Deoxygenated Groundwater Upwelling in the Atlantic Salmon Incubation zone: Implications for Embryonic Survival and an Opportunity for Adaptation

by

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Thesis for the degree of Doctor of Philosophy

November 2016
There is increased recognition that groundwater upwelling causes low oxygen in Atlantic salmon nests (redds), and reduces incubation success. Field data suggest patterns of groundwater upwelling are driven by seasonal hydrological variation. However, these data are currently limited to a small number of sites and are predominantly descriptive. Consequently, wavelet analysis was performed on a range of published and unpublished datasets that monitored oxygen concentration in the incubation zone of Atlantic salmon spawning rivers throughout the UK. This analysis demonstrated episodes of groundwater induced oxygen depletion in all locations and that groundwater upwelling was linked to antecedent and prevailing weather conditions.

To test the possible effects of episodic periods of hypoxia on Atlantic salmon eggs, such as those caused by groundwater upwelling, an incubation facility was designed that enabled fine-scale control of oxygen depletion. Hypoxia in the earlier stages of development did not affect survival but caused alevin to hatch later in an underdeveloped state. Low oxygen conditions at the later stages of development reduced survival by 15% and caused premature hatching of severely underdeveloped alevin. Therefore, effective management of factors that influence groundwater upwelling is important to limit effects on incubating salmonids.

The membrane surrounding the Atlantic salmon egg is a barrier to oxygen diffusion, so its permeability influences oxygen supply to the embryo. Due to factors such as deoxygenated groundwater upwelling, oxygen supply varies among spawning locations and could drive inter-population membrane architecture variation. Electron microscopy egg membrane permeability of five UK Atlantic salmon populations varied substantially, and eggs with the lowest permeability were most susceptible to hypoxia-induced mortality. Therefore, variation in egg membrane structure could represent adaptation to oxygen stress, such as that caused by deoxygenated groundwater upwelling, and reinforces the importance of maintaining genetic integrity of distinct populations.
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DECLARATION OF AUTHORSHIP

I, Jack Bloomer

declare that this thesis entitled

Deoxygenated Groundwater Upwelling in the Atlantic Salmon Incubation zone: Implications for Embryonic Survival and an Opportunity for Adaptation

and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

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Acknowledgements

Thanks must first go to my primary supervisor, Professor David Sear, for his continuous cheerful support and guidance, which has been invaluable. My second supervisor, Professor Paul Kemp, has provided helpful encouragement and my scientific writing in particular has improved immeasurably thanks to his input.

Thanks are also due to the Environment Agency for part-funding the project and Tim Sykes, Peri Karageorgopoulos and Kerry Sims in particular for their support. Additional funding was also gratefully received from the University of Southampton Department of Geography and Environment through a doctoral training scholarship.

Assistance in the development of the research facility from Dominic Mann was invaluable. While the guidance and generosity of Anton Page, Lizzie Angus and all at the University of Southampton Biomedical Imaging Unit was of enormous help in the processing of samples for electron microscopy.

Thanks must also go to the various hatchery managers who have provided assistance with provision of Atlantic salmon eggs for all the experiments throughout my research. In particular, this includes Kasi Rajan and Keith Drynan at Landcatch Natural Selection Ltd., David Summers of the Tay District Salmon Fisheries Board, Richard Bond at Kielder Hatchery and David Bamford at the Ure Salmon Trust.

Thanks to all my friends in Southampton who have provided welcome distractions, food and drinks on a regular basis. I also send my best wishes to the Shackleton Geographicals; I’m sure the good times are still ahead! Thanks also to my friends at home for their diverse conversation and unique sense of encouragement. I particularly appreciate never being allowed to forget what a proper pint should taste like.

Finally, thanks must go to my family. My sister and Emily have been a welcome source of northern camaraderie in the south. My Mum and Dad, who have provided unquestioning support in every way imaginable. Lastly, to Sharon, whose unrelenting encouragement and inexhaustible patience has been beyond anything I could have asked for.
Thesis Structure

This thesis is separated into nine chapters that cumulatively identify and address critical research questions outlined in the title: “Deoxygenated groundwater upwelling in the Atlantic salmon incubation zone: implications for embryonic survival and an opportunity for adaptation”.

Chapter 1 comprises of a comprehensive review of literature, broken down into three sections. The first considers studies that have investigated causes of low oxygen in the Atlantic salmon incubation zone, with a particular focus on the effect of deoxygenated groundwater (GW) upwelling. The second section discusses the potential effects of deoxygenated GW upwelling on the survival of Atlantic salmon during incubation, and at later life-stages. The final section considers whether Atlantic salmon eggs could adapt to oxygen stress in the incubation zone through structural variation of the egg membrane. Throughout chapter one, gaps in the literature are identified and used to develop three research aims, which are also presented at the end of each section. These aims and objectives were addressed using novel methods, which were developed using a multi-disciplinary approach described in chapter 2. Each research aim outlined in chapter one was subsequently explored through three independent research papers (chapters 3 to 5). The research papers consist of an introduction that re-emphasises the critical aspects of the literature presented in chapter one, a methodology that gives detailed descriptions of the processes used to address the research aims, followed by key results and conclusions. Chapter 6 consists of overarching conclusions that directly addresses each of the aims and objectives outlined in chapter one and discusses potential avenues for future research. Finally, chapter 7 synthesises the findings to explain how the thesis represents a cohesive body of work.
Chapter 1: Introduction

From New England to Northern Russia and the Iberian Peninsula to Northern Scandinavia, Atlantic salmon (*Salmo salar*) is an iconic species of great cultural, ecological and economic importance. The cultural appeal of Atlantic salmon lies in their unique life cycle and historical value as a source of food for communities throughout their range (WWF 2001). From an ecological perspective, Atlantic salmon are key indicators of riverine health (Parrish *et al.* 1998) and provide a critical source of food for land, marine and freshwater organisms (Metcalfe *et al.* 1987; Carss *et al.* 1990). Finally, the recreational Atlantic salmon fishery in England and Wales is valued at £10million per year (Environment Agency 2009a), a value matched by a single catchment (River Spey) in Scotland (Butler *et al.* 2009).

Despite their importance, anthropogenic influence at every stage of their life cycle has precipitated substantial declines of Atlantic salmon populations. In 2001, 27% of populations worldwide were extinct or at risk of extinction and a further 30% were considered endangered or vulnerable (WWF 2001). Furthermore, in the UK alone, numbers returning to spawn in 2014 were nearly 60% lower than the 1970s average (ICES 2015). Population declines have been attributed to changing climatic patterns that reduce survival in the marine phase by limiting their thermal habitat (Otterson *et al.* 2001). Adults returning to rivers to spawn often faced fishing pressure that caused population crashes in many regions, and led to an almost global ban on commercial fishing of wild Atlantic salmon (Parrish *et al.* 1998). Finally, individuals that successfully complete their spawning run often deposit their eggs in degraded environments that limit embryonic survival and population recovery (Rosenberg *et al.* 2000). The diverse nature of the stressors that Atlantic salmon face means that populations have declined rapidly, and restoration attempts are highly complex.

Due to the value of Atlantic salmon, much research has been conducted into the causes, effects and potential mitigation of stressors that drive population declines. However, the way that stressors must be studied and addressed varies depending on their nature and scale. While research into the potential effects of climate change on Atlantic salmon is critical, mitigation of climate driven population decline cannot be species-specific. Changing climatic patterns are global in extent and, as such, require a global solution that extends beyond the needs of a single species and the borders of individual nations. Stringent fishing quotas and careful management of commercial fisheries have been successful in reversing the damage caused by overfishing for a range of fish species (Marchal *et al.* 2016). However, if the habitats of these species have been degraded so much that they are unable to support viable populations, management of
commercial fisheries alone is insufficient. As a result, successful management of every life-stage is essential to ensure successful rehabilitation of depleted stocks.

The embryonic phase of the Atlantic salmon life cycle, which encompasses the egg and alevin stage, is critical and often represents a population bottleneck (Milner et al. 2003). Higher rates of mortality frequently occur at this stage than at any other in the Atlantic salmon life-cycle (Einum & Fleming 2000a), and this can be exacerbated by reductions in habitat quality (Greig et al. 2007a). Indeed, low rates of embryonic survival can reduce the strength and limit the recovery of depleted populations (Chadwick 1982). In particular, the egg stage of the Atlantic salmon life cycle has received considerable scientific attention. In recent decades, anthropogenic activity in rivers and catchments has been increasingly linked to reduced incubation success (Thorstad et al. 2008; Kemp et al. 2011). As a result, improvement of spawning conditions is a key goal of many fisheries management strategies.

With the aim of enhancing wild populations, management techniques such as supportive breeding have been introduced to alleviate incubation stress. Supportive breeding involves extraction of gametes from wild individuals and rearing the fertilised eggs in a hatchery environment, before releasing alevin, fry or smolts back into native rivers (Tessier et al. 1997). While supportive breeding has merits, particularly in terms of short-term enhancement to severely depleted stocks (Milner et al. 2004), it can mask long-term threats to population recovery in the natural environment (McClure et al. 2008). Furthermore, shifts in key traits at the egg stage have been observed in salmonid populations influenced by supportive breeding (Heath et al. 2003). Due to the possible negative effects of direct management techniques such as supportive breeding, restoration of natural Atlantic salmon incubation zone should be the long-term goal of population recovery programmes.

To ensure restoration projects are effective, information on the nature of stressors found throughout the life cycle of Atlantic salmon is essential. Monitoring of freshwater habitat quality for salmonids has been conducted extensively, and a wide range of stressors have been identified (Finn 2007). While the nature of the effects and method of introduction of stressors to river systems are complex, factors that influence the quality of freshwater habitats are often simpler to monitor than those in the marine environment. This is because their source, pathway for introduction and effects are usually contained within a smaller area. This also means that the management of processes that influence incubation quality can take place on a smaller-scale and often do not require international cooperation, which frequently hinders environmental enhancement projects (Bang 2015). Therefore, improved knowledge of the Atlantic salmon
spawning environment and long-term effect of stressors on population strength would greatly benefit future management strategies.

To address the importance of incubation quality on Atlantic salmon population strength, the remainder of this chapter consists of a review of the current literature broken into three distinct sections:

1. Stressors found in the Atlantic salmon incubation zone. This section focuses on causes of oxygen stress during incubation, with a particular focus on the effect of groundwater upwelling;
2. The effects of insufficient oxygen supply on the survival and development of Atlantic salmon eggs;
3. The potential for different populations of Atlantic salmon exposed to differing levels of oxygen stress to develop adaptations to enhance uptake.

At the end of each section, gaps in the literature are identified and are used to guide the development of a distinct set of aims and objective, which are subsequently presented.

1.1 Oxygen stress in the incubation zone

1.1.1 Atlantic salmon incubation zone

Throughout their range, female Atlantic salmon deposit their eggs in nests, known as redds, in gravel riverbeds (Fig. 1.1) where they are fertilised by one or more males. Typically, eggs are buried to a depth of 15-30cm (Crisp & Carling 1989), which means they are located in the hyporheic zone, a substreambed region where groundwater (GW) and surface water (SW) mix (Malcolm et al. 2008; Tonina & Buffington 2009a). While this location within the hyporheic zone reduces their risk of predation, it increases their vulnerability to mechanical damage and poor water quality (Wootton 1990).
Figure 1.1. Diagram of a typical salmonid redd. Note numerous egg packets at the tail of the redd.

Taken from Washington Department of Fish and Wildlife (2015).

Within the United Kingdom, Atlantic salmon spawning takes place from October to early January, depending upon river temperature. Earliest spawning takes place in the cooler rivers of northern Scotland and the highlands (Calderwood 1901; Webb & McLay 1996), while the latest occurs in the warmer chalkstreams of southern England (Welton et al. 1999). This thermal gradient of spawn timing arises because temperature positively correlates with embryonic developmental rate (Alderdice & Velsen 1978; Beacham & Murray 1985, 1990). As a result, Atlantic salmon populations native to cooler rivers tend to spawn earlier to compensate for slower development. This ensures juveniles hatch and subsequently emerge from gravels at a time of greater food availability (Schindler et al. 2005). Therefore, in the coolest rivers, Atlantic salmon eggs can incubate for up to 4 months (Malcolm et al. 2006). This extended period means that there is a high likelihood of exposure to one or more stressors.

Four of the main stressors experienced by Atlantic salmon eggs that can reduce incubation success are: (1) accumulation of metabolic waste. Metabolic waste such as ammonia and carbon dioxide that are produced by the embryo during respiration can accumulate in the perivitelline fluid (PVF) within the egg capsule and the microenvironment surrounding the egg surface (Dhiyebi et al. 2013). These substances are deleterious to salmonids in high concentrations (Burkhalter & Kaya 1977). In addition, hydrogen ions produced during respiration reduce the pH of the PVF, which can further increase mortality rates (Lacroix 1985); (2) Mechanical disturbance of the incubation zone, which generally occurs due to scour, can cause 100% mortality of eggs in the redd (May et al. 2009; Rollinson & Hutchings 2011; Roni et al. 2015). Scour occurs most frequently during high flow events (Lisle 1989; Lapointe et al. 2000) and in areas of greater bed mobility.
(Montgomery et al. 1999); (3) Exposure to toxic substances such as heavy metals (Armitage et al. 2007; Jezierska et al. 2009) and pesticides (Biggs et al. 2007), which are found in rivers due to anthropogenic activities in the catchment such as mining and agriculture. These substances can interfere with the physiology of fish and eggs, and can cause high rates of mortality (Jezierska et al. 2009). Finally, (4) insufficient supply of oxygen is perhaps the best-studied determinant of incubation quality (e.g. Meehan & Swanston 1977; Chapman 1988; Reiser et al. 1998; Crisp 2000; Malcolm et al. 2006; Greig et al. 2007a; Soulsby et al. 2009; Sear et al. 2014) can cause very high rates of mortality (e.g. Malcolm et al. 2003; Greig et al. 2007b). The remainder of section 1.1 addresses the causes of oxygen stress in the Atlantic salmon incubation zone.

1.1.2 Oxygen stress

As with all respiring organisms, incubating Atlantic salmon require a consistent supply of oxygen from their environment to maintain metabolic function. Embryos obtain this oxygen from a thin film of water surrounding the egg, known as the boundary layer (. 1.2, Daykin 1965). Therefore, oxygen supply is determined by the concentration gradient from the boundary layer to the embryo. In turn, the oxygen concentration of the boundary layer is determined by (1) oxygen demand of the embryo, and (2) oxygen supply to the boundary layer. The oxygen demand of the embryo is influenced by temperature and embryonic developmental state, and is discussed in detail in section 1.2.1. The rate of oxygen supply to the boundary layer, which forms the focus of this section, is largely determined by: (A) intragravel velocity and (B) oxygen concentration of the water (Daykin 1965; Wickett 1975; Greig et al. 2007a; Fig. 1.2). In ideal conditions, the redd allows clean, cool, well oxygenated water to continuously flow past the eggs, ensuring embryonic metabolism can be maintained. However, factors such as fine sediment infiltration and upwelling of deoxygenated GW can reduce oxygen supply to the boundary layer.
Figure 1.2. Diagram displaying oxygen supply to an individual salmonid egg. Note the boundary layer surrounding the egg. Oxygen supply is determined by the rate of flow and oxygen content of the water.

1.1.3 Fine sediments

During redd construction, the hydraulic action of the female sorts gravels, resulting in the removal of fine sediments (Montgomery et al. 1996). The typical diameter of gravels within Atlantic salmon redds varies, depending on female body size, but has a median value of 22mm (Kondolf & Wolman 1993). Well-sorted gravels with low fine sediment (henceforth described as sediments of diameter <2mm [Wood & Armitage 1997]) content ensure redds have high intragavel permeability, maintaining continuous flow of water past the eggs. This enhances oxygen supply and waste metabolite removal.

While fine sediments naturally occur in rivers through channel (river bank and bed) and non-channel (catchment) sources (Wood & Armitage 1997), human activity can increase their input considerably. In particular, non-channel input can be high in rivers flowing through catchments subject to intensive cropping (Naura et al. 2016), overgrazed pasture (Russell et al. 2001), deforestation (Motha et al. 2003), construction and mining (Owens et al. 2005). In addition, the recent European trend to switch from spring to autumn-sown cereal crops means that sediment input could be highest during the Atlantic salmon spawning season (Kemp et al. 2011; Fig. 1.3). This exacerbates the impact of fine sediment on incubation quality.
Figure 1.3. Representation of the annual peaks of sediment yield in UK rivers combined with common cropping practices and period where eggs (dotted bars) and hatched embryos (hashed bars) of various fish species are present in rivers. In the winter months, fine sediment load is highest because agricultural activity results in bare soils that are most vulnerable to erosion by run-off. This coincides with the period where salmonid eggs are likely to be present in streams, so means they face a high risk of exposure to fine sediments. Figure taken from Kemp et al. (2011).

