the fish with the most extreme weight phenotype, we hoped to explore whether this was linked with colour.

3.4. *RCI of selected fish*

In light of the bimodal weight distribution phenotype that presented towards the end of this study, the weight of each fish was ranked within cage at each sampling, and the five largest and five smallest fish combined as ‘big’s and ‘small’s within each cohort EW and LW (Supplementary Table 1). Given the performance of cage replicates was the same (Section 3.2 above), the most extreme phenotypes within each cohort contained 10 fish each. The RCI of these four groups (EW big; EW small; LW big; LW small) were plotted over time (Fig. 6). Significant trend lines demonstrated a distinct difference in the response in the performance of the two cohorts, and of the different sized fish within each cohort. The weight of the largest fish at the beginning of summer (EW big) was not significant, although fish showed a trend for increased weight consistent with the recorded return to feeding for that cohort.

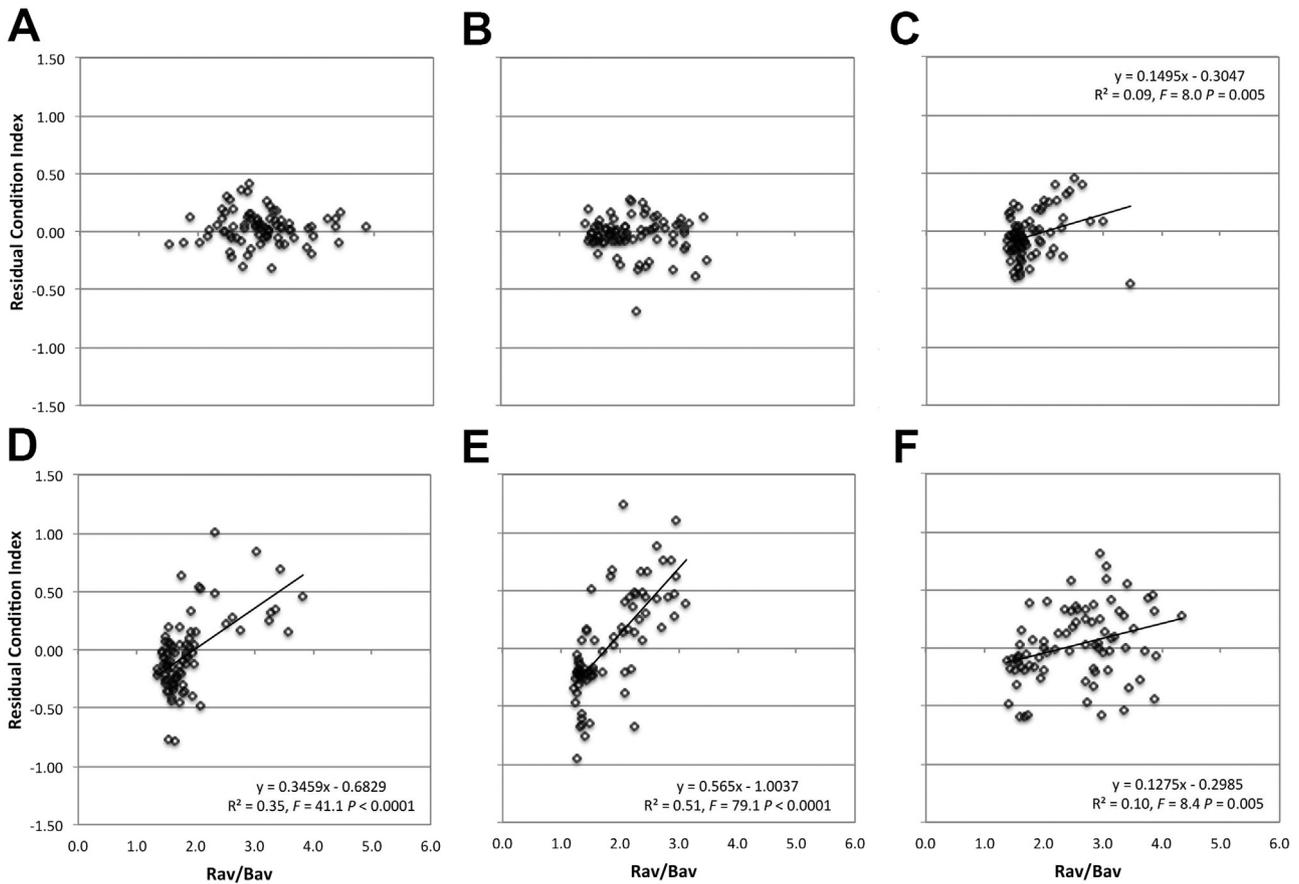


Fig. 4. Relationship between fillet colour and residual condition index. Average RGB colour for all fillet sites for each individual fish sampled was determined (Rav, Gav, Bav) and the ratio of Rav/Bav calculated. This was plotted against residual condition index for each fish in each sampling event (A–F = S1–S6), and allowed comparison of colour from different sized fish. Trend lines are shown only for significant regressions with corresponding significance values.

Weight of the EW smalls had a mild but significant linear decrease throughout ($R^2 = 0.28$, $F = 22.7$, $P < 0.0001$) suggestive that the smaller fish within each cage never returned to feeding. The LW bigs had a strong significant polynomial relationship ($R^2 = 0.48$, $F = 16.0$, $P < 0.0001$), where fish were of higher relative condition between March and June than at any other fish during the sampling. Although individual feed rates could not be determined, observations of gut fullness and liver condition indicate that this effect was most likely due to the return to feeding (data not shown) and led to the LW bigs outperforming the EW smalls within two months. In contrast, the LW smalls had a significant polynomial relationship ($R^2 = 0.36$, $F = 10.1$,

$P < 0.0001$), and showed a slight increase in RCI in the last month of sampling. This result was likely affected by combining graded fish with other cohorts during the final month of sampling. Given the RCI defined differences in the sampled fish, other relationships in the colour and plasma analysis were investigated within the most extreme phenotypes encountered in each cohort.

