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**Identifying the nature, extent and duration of critical production periods for
Atlantic salmon in Macquarie Harbour, Tasmania, during summer**

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Executive Summary

Macquarie Harbour (MH) has been the site of Atlantic salmon (*Salmo salar*) and Ocean Rainbow trout (*Oncorhynchus mykiss*) production for approximately 30 years, with a recent expansion of gross annual production in the harbour. The production environment for salmon throughout the year is broadly suitable, although the summer months have been identified by Huon Aquaculture as a critical production period given the combination of high temperatures and low dissolved oxygen (DO) at production depths within the cages.

In this project, we sought to determine the nature and extent of warm and hypoxic conditions in Macquarie harbour during summer periods to assist in planning future production strategies. Specifically, we: 1) summarised the known oxygen requirements of salmon under culture and the known environmental drivers of their swimming depths and densities; 2) analysed existing long-term data sets of environmental dissolved oxygen levels and temperature with depth at Huon Aquaculture leases to determine suitable production depths and how they vary with season; 3) documented the swimming depths and densities of Atlantic salmon in production cages in Macquarie Harbour during a critical summer production period in February 2016 using a cage-based echo-integration system; and 4) combined the environmental and group-based salmon data to determine the temperatures and dissolved oxygen levels salmon are exposed to during critical summer production periods.

Long-term data of temperatures across all cage depths (0-15 m) indicated temperatures were broadly suitable for salmon production for most of the year (6 - 17°C), with the exception of the surface 3 - 4 m layer in late summer where temperatures exceeded 18°C. Salmon actively avoid water warmer than 17 - 18 °C (Johansson et al., 2006, 2009).

The long-term DO data at Huon Aquaculture's MH lease sites indicates that suitable depths for production depend strongly on season. Based on the assumption that DO levels below 6 mg L⁻¹ are sub-optimal for production, only 4 months of the year have suitable DO levels at 10 - 15 m (July to October) and only 6 months of the year have suitable DO levels at 5 - 10 m (July to December). DO levels at 0 - 5 m depths were consistently high throughout the 2013 - 2016 monitoring period, with the exception of the intrusion of poor DO waters (3 - 4 mg l⁻¹) into the 4 - 5 m depth layers in February 2015.

During the 3 day period from Feb 12 – 14 when salmon were intensively monitored with the echo-integration system, over 75% of the salmon biomass in cages crowded into a narrow depth range between approximately 4 and 6 m depth. Observed fish densities in this depth range were 25 - 35 kg m⁻³ and 15 - 30 m⁻³ in the two monitored cages, with packing factors 3-4 times the overall stocking densities in both cages.

Limiting Oxygen Saturation (LOS) is considered the limit for acceptable DO reductions with respect to fish welfare (Huntingford and Kadri 2008). Known LOS levels for healthy diploid salmon are 47% saturation at 16°C and 55 % at 18°C (Remen et al. 2013). LOS have not been determined for triploid salmon, however, triploids generally perform worse than diploids in warm temperatures and at low DOs (Hansen et al. 2015). As such, LOS limits are likely higher for triploids than diploids.

Based on known LOS levels for salmon (Remen et al. 2013), the swimming depths of salmon in the 4 - 6 m depth range in the period from 12 - 14 Feb, and the environmental DO and

temperatures, we conclude that LOS was consistently breached throughout this period and that welfare conditions were unacceptable. 32 - 52 % saturation at 4 m and 26 - 41 % saturation at 5 m over the course of Feb 7 - 14, 2016, at temperatures of 17 - 19°C were consistently below the known LOS threshold for diploid salmon at 18°C (55%). This is the level at which salmon are no longer able to metabolically regulate to cope with hypoxia. The effects of environmental hypoxia on production in MH will include reduced feeding and appetite, poor growth with the possibility of negative growth rates, elevated levels of ram ventilation to increase water flow across the gills, and elevated levels of mortality.

As environmental conditions were consistently poor throughout mid-Jan to mid-Feb 2016, it is likely that DO consistently breached LOS throughout this period. The long-term environmental data across the 2013-2016 period also indicates that similar periods of unacceptable hypoxia, though less intense in strength and duration, would have also occurred in the 2013/2014 and 2014/2015 summers.

The effects of poor DO levels in cages in summer can be mitigated in three ways: 1) modifying the environmental DO levels through adding oxygen to the cages; 2) only feeding fish when DO levels are above LOS and in the range where they can effectively utilise the feed; and 3) minimising stocking densities to reduce cage-scale draw down of DO levels.

Introduction

Salmon production in Macquarie Harbour

Macquarie Harbour (MH) is an approximately 250 km² embayment on the west coast of Tasmania, Australia (42°18.17'S, 145°23.86'E). The harbour has a narrow entrance to the sea, which creates an enclosed harbour environment with little flushing and long water residence time. The Gordon and Franklin river systems provide large freshwater inputs into MH, with waters high in humic substances and higher plant debris. As such, salinity levels in the harbour are typically low, with a brackish surface layer common. The surface brackish layer is believed to have distinct advantages in limiting the impact of the amoebic gill disease causing agent, *Neoparamoeba perurans*, which is epidemic elsewhere in Tasmanian salmon production systems. Freshwater inputs lead to tannin rich waters and low light penetration in the water column, with low dissolved oxygen below the halocline and in sediments (Cresswell et al. 1989, Carpenter et al. 1991, Koehnken 1996).

MH has been the site of Atlantic salmon (*Salmo salar*) and Ocean Rainbow trout (*Oncorhynchus mykiss*) production for approximately 30 years. The aquaculture industry is currently in a phase of expansion in the harbour, with gross production growing from 5,000 t in 2008 to 18,000 tonnes in 2015. Maximum allowable standing biomass (i.e. maximum biomass allowable at any one time) does not necessarily equate to the total gross annual production in one year, which could be more than this amount. Most salmon production in MH is based on triploid fish, as they provide advantages in terms of minimising undesirable early maturation. While conditions for growth for triploid fish are suitable for the majority of the production cycle in MH, summer cage environments are challenging. During January and February, the surface waters warm, and dissolved oxygen levels at cage depths reach their lowest levels for the year, creating conditions far below optimal levels for growth.

Triploids are more susceptible to poor dissolved oxygen conditions than diploids, particularly at high temperatures (Hansen et al. 2015). Feeding rates and growth performance at 19 °C are reduced in triploid Atlantic salmon compared to diploids, with the greatest effect on mortality rate evident at O₂ saturation levels of <70%. Determining the nature and extent of warm and hypoxic conditions in Macquarie harbour during summer periods will assist in planning future production strategies.