Greater rates of Atlantic salmon embryo mortality have been linked to fine sediment input (Fig. 1.4). This is related to lower rates of oxygen supply to embryos as a result of one or a combination of three key processes: First, sediments can physically block micropores on the egg surface. Oxygen diffuses from the boundary layer to the incubating embryo through micropores on the surface of the egg membrane. Greig et al. (2005b) demonstrated that the introduction of clay particles to an incubator led to a decline of oxygen consumption of Atlantic salmon eggs. An overlap between clay particle size (<4μm) and estimates of micropore diameter (0.5–1.5μm) taken from the egg membranes of chum salmon (Oncorhynchus keta [Bell et al. 1969]) implies that clay particles can enter and physically block micropore canals (Greig et al. 2005b). If this takes place, oxygen is unable to diffuse from the boundary layer to the embryo, which could impede development and cause high mortality rates.
Figure 1.4. Relationship between percentage accumulation of fine sediments and percentage of Atlantic salmon embryos survival to hatch. Taken from Kemp et al. (2011) using data from Greig et al. (2007), O’Connor & Andrew (1998), Julian & Bergeron (2006).

Second, fine sediments gather within pore spaces of the redd, which reduces gravel permeability, intragravel velocity and thereby oxygen supply (e.g. Chapman 1988; Lisle & Lewis 1992; Levasseur et al. 2006; Greig et al. 2007a; Fig. 1.5). The cumulative effect of fine sediment infiltration on oxygen supply varies depending on its total volume (Fig. 1.5), average diameter and organic content (Sear et al. 2008, 2016). Larger sediments such as sands are more permeable to water flow; however fewer particles are required to block pore spaces. By contrast, finer sediments such as clay and silt flocculate and restrict water flow more strongly, but a greater amount is required to reduce flow rates (Chapman 1988). Sediments with high biological content can further restrict oxygen supply to eggs by promoting biofilm development around sediment particles (Sear et al. 2008). These biofilms form cohesive matrices, which further restrict intragravel velocity (Sear et al. 2008). The importance of size and constituent components means that there is not a direct linear relationship between fine sediment concentration and oxygen supply, and could explain some of the scatter observed in figures 1.4 and 1.5.
Third, active elements within sediments consume oxygen and reduce concentration in the redd (Greig et al. 2005a). The total effect of sediments on oxygen concentration of water flowing past salmon eggs is determined by the sediment oxygen demand (SOD). The SOD is the rate at which chemical and biological processes taking place in sediments and the streambed strip dissolved oxygen from river water (Hatcher 1980; Nakamura 2003; He & Liu 2011). In natural conditions, SOD is the combined total of chemical oxygen demand (COD), nitrogen oxygen demand (NOD) and biological oxygen demand (BOD) (Greig et al. 2007a). While the influence of COD on SOD in a salmonid redd is limited as it only occurs in anaerobic conditions, it could influence shallow GW (Krause et al. 2013). However, nonpoint input of biological material, particularly from agriculture, can increase the BOD and NOD of rivers (Sierra & Gomez 2007; Zia et al. 2013), as can point source input, from features such as outflow from sewage treatment plants (Vaquez-Sunyer et al. 2015; Naden et al. 2016). Consequently, the chemical and biological content of fine sediments plays an important role in determining oxygen supply and can reduce embryonic survival (Sear et al. 2016).

While SOD can reduce the oxygen concentration of water flowing through redds, other naturally occurring processes can exacerbate the issue. Of particular interest here, and forming the main focus of this study, is the effect of GW on the oxygen concentration within Atlantic salmon reds.
1.1.4 Groundwater upwelling

Atlantic salmon deposit their eggs in the hyporheic zone of rivers (Crisp & Carling 1989; Malcolm et al. 2003), so are subject to the interactions between GW and SW (Tonina & Buffington 2009a). During periods of high GW – SW connectivity, circulation cells trigger mixing that moves water into the alluvium through downwelling and into the river through upwelling, a process known as hyporheic exchange (Bencala 2005). While short-residence GW is often similar in nature to SW, the hydrochemistry of long-residence GW can be very different (Table 1.1). Therefore, the direction of hyporheic exchange can influence primary productivity and nutrient cycling in the stream (Mulholland & Webster 2010), invertebrate assemblages of the riverbed (Davy-Bowker 2005; Gibbins et al. 2016) and plays an important role in determining salmonid incubation quality (Malcolm et al. 2006, 2010; Soulsby et al. 2009). Of particular interest to the present study is the observation that GW can be severely oxygen depleted (Table 1.1).

Table 1.1. Comparison of some key characteristics of groundwater and surface water.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Surface Water</th>
<th>Groundwater</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>Variable, changes seasonally</td>
<td>Constant. Warmer than SW in winter and cooler in summer.</td>
<td>Burkholder et al. 2008</td>
</tr>
<tr>
<td>Conductivity</td>
<td>Variable but usually low</td>
<td>Usually high</td>
<td>Malcolm et al. 2003</td>
</tr>
<tr>
<td>Turbidity</td>
<td>Variable</td>
<td>Usually low</td>
<td>Ward et al. 1999</td>
</tr>
<tr>
<td>Nitrates</td>
<td>Variable but usually low</td>
<td>Often high</td>
<td>Krause et al. 2013</td>
</tr>
<tr>
<td>Ammonium</td>
<td>Only found in polluted water</td>
<td>Frequently present</td>
<td>Buss et al. 2004</td>
</tr>
<tr>
<td>Oxygen</td>
<td>Variable but high in unpolluted water</td>
<td>Usually low or zero</td>
<td>Malard &amp; Hervant 1999; Malcolm et al. 2006;</td>
</tr>
</tbody>
</table>

The direction of hyporheic exchange in the field can be recorded by monitoring fluctuations of hydrochemistry in the riverbed or through direct measurements of pressure variation. The contrasting hydrochemistry of GW and SW (Table 1.1) means that fluctuations in water quality could be representative of changing patterns of hyporheic exchange. Therefore, temperature (Malcolm et al. 2004; Soulsby et al. 2009), alkalinity (Malcolm et al. 2004) conductivity (Malcolm
et al. 2004, 2010) and dissolved oxygen (Malcolm et al. 2010; Soulsby et al. 2009) have been used as tracers to describe GW – SW interactions. However, by using hydrochemistry to monitor hyporheic exchange patterns, it is likely that upwelling of short-residence GW, with a similar hydrochemistry to SW, will not be recorded. Consequently, direct measurements of pressure variation using piezometers or pressure transducers (Malcolm et al. 2004, 2006; Tonina & Buffington 2007; Ibrahim et al. 2010 Sear et al. 2014) in the riverbed are often preferable. Such monitoring enables calculation of the vertical hydraulic gradient (VHG), which describes the direction of hyporheic exchange (Ibrahim et al. 2010). A positive VHG indicates areas characterised by upwelling, while a negative VHG is indicative of downwelling regions. Correlation between VHG and hyporheic water quality parameters has been observed in the field (Malcolm et al. 2006), and demonstrates that hyporheic exchange influences water quality in the hyporheic zone, and could affect salmonid incubation success.

Intermittent monitoring of brown trout (Salmo trutta) reds in northeast USA first demonstrated that salmonid eggs deposited in GW-dominated regions of the riverbed could experience low oxygen conditions (Hansen 1975). Since then, development of new technologies that enable high frequency monitoring of oxygen concentration (Malcolm et al. 2006) has driven increased interest in the effect of deoxygenated GW on salmonid incubation. Indeed, studies in Scotland (Malcolm et al. 2006, 2010; Soulsby et al. 2009), England (Greig 2004, Greig et al. 2007b; Bateman 2012), Wales (Burke 2011) and continental Europe (Schindler Wildaber et al. 2014) have provided evidence of severe GW-induced hypoxia in reds. Oxygen depletion to a level that is likely to be detrimental to Atlantic salmon incubation success has been frequently observed, so greater research into its potential effects is required.

Oxygen depletion of groundwater

Biological and chemical processes drive rapid oxygen depletion of GW in the aquifer less than 2m below the water table (Trudell et al. 1986; Danielopol & Niederreiter 1987). At this depth, the breakdown of dissolved organic carbon (DOC) by denitrifying bacteria strips oxygen from water, which can reduce concentrations to <1mg l⁻¹ within a few hours (Zarnetske et al. 2011a, b; Fig. 1.5). The type of sediment within the aquifer also influences the extent and rate of oxygen depletion. In particular, peat and clay soils tend to have higher concentrations of labile DOC, which is more bioavailable to denitrifying bacteria (Zarnetske et al. 2011b). This is broken down more readily, leading to greater and more rapid oxygen depletion (Krause et al. 2013). This can be further exacerbated by organic carbon loading through intensive agriculture, which increases the rate at which oxygen is stripped from the water (Foster & Chilton 2003). Oxygen supply to GW is limited due to the low diffusion coefficient in water (Cussler 1997), minimal convection currents
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(Hendricks & White 1995) and slow flux (Freeze & Cherry 1979). Therefore, the rate of oxygen depletion in the aquifer is the main determinant of GW oxygen concentration.

While denitrifying bacteria rapidly reduces oxygen levels to <1mg l⁻¹ (Zarnetske et al. 2011a, b), additional processes strip oxygen from GW if it remains in the aquifer for an extended period. In particular, long-residence GW often undergoes further oxygen reduction through consumption of soil organic carbon (SOC) and reduction of manganese, iron and sulphate (Malard & Hervant 1999). These processes often cause GW to become anoxic (Malard & Hervant 1999; Burow et al. 2010; Fig. 1.5) and are most common in confined aquifers that are overlain by densely packed fine sediments such as clay or impermeable rocks such as granite, which block hyporheic exchange (Malard & Hervant 1999). Consequently, locations exposed to long-residence GW upwelling could be typified by poor water quality that limits incubation success.

![Diagram of groundwater flow system](image)

**Figure 1.5.** Summary of the main physiochemical changes and redox reactions taking place along the flow path of groundwater in a confined aquifer system. DOC = dissolved organic carbon; SOC = sedimentary organic carbon. Taken from Malard & Hervant (1999).

**Effect of geomorphology on hyporheic exchange**

Patterns of hyporheic exchange fluctuate over a range of temporal and spatial scales due to geomorphological and hydrological variation. While lateral (between riverbank and stream) hyporheic exchange influences water quality in many rivers (Wroblicky et al. 1998; Kasahara & Hill 2007), this review focuses on vertical (between riverbed and stream) hyporheic exchange.
patterns because this influences the Atlantic salmon incubation zone. Locations of greater GW influence in the hyporheic zone are generally selected by geomorphological features from the basin-wide to micro-scale that vary downstream due to changes in channel confinement, valley slope and structural variation of the channel and floodplain (Baxter et al. 2003; Wörman et al. 2006; Buffington & Tonina 2009; Ibrahim et al. 2010). Such downstream variation led to the development of the ‘hyporheic corridor’ concept (Stanford & Ward 1993), which proposes that community assemblages change downstream in response to variable hyporheic exchange patterns. While broad-scale patterns described in the hyporheic corridor concept are frequently present, variation among and within catchments means that they are not linear and vary over a range of nested spatial scales (Malcolm et al. 2004; Buffington & Tonina 2009; Fig. 1.6).

**Figure 1.6.** Nested diagram of factors influencing groundwater upwelling on a variety of scales. 1: Changes at the valley segment scale e.g. valley confinement, alluvial volume or underlying bedrock; 2: Reach scale exchange in response to changes in alluvial volume, between meander bends etc.; 3: Exchange at the channel unit scale at pools bars or steps; 4: Variation at the microscale around debris, salmonid redds etc. at a scale of channel width. Dashed lines indicate boundaries between scales. Horizontal shaded areas indicate areas of compacted impervious clay. Figure taken from Buffington & Tonina (2009).
Features on the valley-segment scale (hundreds to thousands of channel width units) such as valley confinement, alluvial volume and underlying bedrock elevation influence hyporheic exchange. Confined valleys, which are characterised by bedrock outcrops on both stream banks tend to produce strongly positive VHGs (Ibrahim et al. 2010). By contrast, unconfined aquifers, which are characterised by bedrock overlain by an expansive and thick layer of sedimentary deposits, typically exhibit negative VHG (Ibrahim et al. 2010). However, in some instances, discharge of long-residence GW and a positive VHG occurs in unconfined aquifers where stream incision increases GW-SW connectivity (Ibrahim et al. 2010). The interface of an unconfined and confined aquifers frequently exhibits variation of hyporheic exchange patterns with intense upwelling arising at the transitional zone from an unconfined to a confined aquifer (Ibrahim et al. 2010). In locations where bounded alluvial segments widen or deepen, downwelling dominates hyporheic exchange patterns, and the reverse is true where they become shallower or narrower (Tonina & Buffington 2009a). Where impervious bedrock is irregular in height, particularly in locations with a shallow alluvium, the direction of hyporheic exchange is likely to vary in response to fluctuating bedrock elevation (Buffington & Tonina 2009; Fig. 1.6). Due to reduced valley gradient and greater sediment supply, alluvial volume tends to increase downstream, leading to reduced GW – SW connectivity and greater net downwelling (Buffington & Tonina 2009a). However, the depth of the alluvium is also influenced by its sedimentary composition. In particular, large quantities of compacted fine sediments at depth increase the overall extent of the impervious layer, reducing alluvial volume (Vaux 1968). In these instances, the depth of the permeable section of the alluvium, as opposed to its total depth, determines patterns of hyporheic exchange (Storey et al. 2003; Tonina 2005).

At the channel reach scale (up to tens of channel widths in length) variation of alluvial volume, changes in slope and formations on the riverbed affect patterns of hyporheic exchange (Fig. 1.6). Small-scale variation of alluvial volume, primarily driven by compaction of impermeable sediments such as clays, influence hyporheic exchange at the channel reach scale in a similar manner to variations of alluvial volume at the valley-segment scale described above (Buffington & Tonina 2009). River slope plays an important role in determining patterns of hyporheic exchange through variation in hydraulic head. In particular, upwelling is most common at the transitional zone from a steep to a shallow river gradient, while downwelling occurs where channel gradient steepens (Harvey & Bencala 1993; Crispell & Endreny 2009). In addition, transitional zones from high-pressure to low-pressure systems, such as from a pool to a riffle can drive downwelling, while upwelling is more common at riffle tails (Baxter & Hauer 2000; Tonina & Buffington 2007; Environment Agency 2009b; Ibrahim et al. 2010; Fig. 1.6). The sedimentary characteristics of the downstream zone of riffles make it a suitable incubation zone for salmonids (Crisp & Carling
1989). However, the frequency with which GW upwelling occurs in this region could have implications for incubation success.

At the channel unit scale (up to several channel widths in length), step-pool sequences drive predictable patterns of hyporheic exchange. Downwelling frequently occurs in the high-pressure section above steps, while upwelling occurs at the low-pressure section below steps (Gooseff et al. 2006; Kasahara & Hill 2006; Huber et al. 2013). Consequently, anthropogenic construction of artificial log-steps to increase riverbed heterogeneity can influence patterns of hyporheic exchange. This can produce unfavourable conditions for Atlantic salmon embryos where GW upwelling reduces oxygen supply in redds found in regions below steps (Schindler Wildhaber et al. 2014).

At an even smaller scale (up to a single channel width in length), small riverbed features such as large woody debris (LWD), boulders or salmonid redds affect hyporheic exchange patterns (Buffington & Tonina 2009; Fig. 1.6). Rapid hyporheic exchange can occur under boulder obstructions and similar obstacles in the riverbed (Buffington & Tonina 2009). In addition, LWD and boulders can induce near-bed turbulence and pressure fluctuations, which trigger variable exchange patterns (Tonina & Buffington 2007). LWD removal can homogenise the riverbed and reduce the extent of hyporheic exchange in mountain locations (Kasahara & Wondzell 2003; Wondzell 2006). However, in lower gradient rivers, such obstructions can reduce hyporheic exchange by limiting the formation of alternating bars (Wondzell et al. 2009). Micro-scale modifications to the riverbed through redd construction by maternal salmonids also affect hyporheic exchange. In particular, the tailspill (Fig. 1.1) causes a high-pressure zone which forces downwelling SW into the redd where eggs would typically be located (Buffington & Tonina 2009). Therefore, the process of redd construction could override natural patterns of upwelling frequently observed in locations favoured for spawning (Baxter & Hauer 2000; Geist 2000; Tonina & Buffington 2007; Environment Agency 2009b). This could reduce the impact of low-oxygen GW and provide more favourable conditions for incubation.