3.5. Colour loss of selected fish

Analysis of the most extreme fish also showed a strong colour phenotype between fish size within cohorts and across cohorts. The

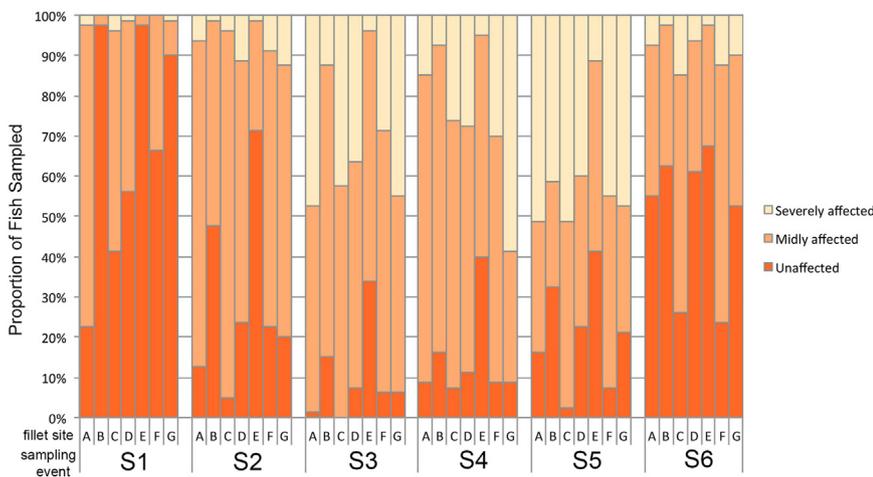


Fig. 5. Proportion of severely, mildly or unaffected fish combining all fillet sites (Supplementary Fig. 2, A–G) for all fish over each sampling event (S1–S6). The ratio of Rav/Bav was used from each site from quantified RGB values from digital images to create a threshold of severely ($Rav/Bav < 1.5$), mildly ($Rav/Bav > 1.5 < 2.5$) or unaffected ($Rav/Bav > 2.5$). The colour of each category approximates the severity of the colour loss.

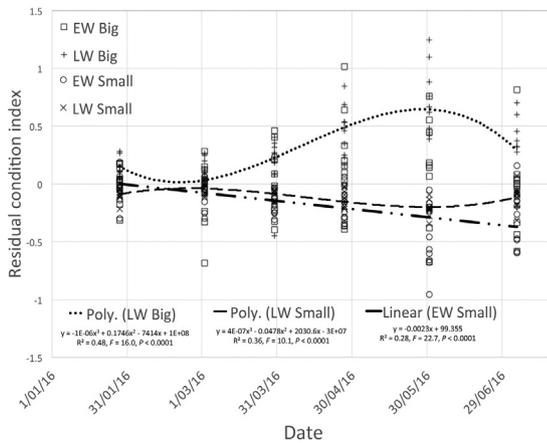


Fig. 6. Changes in residual condition index (RCI) over time for the ten largest (big) and ten smallest (small) fish from each early winter (EW) and late winter (LW) cohort. Trend lines are shown only for significant regressions with corresponding significance values.

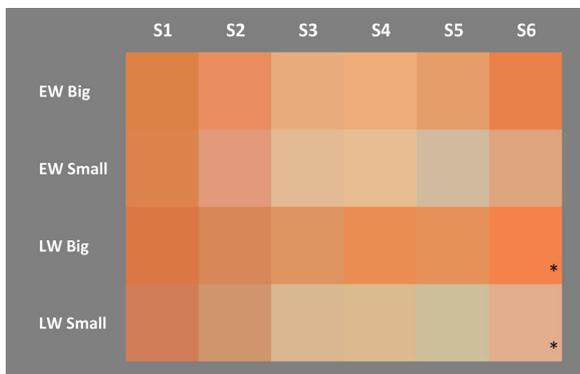


Fig. 7. Average RGB colour for all fillet sites from the ten largest (Big) and ten smallest (Small) fish sampled from each cohort (EW – early winter; LW – late winter). Average RGB values were converted to a single colour square that represents the average fillet colour of large and small fish from each cohort at each sampling event (S1–S6). Asterisk indicates that grading and mixing within cage replicates that occurred in June 2016 as outlined in the Methods.

average RGB value for all seven fillet sites was determined for the 10 largest and smallest sampled fish within each cohort, the colour expressed within a single box for each month over the seven sampling events (Fig. 7) and the Rav/Bav ratio used for statistical comparisons. Several factors were significant in determining colour, including size ($F = 158.6, P < 0.0001$), sample event ($F = 30.4, P < 0.0001$), size and cohort ($F = 12.4, P < 0.001$), size and sample event ($F = 9.2, P < 0.0001$) and cohort and month ($F = 4.4, P < 0.001$). Colour was significantly affected by fish size in both the EW ($t = 4.7, P < 0.0001$) and LW ($t = 6.4, P < 0.0001$) cohorts. Similar to growth performance data, LW bigs showed the best average colour score of all cohorts (Fig. 7), with significant difference in colour of big LW fish at S4 ($t = 2.1, P < 0.05$), S5 ($t = 4.7, P < 0.0001$) and S6 ($t = 5.9, P < 0.0001$) compared with big EW fish. Although average colour decreased during February and March, colour loss was significantly less than that of the EW bigs and returned to harvest quality faster as demonstrated by their earlier performance data. Meanwhile, small fish colour was not significantly different between the EW and LW cohorts (Fig. 7), and the most severely colour affected fish were all within the smallest of those sampled (data not shown).