Salmon swimming depths in sea-cages

Environments within fish farms are variable in space and time, with great variation occurring with depth. Salmon sense and respond to a range of environmental influences on farms (Oppedal et al. 2011a), and in doing so choose the depths at which they swim and the densities in which they occur. Preferred swimming depths and densities of salmon are the result of active trade-offs among temperature, light, the entry of feed, oxygen levels or the presence of treatment chemicals, and an array of internal motivational factors, such as hunger and perceived threats. Behavioural trade-offs in response to environmental and internal drivers typically result in schooling at specific depths at densities 1.5 to 5 times their stocking density, and up to 20 times in extreme cases (Oppedal et al. 2011b). Understanding the behaviour-environment interactions of fish is critical in determining when they will be exposed to sub-optimal or even lethal conditions. Further, it can help farmers evaluate mechanisms to modify environments during critical periods or implement other management practices to improve production.

Known effects of temperature on salmon swimming depths

At stratified sites where temperature and other environmental variables have been measured in high spatial and temporal resolution, salmon clearly position themselves vertically in relation to temperature within sea-cages (Johansson et al., 2006, 2007, 2009; Oppedal et al., 2007; Dempster et al., 2008, 2009a; Korsøyen et al., 2009). Seasonal changes in the vertical distribution of salmon have occurred concurrent with temperature shifts, suggesting that salmon avoid colder temperatures (Oppedal et al., 2001a).

Johansson et al. (2006) performed a multivariate analysis to determine which environmental variables most influenced the vertical distribution of salmon; temperature emerged as the key environmental factor associated with density and swimming depth. The preferred temperature range was 16 - 18 °C within a range of 11 - 20 °C. Salmon individuals and groups displayed both avoidance to water warmer than 17 °C and water at the cold end of the temperature spectrum, indicating active behavioural thermoregulation (Johansson et al., 2006, 2009). In contrast, in reasonably homogenous environments where temperature varies little with depth, temperature does not influence the vertical distribution of salmon (Juell et al., 2003; Juell and Fosseidengen, 2004).

Critical Dissolved Oxygen thresholds for salmon production

Complex spatial and temporal variations in dissolved oxygen (DO) levels exist within sea-cages stocked with salmon (Johansson et al., 2006, 2007; Vigen, 2008; Stien et al., 2009). Strong vertical gradients in DO typically coincide with the pycnocline, while fluctuating patterns occur over days to weeks (Johansson et al., 2006, 2007). Severely hypoxic conditions (30% saturation at 12 °C) have been recorded over periods of up to 1 h in the centre of a commercial cage and were correlated with periods of low water flow (Oppedal et al. 2011). Seasonal variations in DO levels are also frequently observed at commercial salmon farms.

Adequate DO levels are a key requirement to ensure fish welfare and development (Kindschi and Koby, 1994; Van Raaij et al., 1996; Ellis et al., 2002). Pedersen (1987) showed that at 15 °C, growth rates of juvenile rainbow trout decreased if fixed levels of DO fell below 7.0 mg O₂ l⁻¹ (70% oxygen saturation) and that trout fed less when fixed levels reached 6.0 mg O₂ l⁻¹ (60% oxygen saturation). A recent study with full-feeding Atlantic salmon held in seawater at 16 °C and given fluctuating hypoxic saturation levels of 70% led to reduced appetite; 60% additionally initiated acute anaerobic metabolism and increased skin lesions; 50% additionally initiated acute stress responses, reduced feed conversion and growth; and 40% additionally caused impaired osmoregulation and mortalities (Remen et al. 2012). Growth rates and condition factors gradually decreased and proportions of fish with skin infections gradually increased in severity as hypoxia levels rose. Lack of energy from aerobic metabolism for fish within the hypoxic groups may have led to down-regulation of energy-demanding processes such as feed uptake, growth and immune function (e.g. review by Wu, 2002).

Further work by Remen et al. (2013) established the effects of temperature and hypoxia acclimation on the routine oxygen consumption rate ($\dot{M}O_2$) and the limiting oxygen saturation (LOS, defined as the hypoxia tolerance threshold). They used undisturbed, fed fish kept in groups to resemble commercial aquaculture conditions. $\dot{M}O_2$ was measured using open respirometry where a progressive decline in O₂ was caused by fish O₂ consumption during a period of low water turnover. LOS was defined as the O₂ below which fish were no longer able to uphold routine $\dot{M}O_2$. Both $\dot{M}O_2$ and LOS were increased exponentially with temperature, but no effect of hypoxia

acclimation was found. Mean LOS at 6, 12, 16 and 18 °C were 30 ± 1 (mean \pm SE), 39 ± 1 , 47 ± 1 and $55 \pm 2\%$ saturation, respectively. The lower limits determined in this study for acceptable drops in O₂ are widely used to plan and manage aquaculture production in Norway.

A separate study on the Tasmanian salmon stock (Barnes et al. 2011) determined the critical oxygen threshold (P_{crit}) at 22 °C (4.6 ± 0.3 mg l⁻¹) and 18 °C (3.4 ± 0.3 mg l⁻¹). Above the P_{crit} , fish are able to regulate their metabolic rate, but once DO drops below the P_{crit} this ability ceases and they become dependent upon the environmental oxygen concentration. Whether these values for small (150 g), diploid salmon translate to large (1 - 5 kg), triploid fish remains untested, although theory suggests that both fish size and triploidy will increase the P_{crit} threshold.

Known effects of DO levels on salmon swimming depths

Despite the importance of DO to production parameters and welfare, little specific information exists to determine how salmon modify their behaviours within sea-cages in response to sub-optimal DO levels. Kramer (1987) classified the response of fish to increasing hypoxic conditions as changes in activity and vertical or horizontal habitat changes. Like most other aquatic animals, fish have the capacity to detect and actively avoid low oxygen levels (DO conc 1 - 4 mg l⁻¹/DO sat 15–60% at 25 °C seawater; Wannamaker and Rice, 2000; Wu, 2002) and migrate vertically in the water column to avoid hypoxic zones (e.g. Hazen et al., 2009). However, whether salmon actively avoid depths within sea-cages that have low to intermediate oxygen levels (DO conc 2.5 - 6 mg l⁻¹ or DO sat 30–75% saturation in 15 °C seawater) remains unresolved.

In an investigation of the environmental parameters influencing the vertical distributions of salmon at 4 commercial sites, a multivariate analysis indicated that salmon avoided specific depths in the water column where oxygen saturation levels approached 60% at 15 °C (Johansson et al., 2007). However, minimum levels of oxygen ranging down to 57% saturation at 14 °C in an experimental study of different stocking densities did not implicate DO as significantly affecting fish densities, possibly due to other environmental factors (temperature) exerting greater effect on vertical positioning (Johansson et al., 2006). This was confirmed by an experiment which created low DO present at the surface with a tarpaulin skirt when there was a simultaneous strong temperature gradient with warmest water at surface of 15°C versus 8 °C deeper down. Here, the salmon preferred the warmest temperature despite potentially lethal DO levels, indicating that their strong preference for temperature overruled any hypoxia avoidance (Stien et al. 2012).