While geomorphological features influence patterns of hyporheic exchange, fine sediments within gravels can form an impervious layer that limit GW – SW connectivity (Packman & Salehin 2003; Simpson & Meixner 2012). The nature of this effect varies and can depend upon the type of sediment that is present. Finer sediments such as clays that are smaller than intragravel spaces infiltrate and settle beneath the riverbed. This is known as ‘bottom-up’ sedimentation (Grieg et al. 2007b) and creates an impervious layer at the base of the riverbed gravels that restricts GW upwelling, resulting in a SW dominated hyporheic zone (Malard & Hervant 1999; Fig. 1.7a). By contrast, sediments that are larger in diameter than intragravel spaces settle on the surface of the
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riverbed. This reduces the diameter of intragravel spaces and leads to the entrapment of successively smaller sediment particles (Lisle 1989; Greig et al. 2007b). In sufficient quantity, the riverbed surface can become plugged (Beschta & Jackson, 1979) and restrict SW downwelling, leading to a GW dominated hyporheic zone (Malcolm et al. 2010; Fig. 1.7b). During the process of redd construction, the maternal salmonid cleanses fine sediments from riverbed gravels, which maximises intragravel porosity, and thereby hyporheic exchange. However, fine sediments rapidly re-accumulate within reds (e.g. Greig et al. 2007b; Schindler 2012, 2014), which can restrict hyporheic exchange and lead to GW-induced hypoxia in salmonid reds (Greig et al. 2007b; Malcolm et al. 2010).

A

Low fine sediment content

Fine sediment deposition

B

Sediment clogs riverbed surface

Low fine sediment infiltration

Figure 1.7. Effects of fine sediment deposition on hyporheic exchange. (a) Fine sediments filter through gravels and create an impervious layer that inhibits upwelling. (b) Formation of a sediment seal at the riverbed surface could limit surface water input, resulting in groundwater domination of the riverbed. Adapted from Greig et al. (2007b).

Effect of hydrology on hyporheic exchange

While hyporheic exchange patterns are linked to geomorphological features, hydrological conditions could influence the timing, duration and intensity of long-residence GW upwelling. In particular, catchment wetting and water table elevation influences GW – SW connectivity (Cardenas & Wilson 2006; Soulsby et al. 2009). Following prolonged dry spells, GW depletion (Burt et al. 2002) means that the water table is below the river substratum so GW – SW connectivity is low. Therefore, SW and short-residence GW dominate the hyporheic zone (Fig. 1.8 [December]; Fig. 1.9A). However, catchment wetting (often observed in datasets through greater river discharge levels e.g. Fig. 1.8) elevates the water table relative to the riverbed and drives greater
connectivity between SW and long-residence GW. Greater connectivity is often first observed through episodic phases of oxygen depletion on the recession limb of high flow events (Soulsby et al. 2009; Fig. 1.8 [January]; Fig. 1.9A). Greater hydrostatic pressure driven by high flow events give rise to a negative VHG and SW downwelling (Fig. 1.9). As the event subsides, the water table increases relative to stream stage, resulting in a more positive VHG, greater GW – SW connectivity, and can give rise to episodic periods of oxygen depletion (Fig. 1.8, 1.9A). The high degree of GW – SW mixing at this stage means that pulses of oxygen depletion could be short and relatively mild (Fig. 1.8).

Following further precipitation, the water table rises further relative to the stream subsurface, leading to greater influence of GW (Fig. 1.9B). In some cases, water table elevation is greater than the river level (Soulsby et al. 2009) and can result in GW dominance of the hyporheic zone, which reduces connectivity, blocks SW input (Cardenas & Wilson 2006; Soulsby et al. 2009; Fig. 1.9B), resulting in prolonged periods of total anoxia (e.g. Soulsby et al. 2009; Sear et al. 2014). GW domination of the hyporheic zone can be occasionally punctuated by short periods of connectivity and oxygen recharge that coincide with high flow events due to greater hydrostatic pressure on the riverbed and SW downwelling (Soulsby et al. 2009; Sear et al. 2014; Fig. 1.9B). The importance of water table elevation on GW – SW interactions implies a seasonal pattern of hyporheic exchange that could potentially be estimated based on rainfall patterns and monitoring GW levels. Furthermore, GW influence is likely to be greatest during winter, when water table elevation is at a maximum. As this coincides with the period of Atlantic salmon incubation, their eggs could experience GW-induced hypoxia throughout their range.
Figure 1.8. Dissolved oxygen concentrations of river water and at 150mm and 300mm depth in the riverbed compared with river discharge levels at the Girnock Burn, Scotland. From Malcolm et al. (2006).
Figure 1.9. Conceptual diagrams of the interactions between river discharge and hyporheic dissolved oxygen concentration based on seasonal changes of groundwater – surface water connectivity. (A) shift from a surface water dominated system due to a low water table to periods of high groundwater – surface water connectivity as repeated catchment wetting increases the influence of groundwater in the hyporheic zone. (B) groundwater dominated hyporheic zone represented by persistent anoxia occasionally interrupted by high flow events that trigger surface water intrusion.

1.1.5 Conclusions

The hydrochemistry of GW and SW can vary substantially, so patterns of hyporheic exchange are an important determinant of water quality in the Atlantic salmon incubation zone. Indeed, a range of studies have demonstrated that riverbed zones subject to high GW input can display intense and prolonged hypoxia that is likely to reduce incubation success. The location and direction of hyporheic exchange is determined by a variety of geomorphological features at a range of nested spatial scales. Furthermore, the timing, duration and intensity of deoxygenated GW upwelling can be influenced by hydrological features. In particular, GW – SW connectivity is likely to be greatest following prolonged catchment wetting, when the water table elevation is highest. The Atlantic salmon incubation period encompasses the wettest time of the year, so their eggs are likely to be exposed to low oxygen conditions in areas susceptible to deoxygenated GW upwelling.

1.1.6 Literature gap

Several studies have been conducted that monitored the effects of GW upwelling on water quality in the incubation zone. Such studies have provided valuable data regarding oxygen regimes in the Atlantic salmon incubation zone and described a relationship between river discharge and hyporheic dissolved oxygen concentration. However, most studies have focused on a single
location, so there is a need to consider a range of datasets to determine how widespread the issue of GW upwelling is. Furthermore, while descriptive analysis of the relationship between river discharge and hyporheic exchange is valuable, statistical support for these observations could enhance understanding and identify periods of greatest vulnerability of Atlantic salmon eggs to GW-induced hypoxia.

1.1.7 Research aims and objectives

Aim: Quantify seasonal patterns of hyporheic exchange at a range of spawning sites, to show how these affect oxygen levels in the Atlantic salmon incubation zone and consider the potential effects on incubation success.

Due to an absence of statistical support in the literature for the effect of antecedent and prevailing weather conditions on hyporheic exchange patterns, the aim of describing such a relationship was developed. Such statistical support will enhance understanding of seasonal and annual fluctuations of hyporheic exchange, and could help to predict oxygen concentrations experienced by Atlantic salmon eggs based on weather patterns in different catchments. This aim was addressed through two key objectives:

Objective one: Using wavelet analysis to detect seasonal variation in interactions between river discharge and hyporheic dissolved oxygen content at numerous sites

Wavelet analysis was performed to determine the statistical relationship between river discharge and hyporheic dissolved oxygen levels. This enabled co-variance between the two variables to be observed at a range of scales at numerous locations. In particular, changes in the nature of the co-variance could be studied to determine how the pattern changes in response to antecedent and prevailing weather conditions. Furthermore, by studying numerous sites, it was possible to determine how frequently deoxygenated GW upwelling influences incubation quality.

Objective two: Use data from the Atlantic salmon incubation period to determine the effect of groundwater upwelling on oxygen regimes experienced by embryos

The likelihood that embryos experience GW-induced hypoxia was estimated by observing oxygen concentrations throughout the Atlantic salmon incubation period. Furthermore, this could be compared with estimates of oxygen demand to determine possible effects on incubation success.
1.2 Effects of low oxygen on salmon incubation success

Atlantic salmon require a consistent supply of oxygen in order to support metabolism and development at all life-stages. In the free-swimming phases of the life-cycle, fish can escape and avoid zones of hypoxia (Wu 2002), but the sedentary nature of the egg-stage means that oxygen availability is driven by the surrounding environment. If oxygen supply falls below the demand of the embryo, then incubation success can decline. The three main indicators of incubation success are (1) survival of eggs to hatch (2) hatch timing and (3) post-hatch fitness of individuals (Alderdice et al. 1958; Schreck et al. 1997; Youngson et al. 2004; Geist et al. 2006; Roussel 2007; Fig. 1.10). The frequency with which hypoxia influences these variables depends on a range of factors related to oxygen supply and embryonic demand. While section 1.1 focused on the factors that influence oxygen supply, this section considers factors that influence demand followed by a review of literature on the effects of insufficient oxygen supply to salmonid embryos.

![Flow chart describing salmonid juvenile response to an environmental stressor during incubation. Adapted from Schreck et al. (1997).](image-url)

**Figure 1.10.** Flow chart describing salmonid juvenile response to an environmental stressor during incubation. Adapted from Schreck et al. (1997).
1.2.1 Embryonic oxygen demand

The oxygen demand of Atlantic salmon embryos increases with metabolic rate (Gillooly et al. 2001), so individuals with a greater metabolism could be more sensitive to hypoxia. As with all ectotherms, temperature controls the kinetic energy present in cellular components of Atlantic salmon, resulting in faster metabolism at higher temperatures (Gillooly et al. 2001; Clarke & Fraser 2004), and greater oxygen demand (Hamor & Garside 1976; Fig. 1.11). In some instances, faster metabolism is considered advantageous because it shortens the incubation phase of the life cycle, thereby reducing vulnerability to stressors such as insufficient oxygen supply, siltation, mechanical damage and predation (Wootton 1990). However, greater oxygen demand driven by higher metabolic rates could increase embryonic sensitivity to hypoxia (section 1.2.2).

The temperature experienced by incubating embryos can vary within and among populations. Differences in shading patterns (Rutherford et al., 2004), water depth (Webb et al. 2008) and GW input (Malcolm et al. 2008) mean that water temperatures within a single river can vary substantially. Furthermore, variation in temperature among rivers is driven by differences in altitude and latitude (Webb & McLay 1996). Due to this variation the temperatures experienced, and consequently, the oxygen demand of eggs within and among populations is likely to differ substantially. This leads to differential rates of development, different levels of oxygen demand (Hamor & Garside 1976) and could influence sensitivity to hypoxia.

The developmental stage of the embryo also plays a key role in oxygen demand, because it determines rate of tissue formation, body mass and oxygen uptake efficiency (Alderdice et al. 1958; Hamor & Garside 1976; Gorodilov 1996; Danner 2008). While oxygen consumption shows a general trend of increasing with developmental stage, this pattern is not linear and spikes of demand occur at specific periods throughout incubation (Fig. 1.11). Proliferation of tissue formation during organogenesis from approximately 80 degree days (DDs) corresponds with spikes of oxygen demand (Gorodilov 1996; Hamor & Garside 1976; Fig. 1.11). Following organogenesis, a functioning circulatory system develops that enhances oxygen uptake efficiency (Alderdice et al. 1958; Danner 2008), leading to a decline of demand. Between 300 and 450DDs, rapid tissue formation and energetic release takes place that drives oxygen demand maxima until the time of hatching (Hamor & Garside 1976). As a result, periods of greatest sensitivity to low oxygen supply occur could occur during organogenesis and just before hatching (Fig. 1.11).
Figure 1.11. Recorded values of oxygen consumption for Atlantic salmon eggs at 5°C and 10°C by developmental stage using data sourced from Hamor & Garside (1976). Key stages of embryonic development are labelled above the graph using data presented in Gorodilov (1996).

1.1.1 Lethal oxygen limits

For decades, researchers have attempted to define critical oxygen concentrations required to support incubating salmonid eggs (e.g. Alderdice et al. 1958; Silver et al. 1963; Louhi et al. 2008). However, variation in developmental stage of organisms tested, sampling methods and experimental conditions give a range of results with no definitive answer with regards to the concentration required for successful incubation (Table 1.2). In truth, due to the diversity of variables that influence oxygen supply to Atlantic salmon eggs (see 1.1.1), and the variable nature of embryonic oxygen demand, it is not possible to give a single-figure answer to the problem.
Table 1.2. Estimates of oxygen concentrations required for successful incubation of Atlantic salmon at different developmental stages.

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Oxygen requirements (mg O$_2$ l$^{-1}$)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyed</td>
<td>3.1</td>
<td>Hayes et al. (1951)</td>
</tr>
<tr>
<td>Hatching</td>
<td>7.1</td>
<td>Hayes et al. (1951)</td>
</tr>
<tr>
<td>Eyed</td>
<td>5</td>
<td>Wicket et al. (1954)</td>
</tr>
<tr>
<td>Hatch</td>
<td>2.0 - 5.0</td>
<td>Davis (1975)</td>
</tr>
<tr>
<td>Throughout development</td>
<td>6.0</td>
<td>Elson (1975) from Stanley &amp; Trial (1995)</td>
</tr>
<tr>
<td>Throughout development</td>
<td>5.0</td>
<td>Bjornn &amp; Reiser (1991)</td>
</tr>
<tr>
<td>Throughout development</td>
<td>5.0</td>
<td>Gibson (1993)</td>
</tr>
<tr>
<td>Throughout development</td>
<td>&gt;7.0</td>
<td>Crisp (1996, 2000)</td>
</tr>
<tr>
<td>Throughout development</td>
<td>2.0-8.0</td>
<td>Kondolf (2000)</td>
</tr>
</tbody>
</table>

The effect of hypoxia on incubation success depends on the extent of the shortfall between embryonic oxygen demand and supply. Consequently, the greater oxygen demand of embryos in warmer conditions could influence their sensitivity to hypoxia. Indeed, several carp species (Downing & Merkens 1957), sturgeon (*Acipenser oxyrinchus* [Secor & Gunderson 1998]) and isopods (*Armadillidium vulgare* [Klok et al. 2004]) show reduced tolerance to low oxygen in higher temperatures. Further, char (*Salvelinus namaycush*) showed greater rates of hypoxia-induced mortality in warmer temperatures (Gruber & Wieser 1983). The effect of temperature on the sensitivity of Atlantic salmon eggs to hypoxia has not been researched. However, if a relationship exists, predictions of warming temperatures associated with climate change (van Vliet et al. 2013) could influence incubation success in the future. In addition, warmer conditions and low oxygen concentrations associated with areas of GW upwelling (Table 1.1) could synergistically reduce survival. Therefore, the combined effect of temperature and low oxygen warrants further investigation with regards to Atlantic salmon incubation success.

The second key driver of embryonic oxygen demand and, consequently, sensitivity to hypoxia is the developmental state of the embryo. While there is little evidence that high demand from 80 to 130DDs affects sensitivity to low oxygen (e.g. Alderdice 1958), substantial data suggest that hypoxia at the latest stages of development reduces embryonic survival in a range of salmonids...
Predictable patterns of oxygen demand and their effect on embryonic sensitivity to hypoxia could aid estimates of incubation success. In particular, the timing of extreme GW-induced oxygen depletion could have important implications in the wild. If GW dominance is at a maximum at the same time as embryonic oxygen demand, then incubation success could be severely reduced.

In addition to developmental state and ambient temperature, the duration of hypoxic exposure is likely to influence rates of mortality. However, only Miller et al. (2008) has studied the effects of hypoxic duration on salmonid embryo development. While they found no differences in mortality at different exposure durations, it is likely that this was due to the relatively mild (30% and 50% saturation) hypoxia tested. Indeed, work on other species suggests that mortality rates will increase with exposure time in more extreme hypoxia (Collins et al. 2015; Joyce et al. 2016). Furthermore, the sensitivity of salmonids to other stressors increases with exposure duration (Milne et al. 2000). The uncertainty on the subject demonstrates that it would benefit from further research, particularly because the duration of GW induced hypoxia in rivers varies greatly (Soulsby et al. 2009).