3.6. Plasma biochemistry of selected fish

Levels of particular plasma enzymes and ions can provide information about the health status of an animal, although such values are rare in fish. All values have been reported in Supplementary Tables to provide baseline data for future comparison. Plasma sodium and chloride were elevated in January, while they had decreased by February (Supplementary Tables 2 and 3). Anion gap, the difference between serum cations (sodium Na^+ and potassium K^+) and anions (chloride Cl^- and bicarbonate HCO_3^-), was elevated in February, March and April (Supplementary Tables 2 and 3). EW big fish exhibited a gradual accumulation in plasma creatine kinase (CK) throughout the trial (Supplementary Table 2). In contrast, elevated levels of plasma CK were observed in LW big fish during January and February, followed by a decline in April and May (Supplementary Table 3). Observed values for protein, cholesterol, bicarbonate and albumin were similar across LW and EW big fish throughout the study (Supplementary Tables 2 and 3). Higher values were recorded in fish that had returned to feeding in March.

Significant linear (EW) and polynomial (LW) regressions fitted the AST and ALT data over time (Fig. 8A and C), while 3rd order polynomial regressions best fitted the EW and LW AP data over time (Fig. 8E). AST was significantly elevated in LW big fish compared with EW fish during April ($t = 5.2, P < 0.0001$) and May ($t = 3.4, P < 0.001, \text{Fig. 8A}$), while ALT was significantly elevated in LW big fish compared with EW fish only during April ($t = 5.6, P < 0.0001, \text{Fig. 8C}$). AP was also significantly elevated in LW big fish compared with EW fish during March ($t = 4.5, P < 0.0001$) and May ($t = 2.8, P = 0.006, \text{Fig. 8A}$). AST in the range of 500–2000 U/L, ALT in the range of 5–60 U/L and AP in the range of 25–300 U/L was observed during this time. Prior to the April and May sampling events pale livers were observed in this cohort (data not shown), with resumption of feeding noted in March. Significant positive linear regression was determined for AST and ALT with RCI within the LW big cohort (regression values shown on Fig. 8), in comparison the EW big cohort that had no significant relationships. The highest Alkaline Phosphatase (AP) activity was observed in LW big fish from March to May (Fig. 8E). Levels of AP significantly increased with RCI within both LW and EW big cohorts, with the slope of the regression greater for the LW fish than EW fish (Fig. 8F).

4. Discussion

4.1. Farm data

The summer of 2015/16 saw record-breaking sea surface temperatures around Tasmania and across parts of the Tasman Sea (Oliver et al., 2017), driven by a southward extension of the East Australian Current. This was associated with the longest and most intense marine heatwave on record for the southeast Australian region (BOM, 2016). The extreme conditions experienced during this study compromised fish performance, feed intake, condition index and fillet colour, as well as disrupted normal plasma osmoregulatory and renal functions. Past research has shown that behavioural changes occur in salmon production cages with seawater temperatures of between 16 °C and 18 °C, including adjusting swimming depth (Johansson et al., 2006; Stehfest et al., 2017), along with avoidance of < 35% DO saturation and seawater temperatures > 20.1 °C (Stehfest et al., 2017). Such behavioural adjustments may have also occurred in the present study, as thermal tolerance limits are likely to have been exceeded from December through to March. Although in-pen oxygen data is not available for this study, temperature and hypoxia are interacting stressors (McBryan et al., 2013; Vikesa et al., 2017b). Out-of-cage oxygen levels did not fall below 70% saturation (Fig. 2), in-cage DO is likely to be lower due to the combined effects of fish biomass and obstructed water flow due to netting, shielding by surrounding cages (Fig. 1) and tidal cycles.