Need to understand the MH production environment for salmon

For several years, Huon Aquaculture has been monitoring temperature, salinity and DO conditions at its MH sites to understand the spatial and seasonal variability in the environment. This pre-existing, long-term data set clearly identifies the summer months as a critical production period given the combination of high temperatures and low dissolved oxygen at production depths within the cages. Monitoring of the environment in MH in late 2015 and early 2016 indicates that dissolved oxygen levels at cage production depths are the poorest on record over the past 3 years. This pre-existing work has identified a clear need to understand what salmon are exposed to during summer conditions.

High-resolution data on salmon swimming depths and schooling densities in relation to temperature and dissolved oxygen, in particular, will enable determination of how to best manage current and future production levels in MH. This includes implementing cage management strategies to minimise negative impacts upon production, timing harvests to avoid compromising the production potential of its farms, and setting appropriate stocking densities for each cage.

Objectives

Production environments in MH have been monitored at cage depths for several years by the fish farming companies. Temperature, salinity and DO levels and how they vary with depth suggest that there is limited volume in cages suitable for triploids in the summer months. However, there is no corresponding information about how salmon respond behaviourally to cope with the challenging environment and if current stocking densities are appropriate. Visual methods, such as cameras, provide little useful information to assess swimming depths and densities at the scale of commercial operations. This is particularly so in MH as tannin rich waters have poor visibility.

In this study, we sought to:

- 1) Analyse existing long-term data sets of environmental dissolved oxygen levels and temperature with depth at Huon aquaculture leases to determine suitable production depths and how they vary with season
- 2) Document the swimming depths and densities of Atlantic salmon in production cages in Macquarie Harbour during a critical summer production period in February using a cage-based echo-integration system
- 3) Combine the environmental and group-based salmon data to determine the temperatures and dissolved oxygen levels salmon are exposed to during critical production periods
- 4) Recommend options to minimise the effects of poor DO levels on salmon production in summer

Methods

Leases studied

Long-term measurements of environmental data and short term monitoring of salmon swimming depths and densities was done for each of two separate leases (Gordon and Strahan) in MH.

Long-term environmental data (2013-2016)

Huon Aquaculture has been taking daily profiles at 1 m depth intervals of temperature, salinity and dissolved oxygen since 2013.

Fish swimming depths and densities (Feb 12 to 14, 2016)

Two 160 m circumference production cages at each of two separate leases (Gordon and Strahan) were monitored in MH, one with 500 g - 1 kg fish and one with harvest size 3.5 - 5 kg fish.

Data on swimming depths and densities of salmon in all cages were continuously recorded using a PC-based echo integration system (Lindem Data Acquisition, Oslo, Norway) connected to transducers. These were positioned within cages at a quarter-distance (13 m) from the edge at approximately 8.5 - 9.5 m depth (slightly raised off the net bottom), facing upwards with a 42° acoustic beam. This gave measures of echo-intensity, which is directly related to fish density, at depth intervals of 0.5 m between 0.25 - 8.5 m (interference at the surface and 1 m above the transducer was removed). Outputs of echo strength are given as the mean value of echo intensity

per minute, and each cell (in depth and time) is calculated as a proportion of the total acoustic backscatter (sum of echo intensities) received at that time point, across all depths.

Using the data, we calculated the observed fish density (OFD) over all depths at a given hour and the packing factor (PF) as OFD/stocking density of fish per m³. The packing factor describes how many times the actual swimming densities of fish is higher than the average cage stocking density.

Ambient temperature and dissolved oxygen measured at 1 m depth intervals outside of cages within the lease areas were available from Huon's existing monitoring procedures at the sites.

Results

Seasonal patterns in temperature and dissolved oxygen in MH (2013-2016)

For both the Gordon and Strahan leases (Fig. 1 & 2), temperatures across all depths were broadly suitable for salmon production for most of the year (6 - 17°C), with the exception of the surface 3 - 4 m layer in late summer where temperatures exceeded 18°C.

DO levels at 0-5 m depths were consistently high across the 3+ year long-term monitoring period for both the Gordon and Strahan leases (Fig. 3 & 4), with the exception of the intrusion of poor DO waters (3 - 6 mg L⁻¹) into the 4-5 m depth layers in mid-February 2015. DO levels at 5 - 10 m depths were typically sub-optimal for production (range: 3 - 6 mg L⁻¹) across summer and autumn each year (January to June), although poor conditions occurred as early as October 2015. DO levels at 10-15 m depths were sub-optimal for salmon for almost 8 months per year from Oct/Nov to July in most years, with some variability.

Fish swimming depths and densities in MH (Feb 12 to 14, 2016)

Over the 3 day period from Feb 12 - 14, 75% of the salmon biomass in both cages at the Gordon lease crowded into a narrow depth range between approximately 3.5 and 6 m depth (Fig. 5). Observed fish densities in this depth band were typically 25 - 35 kg m⁻³ in cage 12 and 15 - 30 m⁻³ in cage 13, with packing factors 3-4 times the overall stocking densities in both cages. The swimming depth pattern was consistent across both day and night. The typical daily variation in swimming depth (shallow at night, deeper during the day) was not evident. As fish were not fed during this period, which rules out changes in swimming depths related to feeding, it appears that temperature alone governed swimming depth preference, with fish crowding into the temperature range of 17 - 19°C. Avoidance of waters deeper than 6 m could have been driven by either temperature preference, as waters below 6 m were <17°C, avoidance of poor DO levels, as waters below 6 m had even poorer DO levels, or a combination of the two. A similar, though more variable pattern was observed for smaller fish (0.5 - 1 kg) at the Strahan lease (Fig. 6), with fish in the 4 - 6 m depth band on the 12th and 13th Feb, with the biomass moving shallower and centred around 3 m on the 14th.

Dissolved oxygen and temperatures at the main swimming depths of salmon (Feb 7 to 14, 2016)

From Feb 7 - 14, 2016, which included the period Feb 12 - 14 where fish swimming depths centred around 4-5 m depths, DO levels at 4 m dropped as low as 3 mg L⁻¹ (range: 3.0 - 4.8) and at 5 m to 2.5 mg L⁻¹ (range: 2.5 - 3.8; see Fig. 7 for DO levels plotted for Feb 12 to 14). With temperatures of 17 - 19°C and salinities of 20-25 ppt, these readings equate to 32.2 - 52.2 % saturation levels at 4 m and 26.3 - 40.7 % saturation levels at 5 m for the entire week.

Temperatures from Feb 7 to 14 ranged from 18.2 to 20.2°C at 4 m and 17.4 to 19.1°C at 5 m (see Fig. 7 for temperatures plotted for Feb 12 to 14). As diploid salmon are known to actively avoid waters >18 - 19°C (Oppedal et al. 2011), the surface 3.5 m would have been unsuitable, with 4 m at the upper edge of the preference limit.