In isolation, low oxygen (Alderdice et al. 1958; Malcolm et al. 2003, 2006; Greig et al. 2007b), pesticides (Viant et al. 2006), heavy metals (Jezierska et al. 2009) and reductions of pH (Finn 2007) can cause high levels of mortality in the Atlantic salmon egg stage. However, research has not been conducted on the combined effect of low oxygen and other stressors on survival. It is possible that synergism, where the combined effect of low oxygen with another stressor produces greater rates of mortality than expected (Holmstrup et al. 2010), could exist. Work on Daphnia magna (Ferreira et al. 2008) and the shore crab Carcinus maenas (Depledge 1987) displayed the synergistic effect of heavy metals and low oxygen to reduce survival. Further, pesticides and low oxygen synergistically reduce survival of the cladocerans D. magna (Ferreira et al. 2008) and D. pulex (Hanazato & Dodson 1995). The physiological way in which these toxins influence hypoxic sensitivity varies but is often associated with disruption of oxygen uptake apparatus (e.g. Pilgaard et al. 1994). The frequency with which low oxygen and a range of other stressors are present in the Atlantic salmon incubation zone suggests that this topic is worthy of much greater consideration. In particular, high ammonia and nitrate concentrations in GW (Table 1.1) could work synergistically with low oxygen levels to reduce embryonic survival (Heugens et al. 2001).
1.2.2 Sublethal effects

While mortality in the egg stage is the most frequently used indicator of incubation quality, poor conditions can induce sublethal responses in embryonic Atlantic salmon. These vary in nature but are likely to reduce rates of survival in later life-stages. The following is a review of the literature examining the potential sublethal effects of low oxygen during incubation on salmonids, with a focus on Atlantic salmon where possible.

Altered hatch timing

Teleost (ray finned fish) hatching occurs when the embryonic oxygen demand exceeds the rate at which it can diffuse through the egg membrane (Fuiman & Werner 2002) and is driven by a combination of biochemical and behavioural processes (Fraysse et al. 2006). The biochemical aspect is related to the development of hatching gland cells (HGCs), which produce chorionase, a key chemical associated with egg membrane digestion (Korwin-Kossakowski 2012). The density of HGCs reaches a maximum just prior to hatch, as does the concentration of chorionase within them (Luczynski & Ostashewska 1991). This degrades the egg membrane, creating a groove through which the embryo can tear (Korwin-Kossakowski 2012). The behavioural aspect of hatching is associated with the greater movement and physical strength of the embryo at later stages of development (Miller & Kendall 2009). These factors combine to ensure that the embryo can break through the groove created by the chorionase and successfully hatch.

Deviation in hatch timing is a common occurrence and naturally observed within and among salmonid redds. This deviation is generally caused by (1) innate variation in developmental rate among individuals related to their metabolic rate (Einum & Fleming 2000a) or; (2) exposure to stressors during incubation that affect development. In particular, oxygen depletion interferes with development and causes sub-optimal hatch timing (Alderdice et al. 1958; Oppen-Berntsen et al. 1990; Youngson et al. 2004; Geist et al. 2006; Roussel 2007).

The direction of hypoxia-induced hatch timing shift is related to the physiological state of the embryo at the time of oxygen depletion. At the latest stages of development, the higher oxygen demand of the embryo (Hamor & Garside 1976; Fig. 1.11) means that the threshold at which oxygen demand exceeds supply (Fuiman & Werner 2002) is achieved more readily. In addition, the biochemical and behavioural conditions of late-stage embryos mean that they are physically capable of hatching. Premature hatching of embryos exposed to hypoxia is advantageous as it removes the membrane, which is a barrier to oxygen diffusion (Matschak et al. 1997, 1998; Ninness et al. 2006; Ciuhandu et al. 2007; Dhiyebi et al. 2013), thereby enhancing oxygen uptake. Furthermore, hatching mobilises the embryo, enabling it to escape patches of low oxygen
(Czerkies et al. 2001). At earlier stages of development, embryos have fewer HGCs with lower concentrations of chorionase (Luczynski & Ostaszewska 1991). In addition, their small body size means that they are unable to break free of the egg capsule. Therefore, they exhibit an alternative strategy to survive periods of hypoxia. This involves reduction of oxygen uptake in oxygen stressed environments (e.g. Hamor & Garside 1976; Padilla & Roth 2001; Fig. 1.12). While this enables embryos to survive periods of low oxygen supply, reduced uptake is representative of low metabolic and developmental rate (Polymeropoulos et al. 2016). This results in a developmental lag, which is frequently observed as delayed hatch in Atlantic salmon (Youngson et al. 2004) and other salmonids (Alderdice et al. 1958; Geist et al. 2006; Roussel 2007). Salmonids that experienced hypoxia in the laboratory (e.g. Alderdice et al. 1958; Roussel 2007) and the field (Youngson et al. 2004) during incubation have displayed both premature and delayed hatch. Therefore, GW-induced hypoxia could be a key driver of altered hatch timing in the wild.

![Figure 1.12](image)

**Figure 1.12.** Recorded values of oxygen consumption for Atlantic salmon eggs at 5°C at 100%, 50% and 30% oxygen saturation values. Data taken from Hamor & Garside (1976).

Oviparous (egg depositing) organisms of a single species or population frequently aggregate the time that they spawn and, therefore, when their offspring hatch (Pulliam & Caraco 1984). This is considered to be a functional response that limits the impact of predation on population strength (Holling 1959). Simultaneous hatching means that alevin hatch in high densities, so can exceed the handling capacity of predators such as the bullhead (Cottus gobio [Roussel 2007]) and burbot (Lota lota [Louhi et al. 2011]) resulting in appetite satiation (Begon & Mortimer 1986). Therefore, deviation from the time of peak hatch could increase predation risk (Brännäs 1995).
A second period of elevated Atlantic salmon predation risk is associated with the swim-up fry stage. This is where the juvenile has completed yolk-sac absorption and must emerge from the gravels to establish a territory and commence exogenous feeding. This stage starts with numerous trips from the gravels to the surface of the river to take in air and establish neutral buoyancy (Bardonnet et al. 1993). While emergence from the gravels tends to occur at night to reduce exposure to visual predators (Garcia de Leaniz et al. 2000), substantial predation from species such as brown trout still occurs (Brännäs 1995). Therefore, aggregation of emergence could also help to limit long-term population impacts of predation.

The timing of emergence from gravels can influence the long-term survival of Atlantic salmon by affecting territory availability and susceptibility to predation. Premature emergence can be advantageous due to first access to territories, which can enhance survival, growth rates, ability to maintain territory (Cutts et al. 1999) and enable earlier seaward migration (Larsen et al. 2015). However, these advantages are associated with individuals that hatch or emerge prematurely due to greater developmental and metabolic rate (Brännäs 1995; Einum & Fleming 2000a). By contrast, alevin that hatch prematurely due to exposure to hypoxia might not be at an advanced developmental state, so will not necessarily emerge from gravels early. In addition, they do not experience the benefits of prey-dilution and predator appetite satiation associated with peak hatching. There are limited advantages associated with delayed hatch and emergence, even for individuals not experiencing hypoxia during incubation. While there is a reduced risk of emerging when food resources are low (Curry et al. 1995), the majority of territories will be occupied so individuals will have to travel further distances to find resources. This increases the risk of mortality through starvation (Einum & Fleming 2000a; Einum & Nislow 2005). Individuals hatching late because of low oxygen are likely to experience similar disadvantages that could be exacerbated by reduced physical condition.

Biometrics

The biometrics of alevin refers to their physical characteristics, so they can play an important role in determining their chances of survival into later life-stages. Two of the most obvious and well-studied aspects are their body length at hatch, and the size and mass of the yolk sac (Fig. 1.13). Body length at hatch shows natural variation among individuals, but can be reduced by low oxygen levels during incubation (Silver et al. 1963; Shumway et al. 1964; Hamor & Garside 1977; Youngson et al. 2004; Miller et al. 2008). Brown trout hatching in a hypoxic environment were 17% smaller than conspecifics in normoxic conditions (Roussel 2007), and similar results have been observed in Atlantic salmon (Hamor & Garside 1977). As with hatching, the time of oxygen depletion influences the magnitude of the effect, with greater impacts observed in rainbow trout
(Oncorhynchus mykiss) when oxygen depletion occurs during times of higher demand (Miller et al. 2008).

Figure 1.13. Atlantic salmon alevin with key biometric features labelled.

While smaller individuals arising from hypoxic incubation conditions have been frequently described, the physiological reason for this has received relatively little attention. Explanations include greater embryonic movement, which was thought to limit embryonic growth (Olsson & Persson 1986; Johnston et al. 1999). However, evidence suggests that movement actually stimulates growth (Ciuhandu et al. 2005). Alternatively, production of a growth suppression gene in response to hypoxia such as seen in the goby (Gillichthys mirabilis) could be related to restricted growth (Gracey et al. 2001). However, the most likely explanation of reduced growth in hypoxia is related to metabolic depression in hypoxia and a switch to anaerobic processes that reduce growth efficiency (Hochachka 1997; Wu 2002, Kamler 2008). This means that conversion of the yolk sac to body mass becomes less efficient in low oxygen conditions and the total body mass is reduced (Kamler 2008).

During incubation low oxygen supply can influence the metabolism of the yolk sac mass into body tissue. As a result, embryos that experience hypoxia during incubation frequently hatch with a larger yolk sac relative to their total mass (e.g. Geist et al. 2006; Roussel 2007). In a similar way to body length, this is likely to be related to a reduced efficiency of yolk sac conversion to body mass (Hamor & Garside 1977; Geist et al. 2006; Roussel 2007; Kamler 2008). This reduced efficiency means that the body tissue produced from a given unit of yolk sac mass is lower than in more efficient, well-oxygenated conditions.
Unfavourable conditions during incubation can also reduce alevin fitness at hatch through the development of physical abnormalities. The nature of these abnormalities can vary but include a deformed yolk sac (Ketola et al. 1999), torsion (twisted body) (Bonnet et al. 2007) and truncation of the body posterior (Alderdice et al. 1958). These features occur naturally, but low oxygen levels can disrupt gene expression and enhance their frequency (Nikinmaa & Rees 2007). Such abnormalities significantly impede swimming ability and are likely to result in mortality.

Other less visible physical features of Atlantic salmon can inhibit the chances of post-hatch Atlantic salmon survival. In particular, low oxygen levels have been linked to a reduction in the number and cross sectional area of white muscle fibres (Matschak et al. 1997). White muscle fibres are important for rapid acceleration over short distances (Valente et al. 1999). Therefore, reduced development could inhibit their escape response from predators, reducing chances of survival.

Alevin with a smaller body size and greater yolk sac mass have poorer swimming ability (Parker 1971; Fresh & Schroder 1987; Sogard 1997); this is likely to be exacerbated by other physiological abnormalities. Cumulatively, these factors can increase the susceptibility of alevin to mortality through predation. Indeed, Atlantic salmon incubated in suboptimal conditions have a poorer escape response than those incubated in more favourable conditions (Burke 2011) and brown trout exposed to hypoxia in their embryonic stage were more susceptible to predation (Roussel 2007). Furthermore, Roussel (2007) found that, following hatch, 33% of brown trout juveniles that were incubated in hypoxic conditions were immobile compared to 13% of juveniles that experienced normoxia. The cumulative sublethal effects of hypoxia during incubation increase the likelihood that a juvenile will encounter a predator and reduce their ability to escape. Furthermore, it could reduce their competitiveness, making them more vulnerable to starvation (Cutts et al. 1999). The frequency and effect of sublethal effects on long-term survival demonstrates their importance as indicators of incubation quality.

1.2.3 Spawning site selection

Spawning site selection by maternal salmonids plays a critical role in the conditions experienced by incubating ova and, consequently, incubation success. Sedimentary characteristics and hydraulic conditions have been the focus of studies observing site-selection by maternal fish (De Vries 1997; Geist & Dauble 1998; Moir et al. 2002) and predictive models of available incubation zone (Moir et al. 2005). However, predictions of spawning site distribution based on these models sometimes deviate from those observed (Dauble & Watson 1990). This implies that factors other
than hydrology and sedimentary composition, such as water quality, influence spawning site-selection (Malcolm et al. 2004; Hauer et al. 2011).

GW – SW interactions play an important role in determining hyporheic water quality, so could influence spawning site-selection. While long-residence GW reduces oxygen availability, salmonid ova that incubate in areas dominated by upwelling of short-residence GW could experience favourable conditions. This is because short-residence GW can protect embryos from unstable thermal regimes, potential freezing (Heggenes et al. 2010) and dewatering (Casas-Mulet et al. 2015). In addition, the hydraulic pressure of GW upwelling can clear sediments from spawning gravels and increase water flow past eggs (Bjornn & Reiser 1991), which can be particularly beneficial in lake spawning salmonids where intragravel velocity is lower (Brabrand et al. 2002). Further, warmer temperatures of GW can speed up embryonic development (Crisp 1988) and shorten incubation time (Saltveit & Brabrand 2013). Faster development could be beneficial as it shortens the immobile life-stage, where the embryo is unable to escape stressors such as hypoxia or toxins (Czerkies et al. 2001). In addition, more rapid development means that juveniles emerge enter the fry-stage earlier than conspecifics incubating in cooler temperatures (Saltveit & Brabrand 2013) so have early access to territories.

While there are particular benefits to more rapid development arising from incubation temperatures driven by warmer GW, there are also potentially negative outcomes. Salmonids incubating in GW dominated locations of the riverbed are essentially sheltered from the conditions in the stream. This means that they could hatch or emerge before the time of peak food availability and could face starvation (Curry et al. 1995). In addition, if they emerge when air and SW temperatures are still low, ice cover could cause direct mortality through freezing (Bradford et al. 2001) and anchor ice can limit habitat availability (Cunjak et al. 1998; Huusko et al. 2007). Further, the warmer temperature and more rapid development associated with GW presence drives an increase of metabolic rate (Hamor & Garside 1976), which could enhance oxygen stress.

Work on a range of salmonid species has demonstrated that hyporheic exchange influences spawning site-selection. Bull trout (Salvelinus confluentus), sockeye salmon (Oncorhynchus nerka), and chum salmon preferentially select spawning sites dominated by upwelling of short-residence GW in North American rivers (Baxter & McPhail 1999; Geist et al. 2002). Furthermore, a preliminary study on the River Feshie in Scotland demonstrated greater spawning intensity of Atlantic salmon in streams fed by short-residence GW (Soulsby et al. 2012). Spawning site selection based on patterns of hyporheic exchange implies that maternal fish have the ability to detect cues associated the distinct hydrochemistry of GW (Baxter & Hauer 2000). While this could
be beneficial, the seasonal variability of long-residence GW upwelling (Soulsby et al. 2009) suggests that, while deoxygenated GW may not be present at the time of spawning, it could infiltrate salmon redds at later stages of development. Consequently, the mechanisms and drivers of spawning site selection by maternal fish would benefit from greater research.

1.2.4 Conclusions

Low oxygen levels during incubation can reduce incubation success of Atlantic salmon through (1) increased mortality, (2) altered hatch timing and (3) reduced post-hatch fitness. The nature and extent of these impacts varies depending on the timing of hypoxia relative to the developmental stage of the embryo. In particular, hypoxia at the latest stages of development is likely to result in greater mortality and premature hatching. By contrast, due to physiological differences of the embryo, hypoxia in the earlier stages of development can lead to a delay of hatch timing. Furthermore, low oxygen during embryonic development results in inefficient conversion of yolk sac to body mass that can cause alevin to be smaller and have poorer conversion of yolk sac to body mass at hatch. Hatching of underdeveloped alevin at a suboptimal time could increase the likelihood that embryos will suffer mortality due to predation. Furthermore, the effect of hypoxia on development during incubation could have longer-term impacts that reduce competitiveness, and therefore survival, at later life-stages.