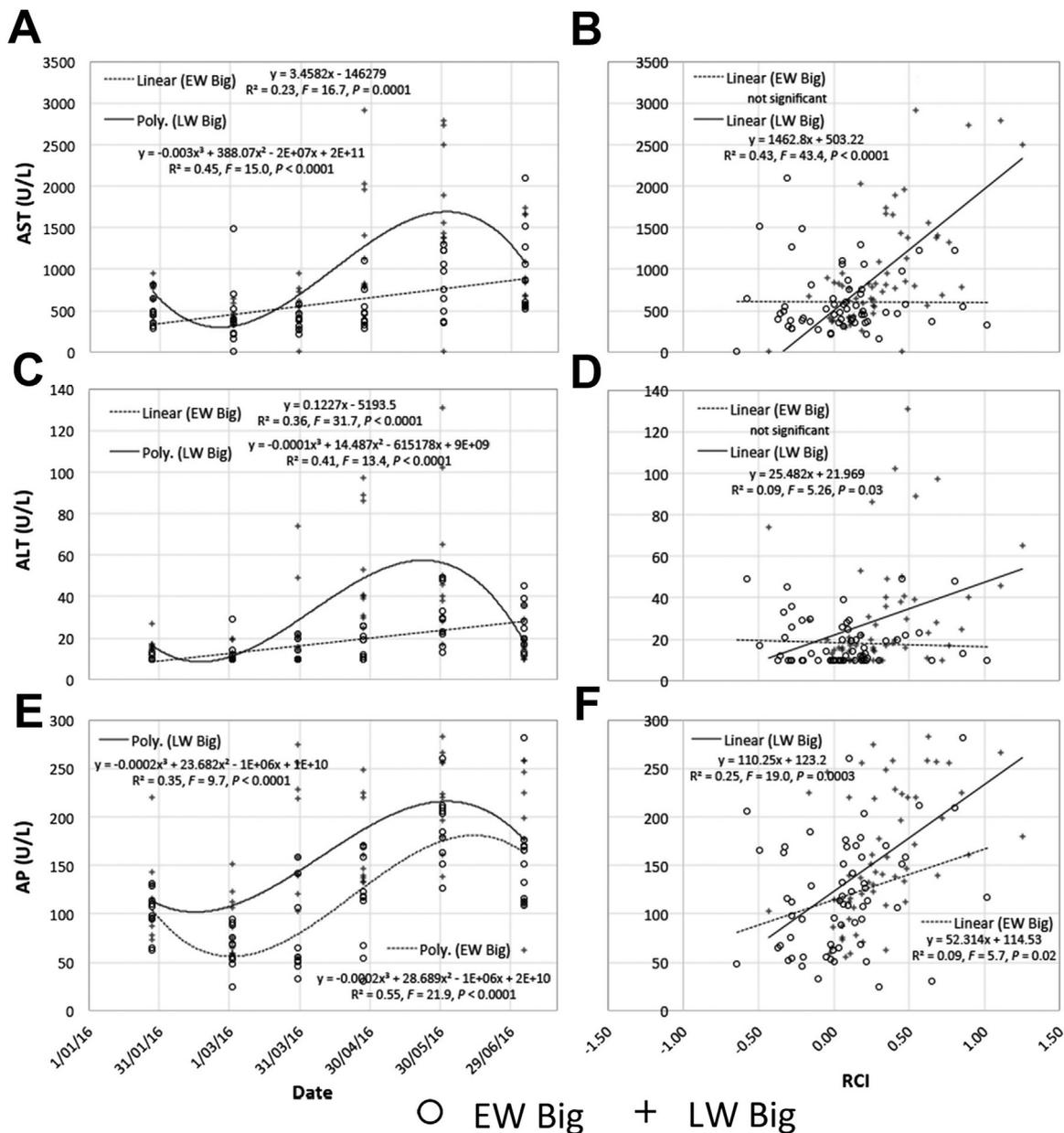


Fig. 8. Time series of plasma muscle and liver health biochemical parameters for (A) aspartate aminotransferase (AST), (C) alanine aminotransferase (ALT) and (E) alkaline phosphatase (AP) that showed an association with thermal stress in the ten largest (Big) fish sampled from each cohort (EW – early winter; LW – late winter). The effect of fish size was removed using residual condition index (RCI) and plotted against AST (B), ALT (D) or AP (F). Trend lines are shown for all regressions with corresponding significance values.

4.2. Feed input and growth performance

One of the main effects of thermal stress in the present study was the complete cessation of voluntary feed intake in both cohorts. Although feed was offered to each cohort daily, no feeding behaviour was observed and hence no feed intake recorded. Most studies of salmon have focussed on how acute thermal stress affected certain physiological or molecular mechanisms (Lund et al., 2002; Nakano et al., 2014; Olsvik et al., 2013; Pan et al., 2000; Pankhurst and King, 2010) or the ability of wild populations to adapt under current or future climate change scenarios (Clark et al., 2012, 2008; Lund et al., 2002; Pankhurst and King, 2010). The chronic effects of thermal stress (up to 19 °C) or low DO (50–70%) for 45 or 56 days resulted in reduced feed intake, reduced growth and loss of body lipids in Atlantic salmon (Hevroy et al., 2013; Olsvik et al., 2013). These effects also triggered the down-regulation of insulin-like growth factors (IGF-1 and IGF-2) in the muscle, and several

pathways protecting against oxidative stress in the liver that may lead to metabolic depression (Hevroy et al., 2013, 2012; Olsvik et al., 2013). The present study demonstrated a significant negative linear relationship between feed intake and temperature, and a complete cessation of feeding in all fish as temperatures rose above 21.5 °C. The development of anorexia observed in this study is likely initiated by similar mechanisms observed in past studies (Hevroy et al., 2012), and further exacerbated by the combined effects of temperature and hypoxia stress (Oppedal et al., 2011; Vikesa et al., 2017a).

4.3. Resumption of feeding, growth and condition

After the peak temperatures, significant negative relationships were maintained between temperature and feed intake, but the smaller LW cohort demonstrated a return of feed intake at 20.2 °C compared with 18.6 °C for the EW cohort. The earlier improvement of key quality

attributes in this cohort were consistent with a return to condition of certain fish. This finding corroborates previous observations of smaller wild fish being more thermally tolerant within a species (Clark et al., 2017; Daufresne et al., 2009; Messmer et al., 2017). While the mechanisms underlying these observations remain speculative (Clark et al., 2012, 2017, 2008), it is clear that our ‘small’ fish within each of the EW and LW cohorts did not benefit from the same size-dependent thermal tolerance. We suggest that the latter finding is a consequence of our ‘small’ fish in each cohort representing underperforming and relatively malnourished individuals, traits that masked any inherent benefit of being small. In any event, this study demonstrates that RCI may be a means by which poor performing fish can be distinguished from those performing well, and that particular size classes may perform better under conditions of thermal stress. In addition, formulation of diets that prolong or promote feed intake may be a highly practical solution to overcoming or alleviating the effects of thermal stress.