Fig. 1 Temperature profile with depth at a reference location outside the cages on the Gordon lease. Temperature was recorded daily at 1 m depth intervals.

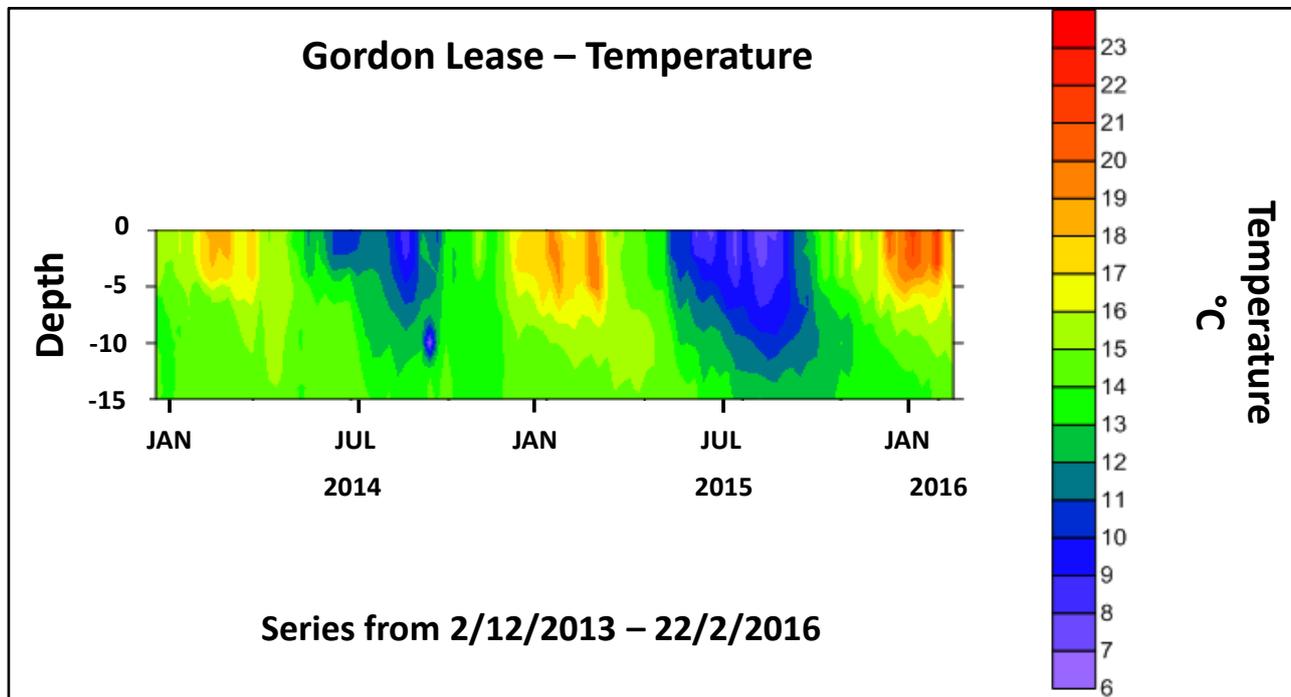


Fig. 2 Temperature profile with depth at a reference location outside the cages on the Gordon lease. Temperature was recorded daily at 1 m depth intervals.

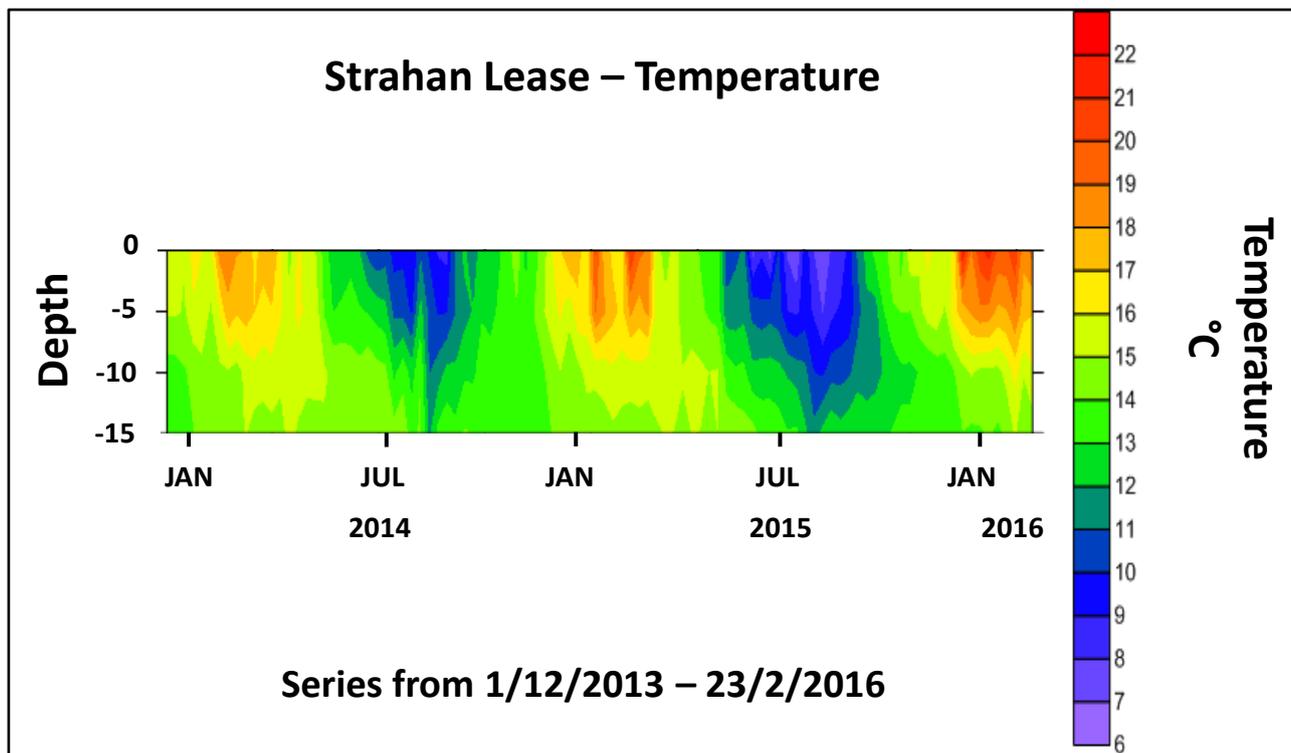


Fig. 3 Dissolved oxygen profile with depth at a reference location outside the cages on the Gordon lease. DO was recorded daily at 1 m depth intervals.