1.2.5 Literature gap

To date there has been little controlled laboratory work conducted on the effects of episodic oxygen depletion, such as that caused by GW upwelling, on the incubation success of any salmonid species. To date, most laboratory studies have focused on the effects of highly regulated periods of oxygen depletion through continuous relatively mild (30-50% saturation) hypoxia (e.g. Roussel 2007; Miller et al. 2008; Côte et al. 2012) or single extreme (<10% saturation) hypoxic events (Alderdice et al. 1958). While high mortality and poor post-hatch fitness of Atlantic salmon embryos has been linked to GW upwelling in the field (e.g. Malcolm et al. 2003; Youngson et al. 2004; Greig et al. 2007b), the nature of field studies makes it impossible to delineate the effects of low oxygen from other characteristics of GW or other stressors potentially found in the incubation zone. Therefore, a laboratory study that isolates hypoxia as the sole incubation stressor would help to determine whether reduced survival in GW dominated incubation zones is a result of low oxygen or a combination of other variables. Such a set-up could be adapted to test the effect of episodic periods of hypoxia of varying intensities on incubation success.
While low oxygen is a critical determinant of incubation success, a range of other stressors such as heavy metals and pesticides stressors can reduce Atlantic salmon incubation success. In isolation these contaminants reduce Atlantic salmon incubation success but could also work synergistically with hypoxia to exacerbate these effects. In addition, and of particular interest in the context of GW upwelling, could be the synergistic effect of features of GW such as high nitrate and ammonia with low oxygen. Laboratory examination of the effect of these stressors in isolation and combination would enhance understanding of this process.

1.2.6 Research aims and objectives

Aim: To determine the effect of episodic periods of low oxygen, such as that caused by deoxygenated groundwater upwelling, on Atlantic salmon incubation success

Section 1.2 demonstrated an absence of data on the effect of transient periods of low oxygen supply on Atlantic salmon incubation success. Therefore, a laboratory study was developed to achieve three key objectives regarding the response of embryonic Atlantic salmon to episodic periods of oxygen depletion:

Objective one: To determine the survival of Atlantic salmon eggs exposed to episodic periods of low oxygen at different periods of development.

The effect of episodic periods of low oxygen on the survival of Atlantic salmon embryos to hatch was investigated using a custom-built laboratory set-up (section 3.2). The timing of oxygen depletion was varied and daily rates of mortality were monitored to identify times of development that were particularly vulnerable to hypoxia.

Objective two: To determine how hatch timing of Atlantic salmon varies when exposed to episodic periods of low oxygen at different stages of development.

The effect of episodic periods of low oxygen on hatch rates was investigated. The effect of variation in the timing of oxygen depletion on hatch rates was determined through the use of Kaplan-Meier estimators.

Objective three: To determine how the post-hatch fitness of Atlantic salmon varies when exposed to episodic periods of low oxygen at different stages of development.

The effect of episodic periods of low oxygen on the length, mass, yolk sac absorption and developmental stage of alevin at hatch were studied. Comparisons among treatments were conducted to determine the importance of timing of exposure to hypoxia. These data were used
to estimate the fitness of embryos at hatch and to determine potential effects on future rates of survival.

1.3 Spatial variability of oxygen supply: Does the egg membrane offer an opportunity for adaptation?

The membrane that surrounds Atlantic salmon eggs (also known as the chorion, egg envelope, shell, zona pellucida, zona radiata) plays a key role in the interactions between the embryo and its external environment. It protects the embryo from mechanical shock (Stehr & Hawkes 1979), reduces the risk of fungal (Songe et al. 2016) and bacterial (Ellis 1999) infection and can limit the uptake of toxic materials (Finn 2007). However, it forms a barrier between the embryo and its environment that limits solute exchange (Ciuhandu et al. 2007). The importance of the membrane in terms of its interactions with the wide variety of potential incubation stressors implies that variation in its structure could have important implications for embryonic survival and could offer an opportunity for adaptation. This section considers the key structural features of salmonid egg membranes and their effect on embryonic survival. In particular, the effect of structural variation on oxygen supply to the embryo is discussed along with the potential influence of hypoxic stress on the development of adaptive traits.

1.3.1 Salmonid egg structure

The salmonid egg membrane is an acellular structure composed of proteinaceous fibres interspersed with micropores (Groot & Alderdice 1985; Fig. 1.14). Three of the most visible structural components of the egg and its membrane that influence the interactions between the embryo and the external environment are: (1) egg size, (2) membrane thickness and (3) micropore diameter and density (Fig. 1.14). Substantial differences among Pacific salmonids in terms of all these features have been observed (Groot & Alderdice 1985) and could represent adaptation to the contrasting incubation conditions that these species experience (Bjornn & Reiser 1991). Consequently, variation in each structural component could influence the survival of embryos exposed to a wide range of stressors. Therefore, this section considers the role each one plays in the interactions between the embryo and its environment.
Figure 1.14. Simplified diagram of a fertilised and water-activated salmonid egg. All features, excluding the subinternus, have been observed in all salmonid species. Features are not to scale. From Groot & Alderdice (1985).

**Egg size**

Atlantic salmon egg size varies within a single clutch of one maternal fish (Songe *et al.* 2016) and within (Songe *et al.* 2016) and among populations (Moffett *et al.* 2006; Songe *et al.* 2016) due to differences in maternal characteristics. In particular, the age (Bernardo 1996; Reid & Chaput 2012) and body length (Rollinson & Hutchings 2010; Kindsvater *et al.* 2016) of maternal fish drive differentiation and positively correlate with egg size. In addition, egg size of repeat spawners tends to vary from one spawning season to another, with a tendency towards larger eggs at the second spawning event (Heinimaa & Heinimaa 2004). Furthermore, greater energy exertion during the spawning migration can result in smaller eggs (Braun *et al.* 2013). This variation is generally associated with the ability of larger individuals with a simpler migration to allocate more energy to gamete production (Reid & Chaput 2012).

Environmental conditions, and temperature in particular, also play an important role in determining egg size. Maternal fish that experience warmer conditions prior to hatch are more likely to produce larger eggs than conspecifics of the same population (Jonsson & Jonsson 2016). Furthermore, populations that spawn at higher latitudes and altitudes tend to have smaller eggs (Fleming & Gross 1990), which indicates selection for larger eggs in warmer environments. Larger eggs compensate for reduced efficiency of yolk sac to body mass conversion in warmer temperatures (Heming 1982) through the provision of greater energetic reserves (Beacham *et al.*
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1985). The effect of temperature on egg size could have particularly important implications in the context of warming river temperatures in response to climate change (van Vliet et al. 2013). In particular, less efficient conversion of yolk sac to body mass could reduce juvenile fitness or drive selection for larger eggs.

While egg diameter is influenced by maternal characteristics and abiotic conditions, energy allocation of the female fish to reproduction is generally considered to be a trade-off between egg size and total fecundity (Roff 1992). Larger eggs are considered advantageous because they are more likely to exceed the gape of potential predators (Foote & Brown 1998) and give rise to larger juveniles (Kazakov 1981) with greater competitive ability (Einum & Fleming 2000b). However, in favourable conditions where incubation and post-hatch survival is high, production of a greater number of smaller eggs could be advantageous as a greater number of offspring could survive to later life-stages, thereby enhancing gene propagation (Heath et al. 2003). The effect of incubation quality on egg size has important management implications, particularly with respect to supplementary breeding, which can remove selection pressure from the embryonic life-stage. While this is unlikely to cause selection-driven loss of egg sizes adaptive to the natural incubation zone (Reisenbichler & Rubin 1999), it could drive selection of traits that increase fecundity as opposed to post-hatch fitness. Indeed, supportive breeding of Chinook salmon in Canada resulted in a shift towards smaller eggs over a few generations (Heath et al. 2003). This could have long-term negative impacts if alevin emerging from smaller eggs are poorly suited to natural conditions, particularly if the supportive breeding programme is ended.

Membrane Thickness

Recorded values of Atlantic salmon egg membrane thickness range from 32μm to 45μm (Songe et al. 2016) and this variation could influence interactions between the embryo and the external environment. Intuitively, it could be expected that thicker membranes provide the embryo with greater physical protection. Indeed, the membranes of demersal eggs tend be thicker than pelagic eggs to provide more protection from greater mechanical stress (Stehy & Hawkes 1979; Davenport et al. 1986; Riehl & Kock 1989). However, inter-species variation in membrane thickness of five Pacific salmonid species (Groot & Alderdice 1985) did not correlate with recorded values of mechanical strength (Jensen & Alderdie 1989). This could be due to differences in biochemical composition of the membrane, which is an important determinant of mechanical strength in avian eggs (De Ketelaere et al. 2002; De Roberts 2004) and could have a similar effect in salmonids. Nevertheless, thicker membranes can be advantageous as they are better able to protect Atlantic salmon embryos from fungal (Songe et al. 2016) and bacterial (Ellis 1999)
infection. Consequently, variation in egg membrane thickness is likely to affect the vulnerability of embryos to a range of mechanical and biological stressors.

*Micropore diameter and density*

Micropore diameter and density have not been studied in detail for Atlantic salmon eggs, but Pacific salmonids show substantial variation in terms of both features. Micropore diameter ranges from 510nm to 830nm, while micropore density varies from $3.45 \times 10^5$ to $4.80 \times 10^5$ pores mm$^{-2}$ (Groot & Alderdice 1985). These values of micropore diameter are at the lower end of previous estimates (Bell et al. 1969) but vary among species and individual eggs (Groot & Alderdice 1985). Micropores are the pathways that enable substances to be exchanged between the external environment and the embryo (Greig et al. 2005b). Therefore, they play a critical role in determining the rate of oxygen diffusion to and waste metabolite excretion from the embryo.

**1.3.2 Egg structure and oxygen supply**

While one of the key roles of the membrane is physical protection from external stressors, this barrier affects the exchange of substances between the embryo and external environment. Studies using oxygen microelectrodes demonstrated an oxygen gradient across the membrane of rainbow trout eggs (Ciuhandu et al. 2007) and that the oxygen concentration in the PVF is substantially lower than the boundary layer (Dhiyebi et al. 2013). Further, removal of the chorion enhanced embryonic growth in low oxygen levels (Matschak et al. 1997, 1998; Ninness et al. 2006). This implies that the egg membrane limits oxygen uptake. Therefore, structural characteristics of the egg and its membrane affect the rate at which oxygen is delivered to the embryo. While these features are unlikely to affect development when intragravel velocity and oxygen concentrations are high (Ciuhandu et al. 2005), they could limit survival if supply rates decline, or at times of demand maxima.

*Egg size*

Egg size is a critical feature in terms of embryonic oxygen supply because it affects delivery to the boundary layer through two key processes. First, larger eggs induce greater advective flow of water from within the redd (Pahlow et al. 1997), thereby increasing rates of oxygen delivery to the boundary layer from the external environment. Second, the spaces between spheres increase with diameter, so the total permeability of the egg packet increases with individual egg size (Seymour 1994). This enhances flow rate past eggs and thereby the rate of oxygen delivery. This could be particularly advantageous for eggs at the centre of the egg packet, where oxygen stress is greatest (Wickett 1975).
While larger eggs have several benefits in terms of oxygen supply, the cumulative effect of egg size on embryonic sensitivity to hypoxia has been the subject of debate. Larger eggs have a higher surface and therefore a greater area over which oxygen can diffuse to the embryo. However, larger eggs also have a greater volume. Previously, it was assumed that egg volume is directly proportional to embryonic oxygen demand (Krogh 1959). Therefore, because egg volume increases at a greater rate than surface area for a given increase of diameter, it was expected that the oxygen demand increased at a greater rate than diffusive surface area, so larger eggs could be more sensitive to hypoxia (Krogh 1959). However, most differences in egg size are driven by yolk sac as opposed to body mass (Beacham et al. 1985), so do not directly correlate with oxygen demand. Indeed, work on brown trout demonstrated that the diffusive surface area of eggs increases at a greater rate than oxygen demand for a given increase of egg mass (Einum & Fleming 2002). Therefore, larger eggs appear to be beneficial both in terms of oxygen supply to the boundary layer and diffusion from the boundary layer to the embryo.

**Membrane thickness**

Total membrane thickness influences the rate of oxygen diffusion from the boundary layer to the PVF by controlling the length of the diffusion pathway, so an increase results in a decrease of permeability (Firpo et al. 2015). Throughout the animal kingdom, the eggs of oviparous organisms experience hypoxic stress, and many demonstrate adaptive responses through modifications of membrane thickness. For example, membrane thickness of salamander eggs reduces in hypoxia to enhance oxygen uptake (Mills et al. 2001). Further, alligator (Warburton 1995), snake (Stahlschmidt et al. 2010), gastropod (Segura et al. 2010), lungfish (Mueller et al. 2011) and cuttlefish (Cronin & Seymour 2000) eggs reduce membrane thickness throughout development in response to greater embryonic oxygen demand. It is not currently known whether salmonid egg membrane structure can change throughout development either temporally or in response to hypoxic stress. However, differences among species (Groot & Alderdice 1985) or populations could influence oxygen supply.

**Micropore diameter and density**

Micropores are the pathways through which oxygen diffuses from the boundary layer to the embryo, so their density and diameter affect the rate of diffusion across the membrane. Furthermore, the proportion of the membrane surface that is composed of micropores determines its porosity. Variability among salmonid species in terms of micropore density and diameter has only received descriptive attention with little explanation provided for differences among salmonid species (Groot & Alderdice 1985). The observation that micropore occlusion by
fine sediments can prevent diffusion to the embryo (Greig et al. 2005b) demonstrates their importance for oxygen supply. Further, they are critical to allow substances such as ammonia and carbon dioxide to diffuse out of the PVF to the boundary layer (Dhiyebi et al. 2013). Therefore, differences in micropore size and density affect the diffusive surface area of an egg and should be considered alongside egg size as a key determinant of embryonic oxygen supply.

While little work has been conducted on teleost eggs, evidence for the importance of micropores on oxygen supply to embryos is available throughout the animal kingdom. Eggs of the Peruvian coot (Fulica americana peruviana) have higher micropore density in low oxygen environments (León-Velarde et al. 1997). Further, aquatic eggs tend to have higher micropore density than terrestrial eggs due to the lower diffusion coefficient of oxygen in water (Cronin & Seymour 2000; Gaino et al. 2001; Koyama et al. 2011). Therefore, differences among species of Pacific salmonids in terms of micropore density and average diameter could influence sensitivity to hypoxia (Groot & Alderdice 1985). While differences between avian populations and comparisons among aquatic and terrestrial species identify potential adaptations to differential oxygen supply, this has not been considered in detail for any teleost species, and would benefit from greater research.

1.3.3 Oxygen stress offers an opportunity for adaptation

Environmental heterogeneity throughout the range of salmonids exerts selective pressure among genetically distinct populations and drives the development of adaptive traits (Garcia de Leaniz et al. 2007). The highly natal homing nature of Atlantic salmon spawning limits gene flow among populations, leading to the rapid development (6-30 generations [Fraser et al. 2011]) of traits that enhance the fitness of local populations to their habitat (Fraser et al. 2011; Bourret et al. 2013). While a wide range of adaptations have been described in Atlantic salmon, most are associated with traits in the free-swimming life-stage (Garcia de Leaniz et al. 2007). However, heterogeneity in the incubation zone of distinct populations (e.g. Greig et al. 2007a) implies that differences in egg structure could influence incubation success. In particular, enhanced membrane permeability could reduce oxygen stress in locations where supply is limited or demand is greater.

Throughout their range oxygen supply to incubating Atlantic salmon differs widely due to variable sedimentation rates (Greig et al. 2007b), patterns of hyporheic exchange (Greig 2004; Malcolm et al. 2006, 2010; Soulsby et al. 2009; Burke 2011; Bateman 2012; Schindler Wildhaber et al. 2014; Sear et al. 2014) and differences in intragravel velocity (Greig et al. 2007a). Furthermore, differences in temperature (section 1.2.1) mean that oxygen demand among locations varies and could affect oxygen stress (Hamor & Garside 1976). These differences could drive variable survival rates among locations, but also offer an opportunity for adaptation. In particular, differentiation
in rates of oxygen uptake could enhance survival of populations that frequently experience oxygen stress.