4.4. Flesh colour

It is generally understood that muscle flesh has a high capacity store and retain carotenoids, and that the tissue depletion of astaxanthin during starvation in Atlantic salmon may proceed at a rate similar to whole muscle degradation (Bjerkeng et al., 2000). The loss of colouration induced by thermal stress has a lasting effect on the perceived quality and therefore price of farmed salmon. Our results indicated that the fish with the highest condition index also had the best fillet colour, and formed the basis by which fish were size graded in the final months to optimise commercial output. These results suggest that, if flesh colour is lost during the summer period, the measurement of RCI could be used as an estimate of flesh colour and therefore overall product quality. The rate at which colouration is lost from salmon fillets during starvation alone has not been determined, even though it is well understood that increased amounts of dietary Axn improve retention in the flesh (Torrisen et al., 1995). No loss of colour was reported in trout after ceasing Axn feeding for up to 55 days (Brown et al., 2016), similarly in trout muscle astaxanthin level was not affected by starvation for up to 48 days under optimal temperature conditions (Choubert, 1985; Foss et al., 1984). The preferential retention of carotenoid pigments along the midline can partially be explained by higher levels of carotenoids in dorsal and midline muscle in normal fillets (Bjerkeng et al., 2000). A recent study showed that the effects of dietary Axn or Vit E supplementation provided no protection against fillet colour loss due to thermal stress (Grunenwald et al., 2016). However, rather than providing carotenoids and other antioxidants well in excess of biological needs, recent research in copepods suggests that the loss of colour better tests their physiological function as an antioxidant in animals (Weaver et al., 2018), implying that rate of colour loss during stressful events may determine antioxidant capacity of carotenoids in salmon. Carotenoids were not quantified from fish in the present study, as the assumption holds that the quantified RGB colour of the fillet can be used as an indicator of the presence or absence of carotenoid pigments. The main challenge remains that poorly coloured fish can only be detected by destructive sampling, although the present study suggests that RCI may allow some ability to discriminate poor quality fish. In instances where changes in RCI have not occurred, the application of near-infrared spectroscopy (NIRS) technology to externally scan and identify potentially affected fish would be of great benefit (Brown et al., 2014).

4.5. Plasma biochemistry

Measured plasma metabolite values are scarce for Atlantic salmon, especially in the Southern Hemisphere. Protein, cholesterol, bicarbonate and albumin were within the baseline ranges reported by Sandnes et al. (1988). Both plasma sodium and chloride remained above the ranges observed for 1 kg fish at 0.5–8.9 °C (Bergheim et al., 1990). The

measurement of plasma sodium and chloride levels is used to indicate osmoregulation efficacy, and this can be one of the first systems to break down with chronic stress (Jeffries et al., 2011). Both parameters stabilised from March onwards, consistent with EW and LW big fish regaining a level of osmoregulatory capacity following a decrease in water temperature and a resumption in feeding. However, no differential effects of temperature or salinity on osmoregulatory capacity during February could be discriminated.

In healthy organisms, AST is found in muscle and liver tissue, while ALT is exclusively found in the liver. Plasma-borne AST and ALT is indicative of the liver “leaking” these enzymes into the bloodstream and is synonymous with liver injury, inflammation or imbalance (Burtis and Bruns, 2014). AST levels of 500–2000 U/L were observed in LW big fish during April and May and June, that greatly exceeded baseline levels previously reported as 202–351 U/L (Sandnes et al., 1988). Results from the present study are consistent with impaired liver function following the peak in summer temperature and the onset of feeding in March, and a pronounced size effect on smaller fish of the LW cohort. A variety of stressors have previously been linked to elevated Atlantic salmon ALT levels (Bowser et al., 1989).

4.6. Alkaline phosphatase as a potential marker of recovering fish

The major functions of AP are assistance with protein breakdown and transportation across cell membranes, with this enzyme family concentrated in the liver, bile duct, kidney, bone and intestinal mucosa under normal conditions (Burtis and Bruns, 2014). AP values in the range 50–300 U/L were observed throughout the study, whereas baseline values are reported to be 647–988 U/L (Sandnes et al., 1988), with overall trends consistent with a decrease in temperature and a resumption of feeding. Reduced AP levels have been associated with anaemia and malnutrition in clinical studies on model species. Decreases in AP similar to those observed in the current study have been observed as a result of nitrite-induced stress in Atlantic salmon (Bowser et al., 1989) and starvation in rainbow trout (Sauer and Haider, 1979). This is consistent with the results of the present study where AP was elevated after a period of starvation, which may have been further exacerbated by environmental stress. Trends in plasma AP from March to May during the present study highlight a potential recovery period and would be a useful focal point for further research. The expression of AP could be non-destructively and quantitatively measured with biochemical or molecular methods (e.g. Skugor et al., 2008), as a potential marker of recovering fish.

It is important to note that the plasma markers assessed in the present study have been developed in mammalian systems, and their relevance in fish has not been fully established. However, this type of analysis allows a quick and cost effective assessment of biochemical parameters, contributes to characterisation of the underlying mechanisms, and may provide non-destructive markers for use in early warning or intervention. In many cases, levels reported here may be within normal ranges for fish, despite significant differences between treatments. The general consistency in plasma lactate within each sampling period (Supplementary Tables 2 and 3) provides support that the standardised plasma collection methods were effective (crowding, culling, sampling technique, time of processing, whole blood and plasma storage conditions, etc.). However, day-to-day variations while the study was undertaken may have a significant effect on absolute plasma values.

4.7. Mitigation of the effects of thermal stress in aquaculture

This study clearly demonstrated the benefit of stocking the smaller late winter fish to tolerate the extremes of summer heat stress, a change in production practice that can be adopted immediately. Given that the main effect of thermal stress was a cessation of voluntary feeding, the ability to prolong the period of feed intake throughout summer is

paramount. Interventions to encourage continued feeding can potentially be achieved through feed attractants (Toften et al., 2003), altering the physical quality of pellets (Oehme et al., 2014), and manipulation of dietary protein and lipid levels (Weihe et al., 2018). Although individual feed intake is possible to quantify under research conditions, it is virtually impossible at commercial scale. In order not to rely solely on cage-level feed intake or RCI to assess fish condition, a key outcome of this study was the possibility that AP may provide a non-destructive and quantifiable marker for the ability of individual fish to recover after a thermal stress event. With further validation under controlled conditions, plasma AP may provide a monitoring tool for overall cage condition, or a means by which to remove fish that show no signs of recovery after a period of thermal stress. Although not directly investigated in the present study, genetic selection for thermal tolerance is a longer-term strategy that could be investigated in Tasmanian stocks. Evidence for significant genetic variation of thermal tolerance has been reported in Atlantic salmon in Canada (Anttila et al., 2013). Australian Atlantic salmon have been shown to be genetically distinct from European and North American populations (Kijas et al., 2018), and may have developed some level of increased tolerance to the high water temperatures experienced in Australian waters during 50 years of reproductive isolation. This concept has been demonstrated in selected lines of trout introduced to Australia (Molony et al., 2004) providing a potential mitigation strategy.