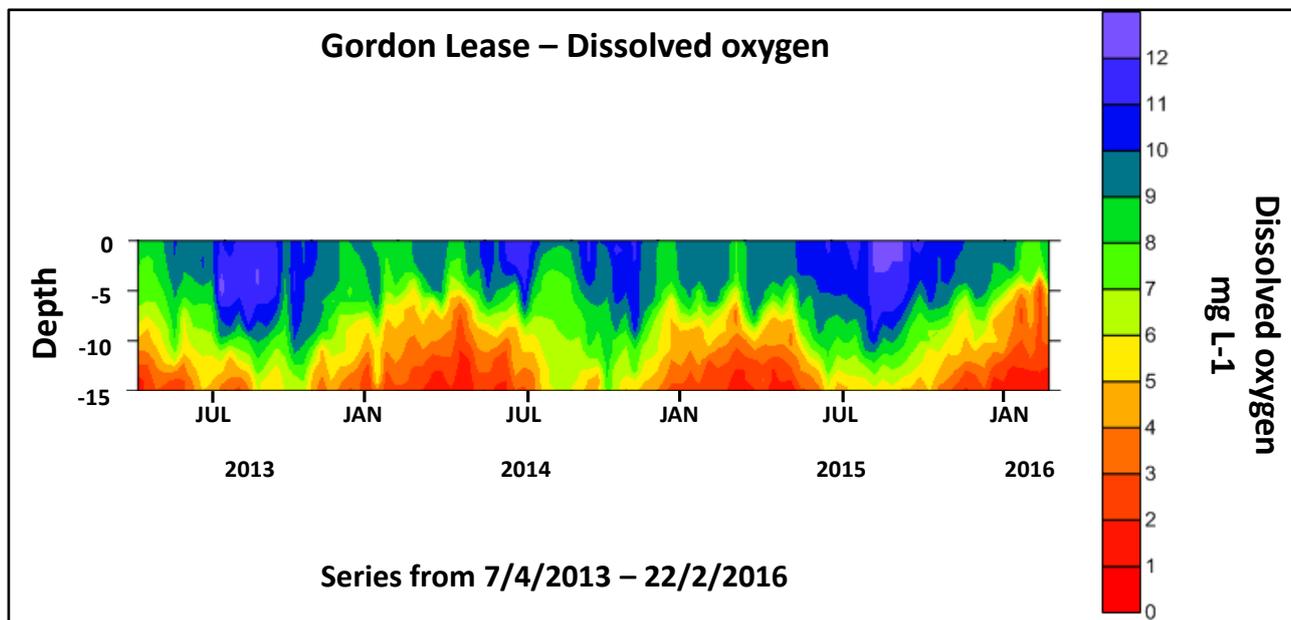


Fig. 4 Dissolved oxygen profile with depth at a reference location outside the cages on the Strahan lease. DO was recorded daily at 1 m depth intervals.

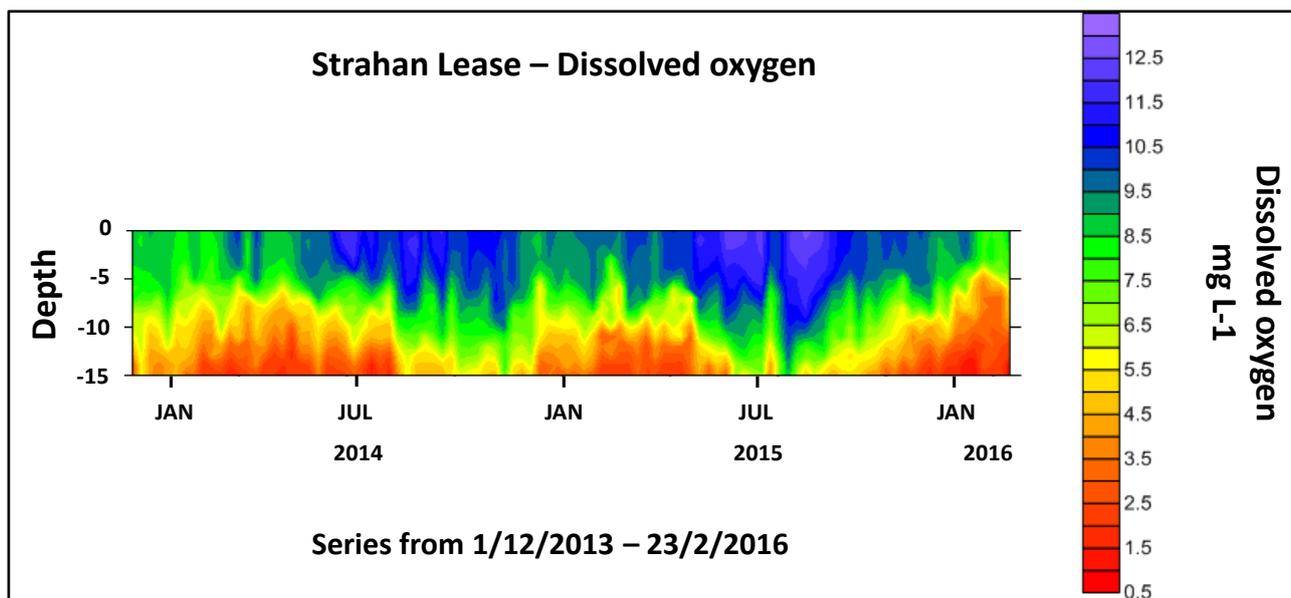


Fig. 5 Observed fish densities (OFD) with depth inside two cages on the Gordon lease. Vertical white bars indicate power outages where no signal was received.