Adaptations to low oxygen have been observed in freshwater invertebrates (Weider & Lampert 1985), marine invertebrates (Childress & Seibel 1998) and even humans (Beall et al. 2002; Yi et al. 2010). However, only relatively recent work on the ability of fish to adapt to low oxygen supply has been conducted. While this has mostly focused on adult African cichlids (Crispo & Chapman 2008, 2009, 2010, Martinez et al. 2009), Côte et al. (2012) studied variability in the embryonic response of four distinct Atlantic salmon populations to low oxygen in France. Populations did not differ in terms of mortality in low oxygen environments, possibly due to the relatively mild hypoxia they experienced (4.79 and 4.42mg l⁻¹). However, differences among populations in terms of post-hatch fitness suggested genetic differentiation in hypoxic tolerance. This could be explained by physiological differences in the response to hypoxia, as suggested by the authors. Alternatively, there could be differences among populations in terms of oxygen uptake ability. This was not investigated, but structural differentiation in the egg membrane among populations could influence sensitivity to hypoxia.

1.3.4 Conclusions

The membrane of Atlantic salmon eggs is a critical component that influences the interactions between the embryo and its environment. However, it presents a barrier to diffusion that could impede development in oxygen stressed environments. Physical characteristics of the egg and its membrane (egg size and membrane thickness and porosity) are likely to influence the rate of oxygen supply to the embryo, so variation in these features could influence sensitivity to hypoxia. The heterogeneous nature of incubation conditions suggests that oxygen supply and demand will vary substantially among Atlantic salmon populations. Consequently, differentiation in egg membrane structure among populations could affect oxygen uptake and therefore embryonic susceptibility to hypoxia. This represents an opportunity for distinct populations of Atlantic salmon to adapt to their incubation conditions.

1.3.5 Literature gap

The Atlantic salmon egg membrane plays an important role in the interactions between the embryo and its environment but has received relatively little scientific attention. In particular, structural features that influence the diffusion of oxygen from the boundary layer to the embryo require greater research due to the widespread nature of oxygen stress during Atlantic salmon incubation. If differences in membrane permeability among populations are present, they could
influence embryonic sensitivity to hypoxia. Therefore, a study that enables observation of membrane structure of Atlantic salmon egg membranes could yield interesting results. Furthermore, the ability to link egg membrane structure to embryonic sensitivity to hypoxia could influence fisheries management practices such as supplementary breeding that remove stressors from the incubation stage of the life-cycle.

Intuitively, it could be expected that membrane thickness correlates with mechanical strength, but evidence from other oviparous organisms suggests that the chemical composition of the membrane could be equally important. Due to the frequency with which Atlantic salmon encounter mechanical stress throughout incubation, particularly due to scour, the importance of structural features on mechanical strength would benefit from greater research. This should focus on the relationship between membrane thickness and chemical composition and mechanical strength.

The importance of temperature on egg size has important implications in the context of warming rivers in response to climate change. Warming temperatures could drive greater embryonic metabolic rate (Dillon et al. 2010) and, consequently, greater oxygen demand. Furthermore, conversion of yolk sac to body mass is less efficient in warmer conditions. Larger eggs enhance oxygen uptake and provide greater energetic reserves that compensate for reduced efficiency of yolk sac to body mass conversion. Therefore, warmer conditions could drive selection for larger eggs. This could reduce maternal fecundity and gene propagation so warrants further research.

1.3.6 Research aims and objectives

Aim: To identify variation in the egg structure of Atlantic salmon eggs and whether this corresponds to differences in tolerance to low oxygen

Section 1.3 identified that salmonid egg membranes inhibit oxygen supply to the embryo and differences in their structure could affect the rate of oxygen diffusion. Therefore, a laboratory study was conducted that aimed to quantify variability of egg membrane architecture among five populations of Atlantic salmon and to determine the effect such variation might have on embryonic tolerance to hypoxia.

Objective one: To determine differences in egg membrane structure among populations of Atlantic salmon, with a particular focus on permeability to oxygen
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Eggs of five distinct Atlantic salmon populations were sampled for egg size and a scanning electron microscope (SEM) was used to measure membrane thickness and micropore diameter and density.

**Objective two: To determine oxygen requirements of Atlantic salmon eggs to meet their metabolic demands based on population differences of membrane structure**

The intragravel velocity required to support embryonic respiration at a range of dissolved oxygen concentrations was calculated based on key structural features of the eggs of each population. This analysis was based on the mass transfer theory (section 3.3.2). Comparisons among populations were conducted to determine differences in oxygen supply requirements.

**Objective three: To determine whether membrane structural differences are linked to spawning conditions experienced by each population**

Differences in standard incubation temperatures were used as a proxy for embryonic oxygen demand for each population and were correlated with membrane permeability.

**Objective four: To determine whether visible differences in membrane structure correlate with differences in the sensitivity of eggs to low oxygen levels.**

Eggs were continuously exposed to low oxygen levels. The membrane structure of those that survived the prolonged hypoxia was compared with those that died to determine whether structural differences in the egg membrane influence embryonic sensitivity to low oxygen.
Chapter 2: Development of Methods

In order to address each aim described in chapter 2, it was necessary to either adapt methodologies employed in other fields or to develop novel techniques that have not been previously implemented. While chapters 4 to 6 provide full methodological detail, the following section describes the rationale and development of selected methodologies that require further explanation.

2.1 Aim one: Using wavelet analysis to assess the co-variability between river discharge and hyporheic dissolved oxygen content

Previous work on the interactions between river discharge and hyporheic dissolved oxygen, although backed up by hydrological theory, has predominantly been descriptive. Following prolonged wet periods, Soulsby et al. (2009) predicted a shift from peak discharge events followed by short periods of oxygen depletion to prolonged periods of oxygen depletion punctuated by oxygen recharge during peak river discharge events. This implies a shift from a hysteretic relationship (high flow event followed by period of oxygen depletion) to an in-phase relationship characterised by continuous hypoxia punctuated by oxygen recharge during high flow events. One of the objectives of this study is to support this previous descriptive work with quantitative analysis and to extend this analysis from a single site (Soulsby et al. 2009) to numerous sites. Several statistical options are available to study these interactions. Basic correlation and cross-correlation analysis could show general trends but do not highlight short-scale variability and the large datasets could lead to Type I (false positive) errors. Moving window correlation or cross correlations could highlight areas of interest and reduce the sample size. However, if the interactions shifted from a lagged correlation to an in-phase correlation, as predicted by Soulsby et al. (2009), this would not be detected. Therefore, these issues were addressed through the use of wavelet analysis (Morlet 1983).

Wavelet analysis is increasing in popularity as a method for identifying the scale and time of temporal patterns in time-series datasets, and to pinpoint periods of coherence between two time-series (Grinsted et al. 2004; Kang & Lin 2007; Keener et al. 2010; Carey et al. 2013; Mengistu
et al. 2013). It has been applied to a wide-range of issues including temperature patterns (Baliunas et al. 1997), wave regimes (Hackney et al. 2013) and the El Niño oscillation (Lachniet et al. 2004). In addition, its use has become widespread in river research, particularly for studies observing the relationship between climate and river discharge (e.g. Carey et al. 2013; Xu et al. 2014).

Wavelet analysis enables observation of transient features and localised patterns of river discharge – hyporheic dissolved oxygen interactions. In particular, it synchronously conducts correlation and cross-correlation analysis over a range of temporal scales from individual readings to the entire dataset. This facilitates identification of short-term coherence between the variables and should help to identify periods where changes in water table levels trigger a different response in hyporheic exchange patterns.

Therefore, the wavelet package developed by Grinsted et al. (2004) for Matlab was used to study the level of coherence between river discharge and hyporheic dissolved oxygen content. River discharge and hyporheic dissolved oxygen content were compared at a range of scales throughout each study period.

2.2 Aim two: Laboratory control of oxygen concentration

Oxygen is a critical environmental variable, so its effect on a wide range of organisms has received considerable scientific attention. As a result, numerous studies have developed and described methodologies that enable manipulation of oxygen levels in the laboratory. These have been conducted on both terrestrial (Baumann et al. 1983; Peck & Maddrell 2005; Harrison et al. 2006) and aquatic organisms (Alderdice et al. 1958; Shumway et al. 1964; Seymour et al. 2000; Geist et al. 2006; Goncalves et al. 2015) using a wide range of methods.

Approaches to oxygen removal from water include boiling (Butler et al. 1994), the use of oxygen scavengers (Charles et al. 2006), and the introduction of carbon dioxide (Macklin et al. 1995) among others (Butler et al. 1994; Sinha & Li 2000). However, these methods introduce additional stressors for salmonid eggs including unfavourable temperature regimes, toxic substances and pH reduction. As a result, alternative methods for the removal of oxygen from salmonid incubating water are necessary. The most widespread method in the literature is the use of nitrogen. As nitrogen dissolves in water, its increased partial pressure removes oxygen (Chawla & Pourhashemi 2004). Therefore, the rate of removal is proportional to the amount of nitrogen introduced, enabling simple manipulation of oxygen concentration.
Nitrogen is a favourable method for biologists to control oxygen content in water as it is inert and, under normal conditions, has no evidence of damage to aquatic organisms. Therefore, several laboratory studies examining the effects of low oxygen on salmonid eggs have controlled oxygen levels through the introduction of nitrogen (e.g. Alderdice et al. 1958; Silver et al. 1963; Einum et al. 2002; Geist et al. 2006; Roussel 2007). These experiments have included the use of closed containers with stationary water (Einum et al. 2002; Hassall et al. 2009) and flow-through chambers (Alderdice et al. 1958; Silver et al. 1963; Geist et al. 2006; Roussel 2007). While stationary containers provide more precise control over oxygen levels and do not require continuous nitrogen input, they are only suitable for short-term experiments as other stressors, particularly waste metabolites, accumulate and affect survival. Therefore, flow-through chambers were considered preferential.

Due to resource constraints it was not possible to construct a new flow-through system, however an existing recirculating egg facility was present in the University of Southampton Chilworth Hydraulics laboratory (Sear et al. 2016). This was a relatively simple set-up whereby water flowed from a recirculating tank featuring biological, physical and ultra-violet filtration systems, through egg chambers (n=60) and back to the filtration system (Fig. 2.1A). This sequence of filtration ensured a highly stable environment, confirmed by continuous monitoring of key water quality parameters (pH, ammonia, nitrate, nitrite, phosphate and copper). This stability and high water quality facilitated successful incubation of Atlantic salmon to hatching. To enable oxygen depletion, an additional step was necessary to allow oxygen levels to be manipulated before water entered the egg chambers (Fig. 2.1B; 2.2A). This additional step involved the introduction of an “oxygen modification chamber” where nitrogen was introduced and oxygen levels were continuously monitored using oxygen probes (Fig. 2.3).

**Figure 2.1.** Simplified diagram of the initial set-up (A) and the modified set-up (B) including the introduction of the oxygen modification chamber and the nitrogen supply. The set-up described in B was replicated 5 times to enable 5 different oxygen regimes to be
studied. Note that multiple egg chambers were present in both A (n=60) and B (n=12) per oxygen modification chamber. Blue lines indicate flow of oxygenated water; red lines show flow of deoxygenated water and black lines show flow of nitrogen gas.

Figure 2.3. Photograph of (A) experimental set-up showing large blue oxygen modification chambers with smaller black perspex egg chambers and (B) egg chambers lined with artificial grass and containing fertilised Atlantic salmon eggs.

Nitrogen was introduced into the oxygen modification chambers (Fig. 2.3) through a ceramic-plate fine-bubble air diffuser (Point Four™). The rate of nitrogen flow was controlled using acrylic flow meters (Omega®, Fig. 2.1B). A flow rate of approximately 0.06m³ h⁻¹ was sufficient to reduce oxygen levels within the oxygen modification chambers to <1% saturation within 20 to 30 minutes. Milder hypoxic conditions were produced using lower flow rates, which were determined through trial and error. Oxygen modification chambers had tight-fitting lids to limit re-oxygenation from the surrounding atmosphere, thereby stabilising the oxygen concentration.
As expected, re-oxygenation occurred between the oxygen modification chamber and the egg chamber. The rate of re-oxygenation was approximately 10% of the quantity of oxygen removed (i.e. water reduced to 50% saturation in oxygen modification chambers would be re-oxygenated to 55% saturation in the egg chamber).

**Figure 2.3.** Diagram of oxygen modification chamber. Water from the filtration system entered chamber through inlet pipes and flowed to the egg chamber through a gravity-fed system at the outlet pipe.

In addition, new egg chambers were introduced. These were smaller than those previously used (Sear et al. 2016; Fig. 2.1A; 2.4) to reduce the amount of water, and thereby nitrogen, used in the system and also increase the number of replicates per treatment. The floor of the egg chambers had a surface area of 375cm$^3$, large enough to hold approximately 1000 eggs in a single layer, based on mean egg diameter of 0.6cm. This was sufficient to statistically test the objectives. Additional features of the egg chambers were designed to ensure oxygen concentration was the only stressor experienced by the eggs. They were opaque black to prevent light damage (Flamarique and Harrower 1999; Fig. 2.2B, 2.4) and lined with artificial grass to reduce physical damage and enable water to circulate around the egg (Hansen & Møller 1985). Water flow rates could be easily controlled using a tap on the inlet pipe and standardised across all egg chambers to ensure water velocity (and thus oxygen supply to embryos) did not affect eggs unequally. The lid of the chambers rested on two plastic supports, enabling easy removal and preventing
disturbance of the eggs. Finally, mesh netting was fitted over the outflow pipe to prevent alevin being lost from the system (Fig. 2.4).

![Diagram of egg chamber](image)

**Figure 2.4.** Diagram of egg chamber with key features to minimise the introduction of external stressors. Water flow in from the oxygen modification chamber through the inlet pipe and returns to the filtration system through the outlet.

### 2.3 Aim three: Egg membrane permeability

#### 2.3.1 Visual estimates of permeability

Previous studies of salmonid eggs (e.g. Stehy & Hawkes 1979; Groot & Alderdice 1985; Songe et al. 2016) have used electron microscopy to describe membrane thickness and micropore size and density. However, little work has been conducted on the combined effect of these structural features on membrane permeability for teleosts. By contrast, the importance of permeability on avian eggs has been recognised for decades (e.g. Wangensteen 1972), with particular focus on permeability to water vapour.

Egg capsule permeability can be measured experimentally using standard diffusion tests (e.g. Kutchai & Steen 1971; Ackerman & Prange 1972; Paganelli et al. 1974; Wickett 1975; Visschedijk et al. 1988). These tests would involve placing an egg membrane fragment between two well-stirred water chambers of differing solute concentration and measuring the rate of diffusion. Due to the small size of the membrane and, consequently, the size of the chambers, oxygen
microelectrodes are necessary to record changing oxygen concentrations. Attempts were made to develop such microelectrodes (e.g. Kagawa et al. 2016), but were insufficient to meet the needs of the investigation.

Permeability can also be measured theoretically using visual measurements of membrane thickness and micropore density and diameter (Solomon 1968; Wangensteen et al. 1972). This method has been used extensively for measurements of natural and artificial membranes and shows strong coherence with experimentally measured values (Kayar et al. 1981). Much of the theoretical work has focused on avian eggs and would not be directly applicable to Atlantic salmon as Krogh’s oxygen diffusion coefficient is 225 000 times lower in water than in air (Cronin & Seymour 2000). Therefore, estimates of oxygen permeability were taken from work on aquatic membranes (Solomon 1968) that incorporates data on membrane thickness and micropore density and diameter (equation 2.1).

$$J = D \varepsilon r \left( \frac{C_o - C_e}{x} \right)$$

where $J =$ membrane permeability to oxygen (mol cm$^{-2}$ s$^{-1}$); $D =$ Diffusion coefficient of oxygen in water (cm$^2$ s$^{-1}$); $\varepsilon r =$ membrane porosity (dimensionless); $C_o =$ required oxygen concentration at Atlantic salmon egg surface at 5°C (2.06×10$^{-7}$mol cm$^{-3}$) to support maximum respiratory requirements; $C_e =$ oxygen concentration within the perivitelline fluid of the eggs (3.34×10$^{-8}$mol cm$^{-3}$); $x =$ membrane thickness (cm)

Due to the microscale of these key structural features, high-resolution microscopy was essential to gain accurate measurements. As a result, embryo fragments were observed using a scanning electron microscope (SEM).

### 2.3.2 Modelling oxygen supply to the embryos

Section 1.1 discussed the effects of oxygen concentration and intragravel velocity in isolation on oxygen availability to Atlantic salmon eggs. However, a complex sequence of processes associated with intragravel supply and concentration determine the total oxygen availability to embryos. Consequently, the process of oxygen supply to embryo’s can be modelled using mass transfer principles of heat and solute transport first described by Daykin (1965). Subsequent refinements to the model (Wickett 1975; Chevalier & Carson 1985; Greig 2004) enabled its application to assess oxygen supply to incubating salmonid embryos.