5. Conclusions

This study quantified the effects of an unprecedented summer heat wave on the growth performance, flesh colour and plasma biochemistry of marine cage-farmed Atlantic salmon (*Salmo salar*). Predictions suggest that summer heatwaves are to become more frequent and more severe (IPCC, 2013), with significant flow-on effects on fish welfare and performance in aquaculture systems. Diets specifically formulated to prolong or re-establish feeding may be assist the fish to tolerate extreme thermal events. Similarly, genetic selection of high performing fish within selective breeding programs will continue to assist in the development of thermally tolerant stocks. In the short-term, residual condition index and plasma alkaline phosphatase levels may potentially be useful as non-destructive indicators of recovery, and assist with removal of underperforming fish from production cages.

Acknowledgements

The study team wish to thank the management and staff of Petuna Seafoods and Van Diemen Aquaculture for their excellent support throughout this project and in-kind contribution of fish and staff expertise. Specifically, thanks to Ross Urquhart, Dianne Maynard, Gianluca Amoroso, Brendan Adair, Hanna Swoboda, Tom Fox-Smith, Phillipa Sims. We would also like to thank Brett Glencross for assistance in design and inception of the study, and Harry King for considered and constructive revision of this manuscript.

Funding

This work was supported by Ridley Aquafeed Pty Ltd, Petuna Seafoods and CSIRO Agriculture and Food.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtherbio.2018.12.021.

References

Amar, E.C., Kiron, V., Akutsu, T., Satoh, S., Watanabe, T., 2012. Resistance of rainbow trout *Oncorhynchus mykiss* to infectious hematopoietic necrosis virus (IHNV)

- experimental infection following ingestion of natural and synthetic carotenoids. *Aquaculture* 330, 148–155.
- Anttila, K., Dhillon, R.S., Boulding, E.G., Farrell, A.P., Glebe, B.D., Elliott, J.A.K., Wolters, W.R., Schulte, P.M., 2013. Variation in temperature tolerance among families of Atlantic salmon (*Salmo salar*) is associated with hypoxia tolerance, ventricle size and myoglobin level. *J. Exp. Biol.* 216, 1183–1190.
- Barton, B., 1996. General biology of salmonids. In: Pennell, W., Barton, B. (Eds.), *Principles of Salmonid Aquaculture*. Elsevier, Amsterdam, pp. 29–95.
- Bergheim, A., Kroglund, F., Vatne, D.F., Rosseland, B.O., 1990. Blood plasma parameters in farmed Atlantic salmon (*Salmo salar* L) transferred to sea cages at age 8–10 months. *Aquaculture* 84, 159–165.
- Bjerkeng, B., Hatlen, B., Jobling, M., 2000. Astaxanthin and its metabolites idoxanthin and crustaxanthin in flesh, skin, and gonads of sexually immature and maturing Arctic charr (*Salvelinus alpinus* (L.)). *Comp. Biochem. Physiol. B-Biochem. Mol. Biol.* 125, 395–404.
- Bolger, T., Connolly, P.L., 1989. The selection of suitable indexes for the measurement and analysis of fish condition. *J. Fish Biol.* 34, 171–182.
- BOM, 2016. Tasmania in summer 2015–2016: record warm, record rain in the northeast, relatively dry in the west. Bureau Meteorol Clim. Data Online.
- Bowser, P.R., Wooster, G.A., Aluisio, A.L., Blue, J.T., 1989. Plasma chemistries of nitrite stressed Atlantic Salmon *Salmo salar*. *J. World Aquac. Soc.* 20, 173–180.
- Britton, G., 1995. Structure and properties of carotenoids in relation to function. *Faseb J.* 9, 1551–1558.
- Brown, K.R., Barnes, M.E., Parker, T.M., Fletcher, B., 2016. Retention of fillet coloration in rainbow trout after dietary astaxanthin cessation. *Fish. Aquac. J.* <https://doi.org/10.4172/2150-3508.1000163>.
- Brown, M.R., Kube, P.D., Taylor, R.S., Elliott, N.G., 2014. Rapid compositional analysis of Atlantic salmon (*Salmo salar*) using visible-near infrared reflectance spectroscopy. *Aquac. Res.* 45, 798–811.
- Burtis, C., Brunts, D., 2014. *Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics*. Elsevier, Philadelphia, London and Toronto.