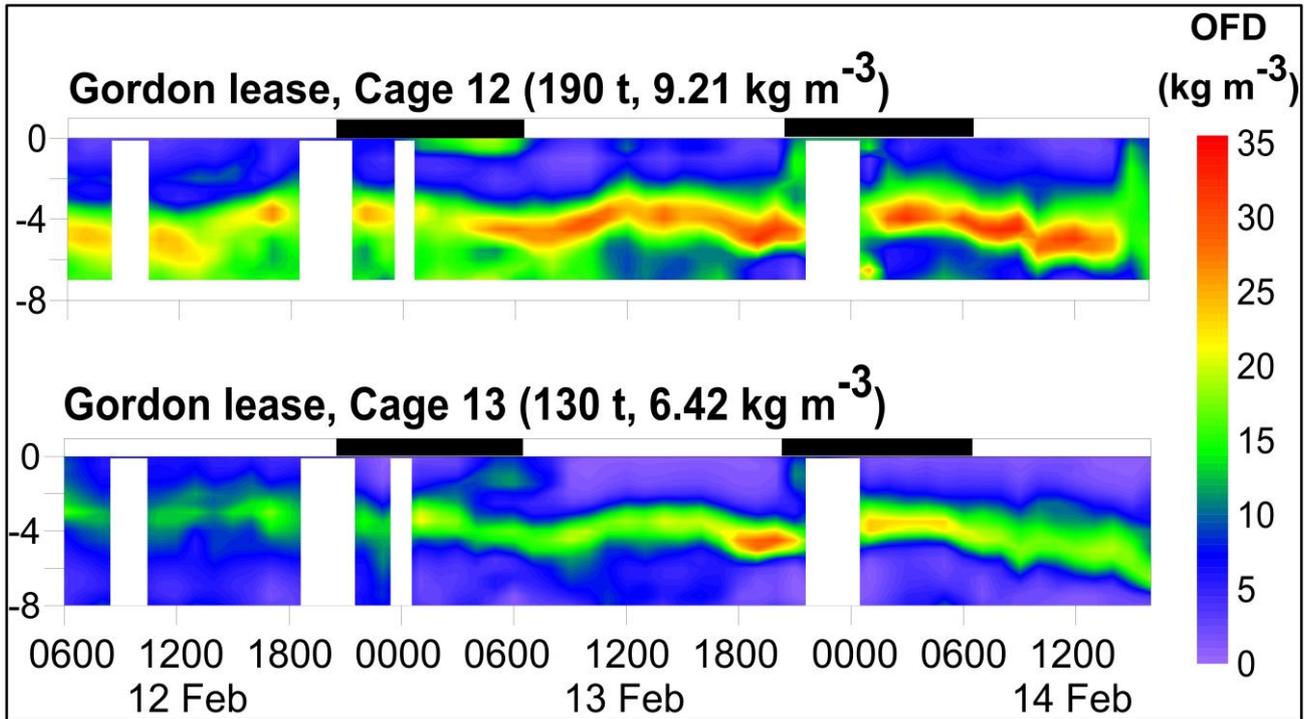


Fig. 6 Observed fish densities (OFD) with depth inside a cage on the Strahan lease. Vertical white bars indicate power outages where no signal was received.

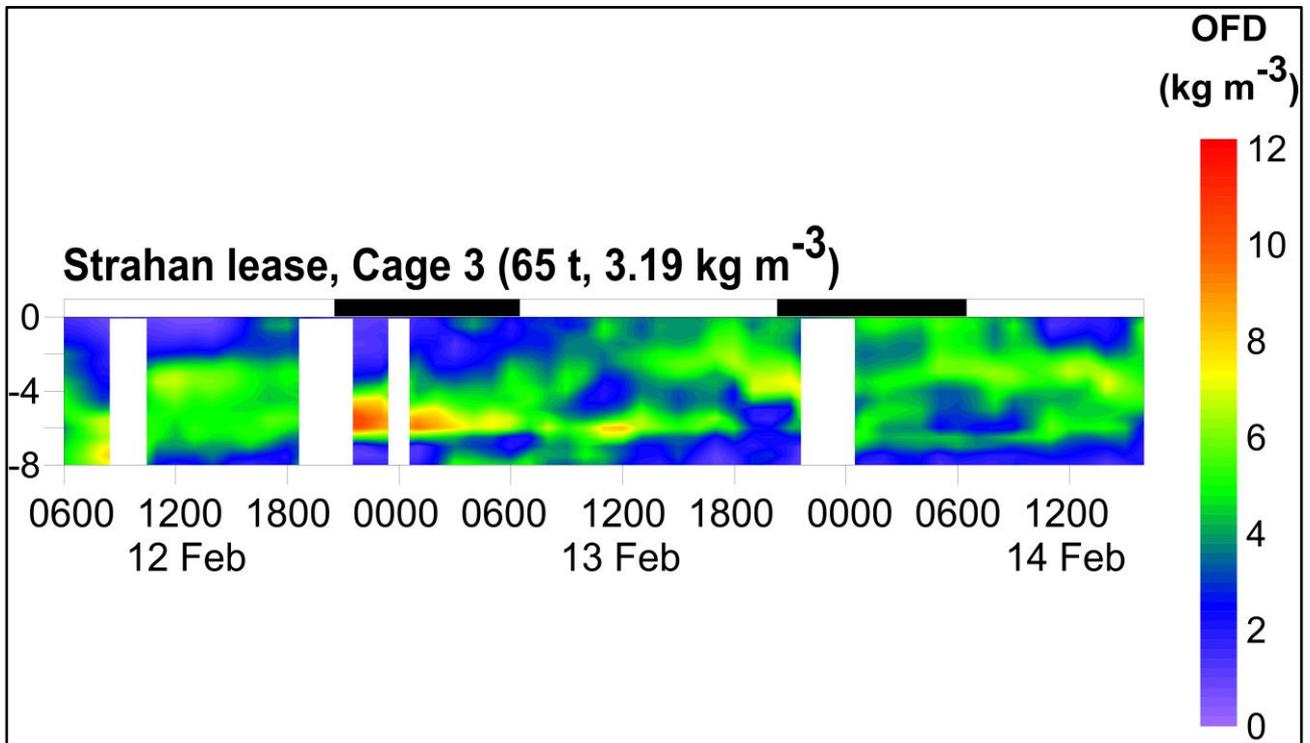
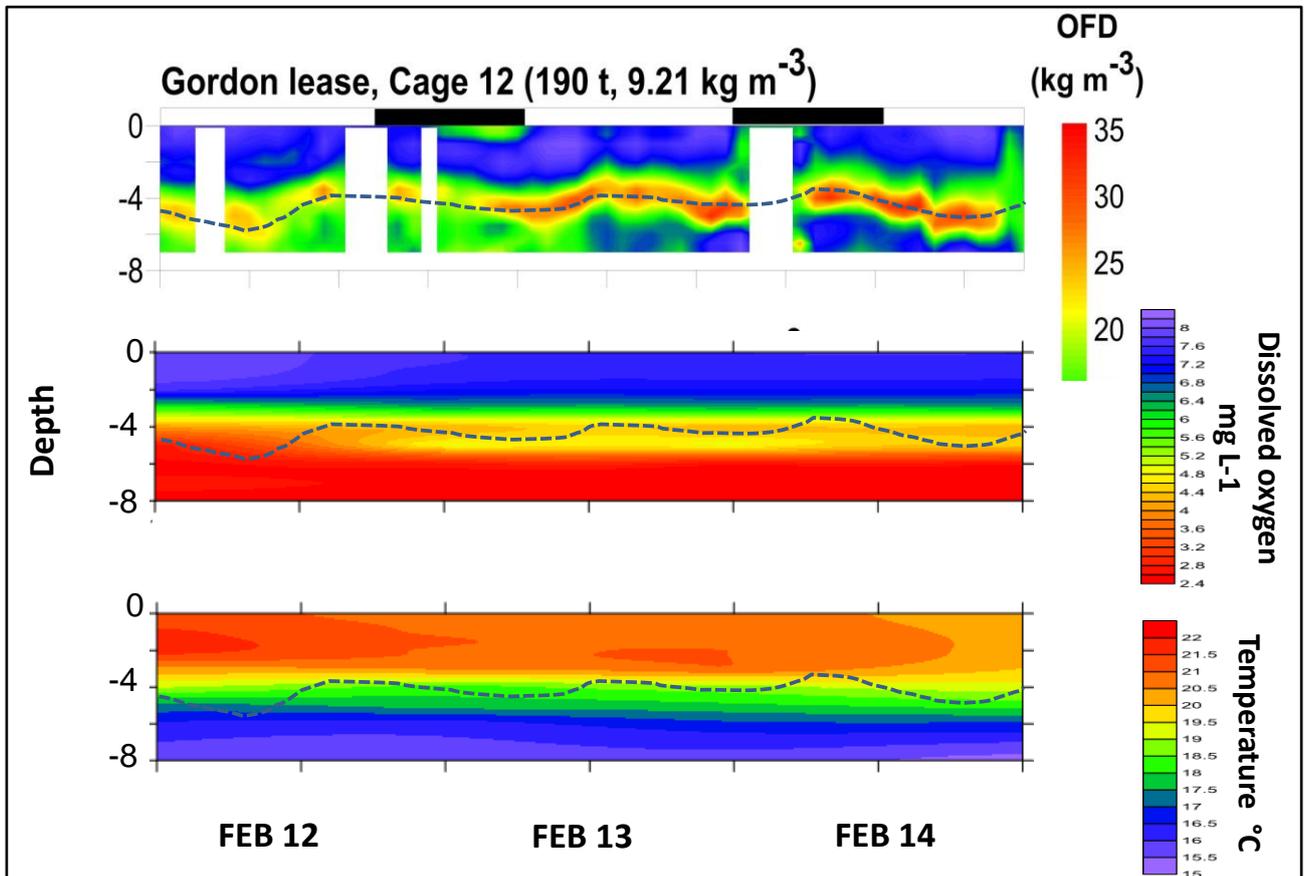