The mass transfer model has two key stages: (1) oxygen supply to the egg boundary layer, which is largely determined by physical conditions within the redd and calculated using equation 2.2
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and; (2) The rate of diffusion from the boundary layer to the embryo, which is influenced by the concentration gradient and the permeability of the membrane and was calculated using equation 2.3.

\[ C_o = \frac{N}{4\pi r^2k} - C_1 \]  \hspace{1cm} (2.2)

where: \( C_o \) = oxygen concentration at the egg surface (mg cm\(^{-3}\)), \( N \) = rate of transfer of solute to the egg (mg s\(^{-1}\)), \( r \) = egg radius (cm), \( k \) = mass transfer coefficient (cm\(^2\) s\(^{-1}\)), calculated from a sequence of equations presented in Wickett (1975) and Greig (2004), and \( C_1 \) = oxygen concentration in the redd (mg cm\(^{-3}\)).

\[ C_o = \frac{Nx}{4\pi r^2D_c} + C_e \]  \hspace{1cm} (2.3)

where \( C_o \) = required oxygen concentration at egg surface to maintain embryonic respiration (mg cm\(^{-3}\)). \( x \) = membrane thickness (cm), \( D_c \) = membrane diffusivity (cm\(^2\) s\(^{-1}\)), \( C_e \) = oxygen concentration within perivitelline fluid of the egg (mg cm\(^{-3}\)).

Membrane diffusivity is calculated using equation 2.4:

\[ D_c = \frac{D\varepsilon\tau\delta}{\tau} \]  \hspace{1cm} (2.4)

where \( D \) = Diffusion coefficient of oxygen in water (cm\(^2\) s\(^{-1}\)); \( \varepsilon \) = membrane porosity (dimensionless); \( \delta \) = constrictivity; \( \tau \) = tortuosity.

The importance of membrane thickness and porosity (determined by proportional micropore coverage) demonstrates the relevance of these structural features to requirements of oxygen supply. Due to an absence of relevant data, previous applications of the mass transfer model to Atlantic salmon (Greig 2004; Sear et al. 2014) have been based on membrane thickness (Wickett 1975) and porosity (Daykin 1965) data for brown trout and Chinook salmon respectively. Therefore, SEM data presented in this study (chapter 6) is used to update values for Atlantic salmon and improve estimates of oxygen supply requirements.

2.4 Summary

This chapter describes a range of approaches that are drawn together from several disciplines to identify the nature and effect of an environmental issue. While this chapter is mainly descriptive it provides important background detail of the development of some of the novel methodologies
employed in this thesis. This background detail is built on in chapters 4 to 6 to give comprehensive information on the way in which each methodological approach was employed.


3.1 Introduction

Located at the interface of the GW and SW dominated regions of the riverbed, the hyporheic zone is a distinct hydrological ecotone where these two water sources mix (Brunke & Gonser 1997; Tonina & Buffington 2009a). During periods of high GW-SW connectivity, circulation cells trigger mixing, moving SW into the alluvium through downwelling and GW into the river through upwelling. This is termed hyporheic exchange (Bencala 2005).

While spatial patterns of hyporheic exchange are influenced by geomorphological features (section 1.1.4), hydrological conditions of the river and catchment play a strong role in determining its timing and direction. In particular, elevation of the water table increases the upward pressure on the riverbed and the VHG (Soulsby et al. 2009), while greater river discharge raises the hydrostatic pressure and can reduce the VHG (Krause et al. 2012). Due to these interactions, the VHG is likely to be most negative when river discharge is high and water table is low, and most positive when the reverse is true. During intermediate periods, GW-SW connectivity could be high, leading to substantial mixing in the hyporheic zone and variability of the VHG (Malcolm et al. 2006). The significance of river and water table levels means that patterns of hyporheic exchange are likely to follow a somewhat seasonal pattern, with greatest periods of GW influence during the wettest times of the year.

The importance of hyporheic exchange is highlighted by the contrasting chemistry of the two water sources. While short-residence GW can be very similar in composition to SW (Malcolm et al. 2004), long-residence (phreatic) GW often has a higher nitrate concentration (Krause et al. 2013), greater conductivity (Malcolm et al. 2003) and a more stable thermal regime (Burkholder et al. 2008; Hannah et al. 2009). In addition, due to the action of nitrifying bacteria, consumption
of soil organic carbon and redox reactions in the aquifer, GW can be severely oxygen depleted (Malard & Hervant 1999; Malcolm et al. 2004; Zarnetske et al. 2011a, b; Krause et al. 2013). As a result, hypoxic and even anoxic conditions have been observed in the hyporheic zone during GW upwelling. This will affect the wide range of organisms that inhabit the riverbed, including the egg stage of Atlantic salmon.

Atlantic salmon eggs are deposited in redds by maternal fish at a depth of 15-30cm in riverbed gravels (Crisp & Carling 1989), so are subject to GW – SW interactions. Intermittent measurements by Hansen (1975) first demonstrated that GW intrusion can reduce dissolved oxygen content in salmonid redds. Since then, advances in monitoring technology have enabled collection of long-term, high-resolution data in the hyporheic zone of Atlantic salmon rivers (e.g. Malcolm et al. 2006, 2010; Soulsby et al. 2009; Sear et al. 2014). These studies have demonstrated that GW upwelling can cause both transient and long-term periods of hypoxia in Atlantic salmon redds. However, the complex nature of the factors that influence hyporheic exchange have restricted statistical analysis of the datasets. Periods of GW-induced hypoxia can cause high rates of egg mortality (Malcolm et al. 2003; Greig et al. 2007b) and reductions in post-hatch fitness (Youngson et al. 2004). In the UK, many Atlantic salmon populations have declined in recent decades (ICES 2015), so there is a need to improve understanding of the nature of stressors that they face at all life-stages.

This paper aims to establish the importance of antecedent and prevailing weather conditions on patterns of hyporheic exchange in several Atlantic salmon spawning rivers in the UK, and to determine the potential consequences for embryonic survival. In particular, the study (1) quantified seasonal variation in river discharge and GW levels; (2) supported previous descriptive work regarding the importance of hydrological conditions on hyporheic exchange through the use of wavelet analysis, a statistical method that enables calculation of covariance between two datasets at a range of temporal scales. This analysis was conducted on datasets from sites throughout the UK to determine the frequency of deoxygenated GW upwelling in Atlantic salmon spawning rivers. Finally, the study (3) considered the effects of deoxygenated GW upwelling on Atlantic salmon incubation success.

### 3.2 Methodology

#### 3.2.1 Data

Data were sourced from published and unpublished studies on rivers within the UK hosting native Atlantic salmon populations (Table 3.1; Fig. 3.1) that met two criteria: (1) simultaneous data were
available for river discharge and hyporheic dissolved oxygen content and; (2) the data were collected at a resolution of ≤1hr. Detailed descriptions of the characteristics of each study site are available in the relevant publication and references therein, key site characteristics are provided in table 3.2.

Where dissolved oxygen and river discharge data was not available in its raw form (Table 3.1), it was digitised from the graphical representation of the data in the published studies using the software GraphClick 3.0. Unfortunately, data from the study by Soulsby et al. (2009) that met the criteria could not be used as it was not possible to extract the data at a sufficient resolution. This is a long-term (40 months) monitoring study of a single site that provides important information about seasonal and annual variation of hyporheic exchange patterns.

In several studies, dissolved oxygen data was collected at multiple burial depths within the hyporheic zone. In these instances, only the data collected at the greatest burial depth was analysed (Table 3.1). This provided consistency among study comparisons and ensured that . Some studies collected dissolved oxygen data in concentration (mg l⁻¹), and others in saturation (%). To enable ease of comparisons among locations, all data was standardised into saturation (%) using equation 3.1 (Lee 1980):

\[
O_2 = \frac{14.6p}{1000e^{-0.0247T}}
\]

where \(O_2\) = maximum oxygen concentration; \(p\) = atmospheric pressure (estimated from elevation data of sites) and \(T\) = temperature. Oxygen saturation was calculated by dividing the recorded concentrations by the values produced from equation 4.1.

### 3.2.2 River discharge and groundwater levels

Seasonal patterns of GW elevation were obtained from the British Geological Survey and compared with river discharge data collected from the National River Flow Archive. While the British Geological Survey monitors an array of boreholes throughout England and Wales, they are not evenly distributed and were not in close proximity to all the study sites. Therefore, due to the differential response between GW and river discharge over very small spatial scales (Blumstock et al. 2016), only GW data gathered within the same catchment as the study river were used. As a result, comparisons between discharge and GW were only conducted using rivers of southern England (Test, Blackwater, Itchen, Arle; Fig. 3.1).

Data for all study sites was limited to the period from 1987-2014 as this was the extent of the GW datasets. Mean discharge and GW levels for each month were calculated over that time frame.
and Pearson’s correlation coefficients were computed to determine the relationship between the two variables.

Figure 3.1. Map of rivers hosting Atlantic salmon within the United Kingdom. Rivers from which data on hyporheic dissolved oxygen content and river discharge was used are highlighted in heavier red lines. Triangles indicate location of boreholes used for groundwater data. Rivers are labelled with the relevant studies from which data was sourced below in italics.
Table 3.1. Details of the studies from which river discharge and dissolved oxygen data were sourced from the present investigation. Hyporheic depth refers to the depth within the riverbed from which dissolved oxygen data in the relevant study was obtained. Digitised dissolved oxygen, river discharge and temperature data was extracted from the publication stated in the reference using GraphClick 3.0.

<table>
<thead>
<tr>
<th>Reference</th>
<th>River</th>
<th>Location</th>
<th>Headwater/Trunk Stream</th>
<th>Time of Study</th>
<th>Hyporheic Depth (cm)</th>
<th>Raw data or digitised from publication</th>
<th>Temperature Data Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burke 2012</td>
<td>Lugg (Meanders)</td>
<td>Tributary of River Wye, Wales, UK</td>
<td>Trunk</td>
<td>16/02/2008 to 26/04/2008</td>
<td>30</td>
<td>Digitised from publication</td>
<td></td>
</tr>
<tr>
<td>Burke 2012</td>
<td>Lugg (Lyepole Bridge)</td>
<td>Tributary of River Wye, Wales, UK</td>
<td>Trunk</td>
<td>26/02/2010 to 23/04/2010</td>
<td>30</td>
<td>Digitised from publication</td>
<td></td>
</tr>
<tr>
<td>Malcolm et al. 2006, 2010</td>
<td>Girnock Burn</td>
<td>Caingorms National Park, Scotland</td>
<td>Headwater</td>
<td>16/11/2004 to 27/03/2005</td>
<td>30</td>
<td>Digitised from publication</td>
<td></td>
</tr>
<tr>
<td>Malcolm et al. 2010</td>
<td>Newmills Burn</td>
<td>North East Scotland</td>
<td>Headwater</td>
<td>14/11/2006 to 20/02/2007</td>
<td>30</td>
<td>Digitised from publication</td>
<td></td>
</tr>
<tr>
<td>Greig 2004</td>
<td>Test</td>
<td>Southern England</td>
<td>Trunk</td>
<td>14/12/2001 to 15/03/2002</td>
<td>25-30</td>
<td>Raw</td>
<td>Raw data</td>
</tr>
<tr>
<td>Sear 2014</td>
<td>River Rede</td>
<td>Tributary of River Tyne, Northern England</td>
<td>Headwater</td>
<td>18/02/2011 to 08/06/2011</td>
<td>20</td>
<td>Raw</td>
<td>Raw data</td>
</tr>
</tbody>
</table>
### Table 3.2. Catchment characteristics of field sites examined in the present study. Unless otherwise stated, catchment information was obtained directly from studies.

<table>
<thead>
<tr>
<th>River (Stretch)</th>
<th>Catchment Area (km²)</th>
<th>GW Dominated/Freshet</th>
<th>Channel Width (m)</th>
<th>Mean Discharge (m³ s⁻¹)</th>
<th>Mean Annual Rainfall (mm)</th>
<th>Dominant local land use</th>
<th>Dominant catchment Geology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lugg (Meanders)</td>
<td>257.77</td>
<td>Freshet</td>
<td>17.3</td>
<td>3.95</td>
<td>852*</td>
<td>Pasture/Arable</td>
<td>Red sandstone</td>
</tr>
<tr>
<td>Lugg (Lyepole Bridge)</td>
<td>231.44</td>
<td>Freshet</td>
<td>12</td>
<td>3.95</td>
<td>852*</td>
<td>Woodland/pasture</td>
<td>Limestone/boulder clay</td>
</tr>
<tr>
<td>Girnock Burn</td>
<td>30.3</td>
<td>Freshet</td>
<td>9</td>
<td>0.53</td>
<td>996*</td>
<td>Heather moorland</td>
<td>Granite and Schists</td>
</tr>
<tr>
<td>Newmills Burn</td>
<td>25.4</td>
<td>Freshet</td>
<td>1.5 to 2</td>
<td>0.2</td>
<td>800</td>
<td>Arable farming</td>
<td>Psammite and Pelite</td>
</tr>
<tr>
<td>Arle</td>
<td>57*</td>
<td>GW Dominated</td>
<td>7.1</td>
<td>1.64</td>
<td>869*</td>
<td>Pasture</td>
<td>Chalk</td>
</tr>
<tr>
<td>Blackwater</td>
<td>10</td>
<td>GW Dominated</td>
<td>2 to 5</td>
<td>0.92</td>
<td>879*</td>
<td>Woodland and Heathland</td>
<td>Barton Sands, Barton Clays and Bracklesom</td>
</tr>
<tr>
<td>Test</td>
<td>104</td>
<td>GW Dominated</td>
<td>12</td>
<td>11.18</td>
<td>827*</td>
<td>Arable agriculture and grazing land</td>
<td>Chalk</td>
</tr>
<tr>
<td>Aran</td>
<td>12</td>
<td>Freshet</td>
<td>5</td>
<td>1.16</td>
<td>1086</td>
<td>Agriculture and heathland</td>
<td>Shale, mudstone, sandstone, limestone</td>
</tr>
<tr>
<td>Ithon</td>
<td>32</td>
<td>Freshet</td>
<td>11</td>
<td>11.89</td>
<td>1125*</td>
<td>Livestock farming</td>
<td>Shale, mudstone, sandstone, limestone</td>
</tr>
<tr>
<td>River Rede</td>
<td>215</td>
<td>Freshet</td>
<td>14.5</td>
<td>5.93</td>
<td>981*</td>
<td>Pasture/moorland</td>
<td>Carboniferous sandstone</td>
</tr>
</tbody>
</table>

**Note.**  
* Data obtained from the National River Flow Archive (NRFA)  
† Data obtained from Schindler Wildhaber et al. (2012)  
‡ Value taken from River Itchen, the river into which the Arle flows.  
§ Value taken from River Lymington, the river into which the Blackwater flows.
3.2.3 River discharge and hyporheic dissolved oxygen

Due to the transient and localised nature of the interactions between river discharge and hyporheic oxygen content, wavelet analysis was selected as the most appropriate tool to examine the relationship (Grinsted et al., 2004; Labat, 2008; Mengistu et al., 2013; Carey et al. 2013). Specifically, wavelet coherence, using the Matlab package provided in Grinsted et al. (2004), was performed. This provides information on the presence and direction of covariance between discharge and hyporheic oxygen content over a range of temporal scales throughout each dataset.

To determine the nature of annual variation in the relationship between river discharge and hyporheic dissolved oxygen concentration, wavelet coherence figures were compared with the raw datasets. When a high flow event (defined as ≥100% increase from base-flow) was observed in the raw dataset, the wavelet coherence figure was checked for statistically significant covariance. The presence or absence of covariation was noted and, if present, the direction of the relationship was also recorded. As the observed datasets encompass different times of year, the month in which these high flow events occurred were recorded. This allows identification of seasonal patterns in the relationship between discharge and hyporheic dissolved oxygen concentration. As the focus of the present study was the effect of hyporheic exchange patterns on oxygen regimes experienced by incubating Atlantic salmon, the data was only analysed at times of the year when their eggs are likely to be present in UK streams (November to April).