- Buttle, L., Crampton, V., Williams, P., 2001. The effect of feed pigment type on flesh pigment deposition and colour in farmed Atlantic salmon, *Salmo salar* L. *Aquac. Res.* 32, 103–111.
- Cheng, C.H., Guo, Z.X., Ye, C.X., Wang, A.L., 2017. Effect of dietary astaxanthin on the growth performance, non-specific immunity, and antioxidant capacity of pufferfish (*Takifugu obscurus*) under high temperature stress. *Fish Physiol. Biochem.*
- Choubert, G., 1985. Effects of starvation and feeding on canthaxanthin depletion in the muscle of rainbow trout (*Salmo gairdneri* Rich). *Aquaculture* 46, 293–298.
- Claireaux, G., Chabot, D., 2016. Responses by fishes to environmental hypoxia: integration through Fry's concept of aerobic metabolic scope. *J. Fish Biol.* 88, 232–251.
- Clark, T.D., Donaldson, M.R., Drenner, S.M., Hinch, S.G., Patterson, D.A., Hills, J., Ives, V., Carter, J.J., Cooke, S.J., Farrell, A.P., 2011. The efficacy of field techniques for obtaining and storing blood samples from fishes. *J. Fish Biol.* 79, 1322–1333.
- Clark, T.D., Donaldson, M.R., Pieperhoff, S., Drenner, S.M., Lotto, A., Cooke, S.J., Hinch, S.G., Patterson, D.A., Farrell, A.P., 2012. Physiological benefits of being small in a changing world: responses of coho salmon (*Oncorhynchus kisutch*) to an acute thermal challenge and a simulated capture event. *PLoS One* 7, e39079.
- Clark, T.D., Roche, D.G., Binning, S.A., Speers-Roesch, B., Sundin, J., 2017. Maximum thermal limits of coral reef damselfishes are size dependent and resilient to near-future ocean acidification. *J. Exp. Biol.* 220, 3519–3526.
- Clark, T.D., Sandblom, E., Cox, G.K., Hinch, S.G., Farrell, A.P., 2008. Circulatory limits to oxygen supply during an acute temperature increase in the Chinook salmon (*Oncorhynchus tshawytscha*). *Am. J. Physiol.-Reg. I* 295, R1631–R1639.
- Daufresne, M., Lengfellner, K., Sommer, U., 2009. Global warming benefits the small in aquatic ecosystems. *Proc. Natl. Acad. Sci. USA* 106, 12788–12793.
- DPWPWE, 2000. Marine Farm Development Plan, Tamar Estuary. Department of Primary Industries, Parks, Water and Environment, Tasmania.
- Elliott, J.M., Elliott, J.A., 2010. Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: predicting the effects of climate change. *J. Fish Biol.* 77, 1793–1817.
- Foss, P., Storebakken, T., Schiedt, K., Liaaen-Jensen, S., Austreng, E., Streiff, K., 1984. Carotenoids in diets for salmonids. 1. Pigmentation of rainbow trout with the individual optical isomers of astaxanthin in comparison with canthaxanthin. *Aquaculture* 41, 213–226.
- Fulton, T.W., 1904. The Rate of Growth of Fishes. 22nd Annual Report of the Fishery Board of Scotland. pp. 141–241.
- Grunenwald, M., Adams, M., Carter, C., Nichols, D., Koppe, W., Adams, L., 2016. Pigment depletion in Atlantic salmon (*Salmo salar*) starved at high temperature: effect of dietary carotenoid type and vitamin E level. In: *Proceedings of the 17th International Symposium on Feeding and Nutrition of Fish*. Idaho, USA.
- Hevroy, E.M., Hunskar, C., de Gelder, S., Shimizu, M., Waagbo, R., Breck, O., Takle, H., Sussort, S., Hansen, T., 2013. GH-IGF system regulation of attenuated muscle growth and lipolysis in Atlantic salmon reared at elevated sea temperatures. *J. Comp. Physiol. B* 183, 243–259.
- Hevroy, E.M., Waagbo, R., Torstensen, B.E., Takle, H., Stubhaug, I., Jorgensen, S.M., Torgersen, T., Tvenning, L., Sussort, S., Breck, O., Hansen, T., 2012. Ghrelin is involved in voluntary anorexia in Atlantic salmon raised at elevated sea temperatures. *Gen. Comp. Endocrinol.* 175, 118–134.
- Higgins, P.J., Thorpe, J.E., 1990. Hyperplasia and hypertrophy in the growth of skeletal muscle in juvenile Atlantic salmon, *Salmo salar* L. *J. Fish Biol.* 37, 505–519.
- IPCC, 2013. *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, United Kingdom and New York, NY, USA.
- Jeffries, K.M., Hinch, S.G., Donaldson, M.R., Gale, M.K., Burt, J.M., Thompson, L.A., Farrell, A.P., Patterson, D.A., Miller, K.M., 2011. Temporal changes in blood variables