Fig. 7 Observed fish densities (OFD) with depth inside one cage on the Gordon lease, with corresponding dissolved oxygen and temperatures measured at a reference location outside the cage. Dashed line indicates the average swimming depth of the population through time, and transposed on the dissolved oxygen and temperature plots, the environmental conditions the fish would have experienced throughout this period. Vertical white bars indicate power outages where no signal was received.



Discussion

Suitability of the production environment in Macquarie Harbour

Temperature and salinities at MH production sites are broadly suitable for salmon production throughout the year at depths of 0 - 15 m, except for the late summer in each of the three years where data was recorded, where surface waters warmed to exceed 18°C. As salmon usually position themselves at the warmest possible temperature up to approximately 17°C (Johansson et al. 2006), temperatures likely strongly influenced their swimming depths in MH throughout the year. Based on the annual profile, they will swim shallower from January to July, but below the 17°C thermocline when water warms at the surface during summer, and swim deeper from April/May to October as surface waters cool. Salmon tolerate a wide range of salinities, and there is limited evidence that fish position themselves vertically with respect to salinity levels once they are beyond the immediate post-smolt stage after sea transfer (Oppedal et al. 2011a).

The long-term DO data at the MH lease sites indicates that suitable depths for production depend strongly on season. Based on the assumption that DO levels below 6 mg L⁻¹ are sub-optimal for production, only 4 months of the year have suitable DO levels at 10 - 15 m (July to October) and only 6 months of the year have suitable DO levels at 5 - 10 m (July to December). DO levels at 0 - 5 m depths were consistently high across the 3 year+ long-term monitoring period for both the Gordon and Strahan leases (Fig. 3 & 4), with the exception of the intrusion of poor DO waters (3 - 4 mg l⁻¹) into the 4-5 m depth layers in February 2015.

As salmon choose their swimming depths principally based on temperature, the depth distributions of DO and temperature have to be considered together to determine if summer conditions are suitable for production. When high surface water temperatures >18°C occur in the upper 3-4 m, this will drive salmon to swim deeper than 4 m most of the time. When warm surface waters coincide with the intrusion of low DO waters as shallow as 4 - 5 m, this severely constrains depths at which salmon can survive during summer in Macquarie harbour to a narrow depth range as little as 2 m wide. Conditions were particularly severe in MH in Feb 2016, with DO conditions extremely poor (3 - 4 mg l⁻¹) at 4 - 6 m depth below the warm surface layer of 0 - 4 m. The long-term environmental data suggests that similar production depth constraints, although less severe in nature and duration, occurred during the 2013/2014 and 2014/2015 summers.

The severity and consequences of environmental hypoxia in summer

Environmental hypoxia compromises the physiological function of fish. Under hypoxic conditions, salmon can maintain their routine metabolic rate (RMR, the metabolic rate of fed fish engaged in voluntary swimming) primarily by increasing gill ventilation and perfusion, until DO reaches a level where this is no longer effective (Perry and Gilmour 1996, Perry et al., 2009). Below this threshold DO, termed the limiting oxygen saturation (LOS), the oxygen consumption rate ($\dot{M}O_2$) begins to decrease with further reductions in DO (Ott et al. 1980, Stevens et al. 1998). At DO below the LOS, anaerobic metabolism increases (e.g. Remen et al. 2012a, Vianen et al. 2001), stress responses occur (e.g. Perry and Reid 1994, Van Raaij et al. 1996) and survival becomes dependent on the availability of fermentable substrates, the ability to suppress metabolism and to withstand a build-up of anaerobic end-products (Nilsson and Östlund-Nilsson 2008, Richards, 2009). LOS is considered the limit for acceptable DO reductions with respect to fish welfare (Huntingford and Kadri 2008).

Known Limiting Oxygen Saturation (LOS) levels for healthy diploid salmon are 47 ± 1 and $55 \pm 2\%$ saturation at 16 and 18°C (Remen et al. 2013). LOS have not been experimentally determined for triploid salmon, however, triploids generally perform worse than diploids in warm temperatures and at low DOs (Hansen et al. 2015). As such, LOS limits are likely higher for triploids than diploids.

Based on these known threshold values (Remen et al. 2013), the swimming depths of salmon in the 4-6 m depth range in the period from 12 - 14 Feb and the environmental DO and temperatures, we conclude that LOS was consistently breached throughout this period and that welfare conditions were unacceptable. 32.2 - 52.2 % saturation at 4 m and 26.3 - 40.7 % saturation at 5 m over the course of Feb 7 to 14, 2016, at temperatures of 17 - 19°C were consistently below the known LOS threshold for diploid salmon at 18°C (55%). This is the level at which salmon are no longer able to metabolically regulate to cope with hypoxia. The effects of environmental hypoxia on production in MH will include reduced feeding and appetite, poor growth with the possibility of negative growth rates, elevated levels of ram ventilation to increase water flow across the gills, and elevated levels of mortality.

As environmental conditions were consistently poor throughout mid-Jan to mid-Feb 2016, it is likely that DO consistently breached LOS throughout this period. The long-term environmental data across the 2013-2016 period also indicates that similar periods of unacceptable hypoxic levels, though less intense in strength and duration, would have also occurred in the 2013/2014 and 2014/2015 summers.

A further important consideration is that wherever comparisons of in-cage DO and reference site DO have been made (e.g. Johansson et al. 2007), conditions within cages are typically 10-50% worse due to local scale oxygen draw down by the fish. As all recorded DO levels used in our analysis came from reference sites 100s of metres from cages, the levels they indicate are likely higher than those experienced by the salmon within cages.

While the echo-sounder data provides group level average swimming depths, it is possible that individual salmon periodically accessed the upper 0 - 3 m depth later where DO levels were higher and obtained some respite from the hypoxic conditions, although the echogram provides little evidence that fish were in surface waters in any great number or period.

Finally, by way of comparison, the poor DO conditions at Huon's leases in MH in summer contrast to its east coast sites. DO measured at cage depths of 0 - 16 m at the Flathead lease in the Huon Channel in February 2016 indicated DO levels were always optimal for production (85 - 110% saturation; Appendix Fig. 1).

Possible management actions to limit the effects of environmental hypoxia

Continuous monitoring of DO and temperature within salmon cages is the most accurate way to determine the likely swimming depths and the DO levels experienced by the fish. Measurements of temperature at 1 m intervals will provide strong predictive capability of the average swimming depths of the cage biomass. Fine-scale DO monitoring should focus on the critical depths at which salmon swim, which is in the waters below their preferred upper temperature limit (19°C), and within the preferred range of 16-19°C. Wherever this temperature range occurs in summer, the greatest biomass of salmon is likely to be found. Dissolved oxygen levels should be measured continuously at a reference point outside of the farm and inside the farm within the most susceptible cage for low DO levels. Such a cage would be positioned between other cages, where the least current flow occurs and/or in the cage with highest stocked biomass.

The effects of poor DO levels in cages can be improved in three ways: 1) modifying the environmental DO levels through adding oxygen to the cages; 2) only feeding fish when DO levels are above LOS and in the range where they can effectively utilise the feed; and 3) minimising stocking densities to reduce cage-scale draw down of DO levels.

Oxygen addition at the correct depth could provide short-term respite when DO conditions are particularly poor. Our data suggests that DO release at the main depths at which fish swim (4-6 m) will have greatest effect.

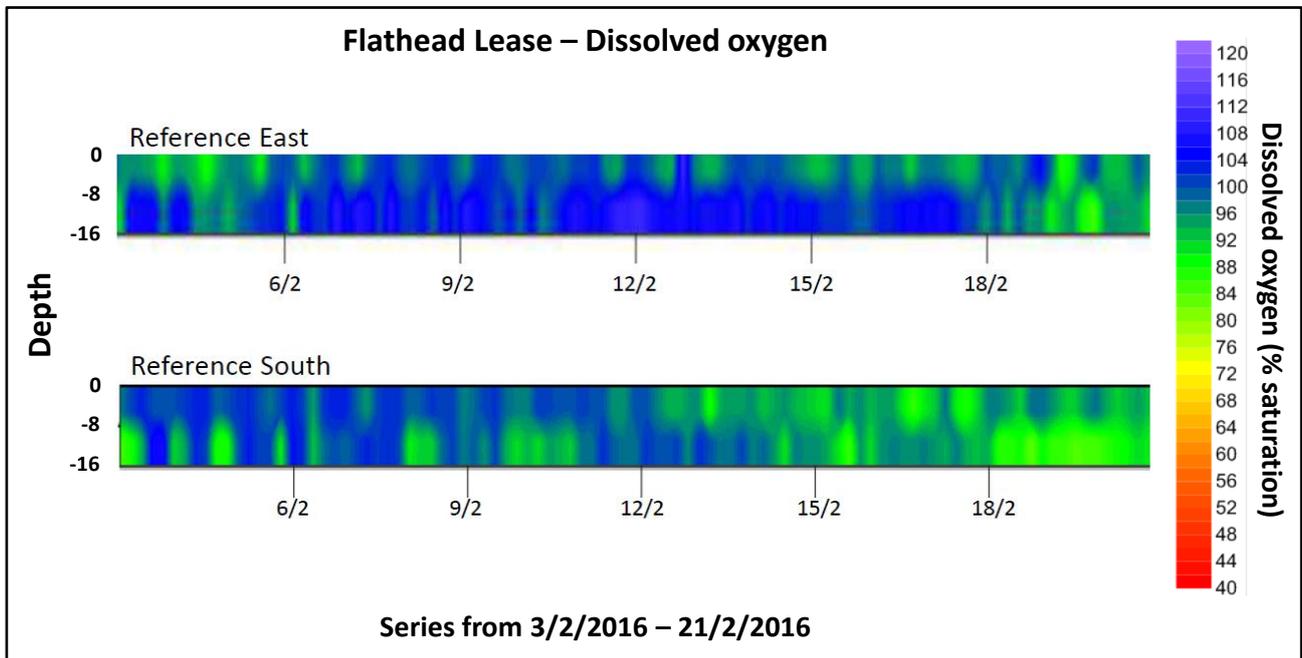
LOS is increased by any factor that increases a fish's metabolic rate, including the quantity of ingested feed (Forsberg, 1997). Thus, feeding when DO saturation levels are approaching the LOS is not recommended. While there is no specific information regarding what the LOS thresholds are for unfed or fed triploid salmon at different temperatures, we recommend a conservative safety margin of 20% above the LOS as a minimum threshold for feeding. This translates to a >70% DO saturation level, which also concurs with the results of Hansen et al. (2015), who determined that triploids fed poorly and had elevated mortality rates at <70% DO saturation at 19°C.

Stocking rates (=salmon biomass/cage volume; kg m^{-3}) will dictate the observed fish densities that fish will pack into when only narrow depth bands are suitable for the salmon. During Feb 2016, fish packed at a factor of 3-4 times their stocking density up to 25-35 kg m^{-3} on the Gordon lease. If stocking density had been higher, the packing factor and observed fish densities would also likely have been higher. In other studies, packing factors as high as 10-20 and observed fish densities >200 kg m^{-3} have been observed (Oppedal et al. 2011a, 2011b), indicating that when pressed by the environment, salmon will pack at very high levels. If stocking densities were any higher in MH, the likelihood of significant cage-scale draw down of DO at the main depths that salmon swim at would increase. In contrast, lower stocking densities minimises the risk of packing and high observed fish densities.

Huon Aquaculture already undertakes these strategies at their MH production leases.

Appendix 1

Appendix Fig. 1 Dissolved oxygen profile with depth at two reference locations outside the cages on the Flathead lease in the Huon Channel. DO was recorded every 1 minute at 3 m depth intervals with *in situ* probes.



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