- during final maturation and senescence in male sockeye salmon *Oncorhynchus nerka*: reduced osmoregulatory ability can predict mortality. *J. Fish Biol.* 79, 449–465.
- Johansson, D., Ruohonen, K., Kiessling, A., Oppedal, F., Stiansen, J.E., Kelly, M., Juell, J.E., 2006. Effect of environmental factors on swimming depth preferences of Atlantic salmon (*Salmo salar* L.) and temporal and spatial variations in oxygen levels in sea cages at a fjord site. *Aquaculture* 254, 594–605.
- Johnston, I.A., Alderson, R., Sandham, C., Dingwall, A., Mitchell, D., Selkirk, C., Nickell, D., Baker, R., Robertson, B., Whyte, D., Springate, J., 2000. Muscle fibre density in relation to the colour and texture of smoked Atlantic salmon (*Salmo salar* L.). *Aquaculture* 189, 335–349.
- Johnston, I.A., de la Serrana, D.G., Devlin, R.H., 2014. Muscle fibre size optimisation provides flexibility for energy budgeting in calorie-restricted coho salmon transgenic for growth hormone. *J. Exp. Biol.* 217, 3392–3395.
- Kijas, J., McWilliam, S., Sanchez, M.N., Kube, P., King, H., Evans, B., Nome, T., Lien, S., Verbyla, K., 2018. Evolution of sex determination loci in Atlantic Salmon. *Sci. Rep.-Uk* 8.
- Lund, S.G., Caissie, D., Cunjak, R.A., Vijayan, M.M., Tufts, B.L., 2002. The effects of environmental heat stress on heat-shock mRNA and protein expression in Miramichi Atlantic salmon (*Salmo salar*) parr. *Can. J. Fish. Aquat. Sci.* 59, 1553–1562.
- Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. *Aquat. Toxicol.* 101, 13–30.
- McBryan, T.L., Anttila, K., Healy, T.M., Schulte, P.M., 2013. Responses to temperature and hypoxia as interacting stressors in fish: implications for adaptation to environmental change. *Integr. Comp. Biol.* 53, 648–659.
- Messmer, V., Pratchett, M.S., Hoey, A.S., Tobin, A.J., Coker, D.J., Cooke, S.J., Clark, T.D., 2017. Global warming may disproportionately affect larger adults in a predatory coral reef fish. *Glob. Change Biol.* 23, 2230–2240.
- Molony, B.W., Church, A.R., Maguire, G.B., 2004. A comparison of the heat tolerance and growth of a selected and non-selected line of rainbow trout, *Oncorhynchus mykiss*, in Western Australia. *Aquaculture* 241, 655–665.
- Nakano, T., Kameda, M., Shoji, Y., Hayashi, S., Yamaguchi, T., Sato, M., 2014. Effect of severe environmental thermal stress on redox state in salmon. *Redox Biol.* 2, 772–776.
- Oehme, M., Aas, T.S., Olsen, H.J., Sorensen, M., Hillestad, M., Li, Y., Asgard, T., 2014. Effects of dietary moisture content of extruded diets on physical feed quality and nutritional response in Atlantic salmon (*Salmo salar*). *Aquac. Nutr.* 20, 451–465.
- Oliver, E.C.J., Benthuyens, J.A., Bindoff, N.L., Hobday, A.J., Holbrook, N.J., Mundy, C.N., Perkins-Kirkpatrick, S.E., 2017. The unprecedented 2015/16 Tasman Sea marine heatwave. *Nat. Commun.* 8.
- Olsvik, P.A., Vikesa, V., Lie, K.K., Hevroy, E.M., 2013. Transcriptional responses to temperature and low oxygen stress in Atlantic salmon studied with next-generation sequencing technology. *BMC Genom.* 14, 817.
- Oppedal, F., Dempster, T., Stien, L.H., 2011. Environmental drivers of Atlantic salmon behaviour in sea-cages: a review. *Aquaculture* 311, 1–18.
- Pan, F., Zarate, J.M., Tremblay, G.C., Bradley, T.M., 2000. Cloning and characterization of salmon hsp90 cDNA: upregulation by thermal and hyperosmotic stress. *J. Exp. Zool.* 287, 199–212.
- Pankhurst, N.W., King, H.R., 2010. Temperature and salmonid reproduction: implications for aquaculture. *J. Fish Biol.* 76, 69–85.
- Portner, H.O., 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J. Exp. Biol.* 213, 881–893.
- Remen, M., Sievers, M., Torgersen, T., Oppedal, F., 2016. The oxygen threshold for maximal feed intake of Atlantic salmon post-smolts is highly temperature-dependent. *Aquaculture* 464, 582–592.
- Sandnes, K., Lie, O., Waagbo, R., 1988. Normal ranges of some blood chemistry parameters in adult farmed Atlantic salmon, *Salmo salar*. *J. Fish Biol.* 32, 129–136.
- Sauer, D.M., Haider, G., 1979. Enzyme activities in the plasma of rainbow trout, *Salmo gairdneri* Richardson – effects of nutritional status and salinity. *J. Fish Biol.* 14, 407–412.
- Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9, 671–675.
- Skrede, G., Storebakken, T., 1986. Characteristics of color in raw, baked and smoked wild and pen-reared Atlantic Salmon. *J. Food Sci.* 51, 804–808.
- Skugor, S., Glover, K.A., Nilsen, F., Krasnov, A., 2008. Local and systemic gene expression responses of Atlantic salmon (*Salmo salar* L.) to infection with the salmon louse (*Lepeophtheirus salmonis*). *Bmc Genom.* 9.
- Stehfest, K.M., Carter, C.G., McAllister, J.D., Ross, J.D., Semmens, J.M., 2017. Response of Atlantic salmon *Salmo salar* to temperature and dissolved oxygen extremes established using animal-borne environmental sensors. *Sci. Rep.-Uk* 7, 4545.
- Storebakken, T., Goswami, U.C., 1996. Plasma carotenoid concentration indicates the availability of dietary astaxanthin for Atlantic salmon, *Salmo salar*. *Aquaculture* 146, 147–153.
- Sutton, S.G., Bult, T.P., Haedrich, R.L., 2000. Relationships among fat weight, body weight, water weight, and condition factors in wild Atlantic salmon parr. *Trans. Am. Fish. Soc.* 129, 527–538.
- Toften, H., Arnesen, A.M., Jobling, M., 2003. Feed intake, growth and ionoregulation in Atlantic salmon (*Salmo salar* L.) smolts in relation to dietary addition of a feeding stimulant and time of seawater transfer. *Aquaculture* 217, 647–662.
- Torrissen, O.J., Christiansen, R., Struksnaes, G., Estermann, R., 1995. Astaxanthin deposition in the flesh of Atlantic salmon, *Salmo salar* L., in relation to dietary astaxanthin concentration and feeding period. *Aquac. Nutr.* 1, 77–84.
- Vikesa, V., Nankervis, L., Hevroy, E.M., 2017a. Appetite, metabolism and growth regulation in Atlantic salmon (*Salmo salar* L.) exposed to hypoxia at elevated seawater temperature. *Aquac. Res.* 48, 4086–4101.
- Vikesa, V., Nankervis, L., Hevroy, E.M., 2017b. High dietary energy level stimulates growth hormone receptor and feed utilization in large Atlantic salmon (*Salmo salar* L.) under hypoxic conditions. *Aquac. Nutr.* 23, 1193–1203.
- Weaver, R.J., Wang, P., Hill, G.E., Cobine, P.A., 2018. An in vivo test of the biologically relevant roles of carotenoids as antioxidants in animals. *J. Exp. Biol.* 221.
- Weihe, R., Dessen, J.E., Arge, R., Thomassen, M.S., Hatlen, B., Rorvik, K.A., 2018. Improving production efficiency of farmed Atlantic salmon (*Salmo salar* L.) by iso-energetic diets with increased dietary protein-to-lipid ratio. *Aquac. Res.* 49, 1441–1453.