3.2.4 Hyporheic dissolved oxygen and incubating Atlantic salmon

To estimate the impacts of deoxygenated GW upwelling on incubating Atlantic salmon, data on the time that their eggs spend in gravels was essential. The time of Atlantic salmon spawning varies among populations and is strongly linked to latitude and altitude (Webb & McLay 1996). Therefore, each dataset was standardised using available information on the spawning time of the populations local to each study site (Table 3.3). Where spawning data for the study sites were not available, they were collected from the nearest location. While factors such as temperature (Webb & McLay 1996) and river discharge (Thorstad et al. 1998) cause annual variation spawn timing, estimates in table 3.3 were considered a useful guideline.

Temperature is an important determinant of developmental rate and incubation duration of Atlantic salmon (Hamor & Garside 1976). Temperature data were collected from the relevant study where possible (Table 3.1) and daily averages were used to calculate the developmental rate of the incubating salmonids in degree days (DDs), also known as accumulated thermal units. Time of hatching was estimated using the threshold of 500DDs for Atlantic salmon (Gorodilov 1996). Using this information, oxygen regimes within the incubation period could be estimated for each location. Some field studies did not last for the duration
Chapter 5

of estimated salmon incubation in their respective locations. In these cases, only the data that encompassed some period of Atlantic incubation was included in this analysis. If the study started after estimated spawning time, then river temperature data sourced from other datasets (Table 3.1) was used to predict the embryonic development state at the time the study commenced.

To determine the conditions Atlantic salmon embryos might experience, patterns of oxygen availability throughout the incubation period were quantified by calculating the likelihood that they experience GW-induced oxygen sags. Oxygen sags were separated into three categories depending on their intensity: (1) mild sags (60-41% saturation); median sags (40-21% saturation) and intense sags (<20% saturation) based on upper, median and lower critical oxygen levels described by Louhi et al. (2008) for successful Atlantic salmon incubation. When one of these boundaries was crossed, a sag was considered to have started, and lasted until the oxygen content crossed the upper or lower boundary of the threshold. The mean number, duration and start time of sags for each location within each category was then calculated.

To establish a general pattern of oxygen availability to incubating Atlantic salmon eggs, the average oxygen content across all studies at each incremental DD was calculated at intervals of 20DDs. This was plotted to observe times during the incubation phase when Atlantic salmon eggs were likely to experience depleted oxygen levels. In addition this was compared to estimates of embryonic oxygen demand at 5°C (Hamor & Garside 1976), which most closely represents average recorded temperatures in the studied locations.

Table 3.3. Estimated Atlantic salmon spawning times for each location with relevant reference.

<table>
<thead>
<tr>
<th>Location</th>
<th>Estimated Time of Spawning (Source)</th>
</tr>
</thead>
<tbody>
<tr>
<td>River Lugg</td>
<td>6th December (Mawddach hatchery data, Environment Agency)</td>
</tr>
<tr>
<td>Girnock Burn</td>
<td>16th November (Webb &amp; McLay 1996)</td>
</tr>
<tr>
<td>River Arle</td>
<td>December 26th (Riley &amp; Moore 2000)</td>
</tr>
<tr>
<td>River Itchen</td>
<td>December 26th (Riley &amp; Moore 2000)</td>
</tr>
<tr>
<td>River Blackwater</td>
<td>December 26th (Riley &amp; Moore 2000)</td>
</tr>
<tr>
<td>River Test</td>
<td>December 26th (Riley &amp; Moore 2000)</td>
</tr>
<tr>
<td>River Aran</td>
<td>6th December (Mawddach hatchery data, Environment Agency)</td>
</tr>
<tr>
<td>River Ithon</td>
<td>6th December (Mawddach hatchery data, Environment Agency)</td>
</tr>
<tr>
<td>River Rede</td>
<td>1st December (R. Bond, Personal Communication)</td>
</tr>
</tbody>
</table>
3.3 Results

3.3.1 River discharge and groundwater levels

In locations where data were available, river and GW levels strongly correlated (Table 3.4) and peaked during the Atlantic salmon incubation period (Fig. 3.2). However, the exact timing of discharge and GW maxima varied among sites. The River Blackwater and Downton borehole peaked from December to January while the Hursley borehole and Rivers Test and Itchen all peaked in February. The River Arle and Preston Candover borehole was at a maximum in March and April.

Table 3.4. Results of Pearson’s correlation coefficient in comparisons of river discharge with groundwater elevation recorded at the nearest borehole.

<table>
<thead>
<tr>
<th>River – Borehole</th>
<th>Correlation coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackwater – Downton</td>
<td>0.880</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Test – Hursley</td>
<td>0.933</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Itchen – Hursley</td>
<td>0.958</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arle – Preston Candover</td>
<td>0.984</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Figure 3.2. Mean monthly river and groundwater levels from 1987-2014 for (A) River Blackwater and Downton borehole; (B) River Test, River Itchen and Hursley borehole; (C) River Arle and Preston Candover borehole. Error bars indicate standard deviation. Shaded grey area indicates
period when Atlantic salmon eggs are present in UK rivers. River data sourced from NRFA (2016); groundwater data from BGS (2016).

### 3.3.2 River discharge and hyporheic dissolved oxygen

All datasets showed periods of oxygen depletion in the hyporheic zone (Fig. 3.3). These oxygen depletion events varied from short periods of mild hypoxia (e.g. River Blackwater) to several weeks of complete anoxia (Newmills Burn, River Aran, River Rede). Furthermore, at several locations, oxygen depletion events increased in intensity and duration as the field studies progressed, particularly into the late winter/early spring months (e.g. River Aran, Girnock Burn 2006 and 2010).

While there was variation among studies, there was evidence of coherence between river discharge and hyporheic dissolved oxygen concentration at all sites that corresponded with high flow events. In total 73 high flow events were observed. Comparison of raw datasets with wavelet coherence figures (Fig. 3.3), demonstrated the response of hyporheic dissolved oxygen concentration to a discharge event followed one of four main patterns: (1) ‘no response, oxygenated’, where oxygen concentration of the hyporheic zone remained high during and following a high flow event. This was observed in 26.0% of discharge events and most frequently in the earlier stages of the studies on the Girnock Burn and the River Ithon. The most common response of hyporheic DO to a high flow event was (2) ‘lagged oxygen depletion’ (Fig. 3.3, 3.4). This occurred when a high flow event was followed by a period of oxygen depletion in the hyporheic zone and occurred in 53.4% of occasions. The extent of oxygen depletion for this response varied among and within sites but the duration was usually relatively short (e.g. River Arle, River Blackwater). High flow events also caused (3) ‘oxygen recharge’, a concurrent rise of hyporheic dissolved oxygen concentration in 11.0% of instances. These occurred during periods of prolonged hyporheic anoxia where high flow events coincided with transient periods of oxygen recharge (e.g. River Rede, Newmills Burn A). Finally, (4) ‘no effect, deoxygenated’ was characterised by continuously anoxic conditions in the hyporheic zone, which were uninterrupted by variation in river discharge (Newmills Burn, River Rede). This response accounted for only 9.6% of observations.

The relative frequency of each response varied substantially when separated by the month in which they were observed (Fig. 3.4). The ‘no effect, oxygenated’ response was most common in November and became progressively less frequent. The ‘lagged oxygen depletion’ response was most frequent from December to March while the response of ‘oxygen recharge’ was only observed from February onwards (Fig. 3.4). Response 4 (no effect, deoxygenated) showed no clear seasonal variation but was most common from January to March and was only observed at the Newmills Burn, River Aran and River Rede sites.
The nature of the interactions between river discharge and hyporheic dissolved oxygen content during periods of high connectivity became clear when they were plotted (Fig. 3.5). Hysteresis associated with the ‘lagged oxygen depletion’ response is displayed (Fig. 3.5a and b). Here, oxygen levels remained high during the rising limb of a high flow event before declining sharply on the recession limb. Periods of oxygen recharge are characterised by initially low dissolved oxygen concentration in the hyporheic zone, which are interrupted by transient increases of oxygen concentration during the rising limb of the high flow event before oxygen concentrations rapidly decline on the recession limb (Fig. 3.5C and D).
Figure 3.3. Wavelet analysis displaying coherence between river discharge and hyporheic dissolved oxygen levels with raw data presented below. The cone of influence delineates edge effects and is indicated by the translucent shading. Areas outlined by heavy black line indicate coherence at the 95% confidence interval. Right facing arrow indicates in-phase relationship, left-facing arrow indicates antiphase relationship, downward facing arrow indicates dissolved oxygen response lags discharge, upward facing arrow indicates discharge lags dissolved oxygen.
Figure 3.3. Continued
Figure 3.4. Frequency and nature of relationships between river discharge and hyporheic dissolved oxygen content following a high flow event. Data is taken from wavelet analysis displayed in Figure 3.3. Values above bars indicate number of high flow events observed per month.

3.3.3 Hyporheic dissolved oxygen and incubating Atlantic salmon

Seventy-seven per cent of study sites displayed oxygen levels below 60% saturation during the time of salmon incubation. 69% experienced oxygen below 40% saturation and 62% exhibited values below 20% saturation. Mild sags occurred most frequently and earliest, whilst intense sags occurred least frequently and latest but generally lasted longer than the mild and medium sags (Table 3.5). However, the number, timing, duration and mean time in each sag category varied substantially among and within studies, demonstrated by standard deviation values often greater than calculated means (Table 3.5).

From estimated time of spawning to hatch, the average oxygen content of the salmon incubation zone decreased from a mean of 90.3% to 57.5%. Therefore, embryos at the latest stages of their incubation are more likely to be exposed to low oxygen levels than those at the earlier stages of development. However, substantial variation among datasets was present, indicated by the high
inter-quartile range values (Fig. 3.6). As Atlantic salmon embryos develop their oxygen demand increases, before reaching a maximum recorded value of 0.216 mg g$^{-1}$ h$^{-1}$ at 415DDs (Fig. 3.6). Consequently, the time of greatest embryonic demand of Atlantic salmon embryos occurs when the likelihood of exposure to deoxygenated GW is at a maximum.

![Figure 3.5](image.png)

**Figure 3.5.** The relationship between hyporheic dissolved oxygen content and river discharge levels during peak flow events for (A) River Blackwater from 31/12/2002 to 2/1/2003; (B) River Lugg (Meanders) from 3/3/2008 to 26/3/2008; (C) Girnock Burn (Malcolm 2006) from 9/1/2005 to 11/1/2005; (D) River Rede from 11/3/20011 to 14/3/2011. A and B indicate periods of a lagged antiphase relationship between river discharge and hyporheic dissolved oxygen concentration. C and D indicate times of positive in-phase covariance.
Figure 3.6. Box plot of oxygen content in Atlantic salmon redds during estimated incubation period for all sites combined at intervals of 20 degree days. Heavy line overlaying data indicates oxygen consumption pattern of individual Atlantic salmon eggs throughout development at 5°C in oxygen saturated conditions (Hamor & Garside 1976). No error values were available for oxygen consumption data.

Table 3.5. General information on hyporheic oxygen conditions in salmonid redds during a single Atlantic salmon incubation period.

<table>
<thead>
<tr>
<th>Sag intensity (% saturation)</th>
<th>Mild (60-40%)</th>
<th>Medium (40-20%)</th>
<th>Intense (&lt;20%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Studies with sags (%)</td>
<td>83.33</td>
<td>75.00</td>
<td>66.67</td>
</tr>
<tr>
<td>Mean number of sags (per incubation period)</td>
<td>7.00 ± 7.29</td>
<td>6.60 ± 7.34</td>
<td>4.40 ± 5.83</td>
</tr>
<tr>
<td>Mean time of first sag (Degree Days)</td>
<td>145.86 ± 124.15</td>
<td>152.14 ± 130.85</td>
<td>154.50 ± 142.84</td>
</tr>
<tr>
<td>Mean sag duration (hrs)</td>
<td>13.80 ± 15.91</td>
<td>8.26 ± 4.94</td>
<td>69.22 ± 66.57</td>
</tr>
<tr>
<td>Mean sag duration (Degree Days)</td>
<td>3.54 ± 3.18</td>
<td>2.06 ± 1.19</td>
<td>18.27 ± 18.49</td>
</tr>
<tr>
<td>Mean total time in each category (%)</td>
<td>3.63 ± 3.13</td>
<td>2.59 ± 2.85</td>
<td>17.72 ± 29.40</td>
</tr>
</tbody>
</table>
3.4 Discussion

All studies demonstrated periods of low oxygen levels in the hyporheic zone and frequent covariation with river discharge provided evidence that this was as a result of GW upwelling. The seasonal and recurring nature of these patterns demonstrated the importance of antecedent weather conditions on hyporheic exchange and suggested Atlantic salmon eggs are exposed to GW induced hypoxia throughout their UK range.

3.4.1 River discharge and groundwater levels

Variation in GW levels was observed at every location and correlated with antecedent weather conditions. Extended dry periods and the subsequent reduction of water supply are responsible for low GW levels in summer. Furthermore, higher temperatures induce evaporation, which drives additional depletion of the aquifer (Haycock & Burt 1993; Burt et al. 2002). In autumn and winter, water levels in the aquifer are recharged due to direct precipitation and water supply from the surrounding catchment (Burt et al. 2002; McMillan et al. 2012). Furthermore, GW is directly discharged into the river from the subsurface (Winter 2007), so its contribution to stream flow is highest when the water table is also at a maximum. In addition, where flooding occurs, the river itself becomes a direct source of water for the catchment and directly recharges the aquifer (Burt et al. 2002). Due to the strong correlation between the two variables, the hydraulic pressure of both water sources is highest during the Atlantic salmon incubation period. This means that connectivity could be greatest at this time and, where long-residence GW is present, Atlantic salmon embryos are likely to experience low oxygen conditions.

3.4.2 River discharge and hyporheic dissolved oxygen

High flow events induced four main responses in hyporheic dissolved oxygen content: (1) no effect, oxygenated; (2) lagged oxygen depletion; (3) oxygen recharge and (4) no effect, deoxygenated. While these patterns have been predicted theoretically (Cardenas & Wilson 2006) and described at a single site (Soulsby et al. 2009), this study provides statistical support for these observations in natural conditions and shows they are widespread and present in a range of river types. Furthermore, seasonal variation in the frequency of each response corresponded with theoretical predictions of changing GW – SW connectivity.
Extended periods of well-oxygenated conditions and the ‘no effect, oxygenated’ response occurred most frequently during November and December due to low GW – SW connectivity. While autumn rainfall recharges the aquifer, water table depletion during drier summer months meant that GW levels are likely below the riverbed in late autumn. As a result, GW influence was minimal and the hyporheic zone was a well-oxygenated, SW dominated system (Fig. 3.7a).

The pattern of ‘lagged oxygen depletion’ most frequently observed from December to March was driven by both antecedent and prevailing hydrological conditions and represented high GW – SW connectivity. Continuous rainfall, represented by high river discharge levels, enhanced aquifer recharge and elevated the water table further. Subsequently, individual high rainfall events simultaneously elevate river and GW levels. During the high flow event, it is assumed that the hydrostatic pressure of SW suppressed GW input to the hyporheic zone and maintained a well-oxygenated environment. However, on the receding limb, the hydrostatic pressure fell and deoxygenated GW seeps into the hyporheic zone (Fig. 3.7B), resulting in oxygen sags. The extent of oxygen depletion as a result of this hysteresis varied depending on water table elevation. Often, in the earlier stages (December to February), GW – SW connectivity was relatively weak, so these patterns of hysteresis were characterised by short periods of mild hypoxia where GW and SW mixed in the hyporheic zone (e.g. River Blackwater, River Ithon). However, continuous catchment wetting elevated the water table further, so these oxygen sags became longer and more intense (e.g. River Arle). Therefore, a trend of increasing oxygen depletion intensity from autumn to spring is frequently observed. This can lead to extended periods of anoxia and a phase-shift from a SW dominated to a GW dominated hyporheic zone.

In late winter and early spring, continuous rainfall led to extended periods of hyporheic anoxia in some locations. This occurred when GW levels exceed the river level (Krause et al. 2012) and upwelling through the streambed blocked hyporheic exchange and limited downwelling (Cardenas & Wilson 2006; Soulsby et al. 2009). Extended anoxia was occasionally punctuated by short periods of oxygen recharge during high flow events when hydrostatic pressure drove well-oxygenated SW into the riverbed (Hanrahan 2008; Soulsby et al. 2009; Fig. 3.7C). However, minor high flow events in the River Aran did not interrupt extended anoxia from January to March, presumably because the hydrostatic pressure was insufficient to reduce the vertical hydraulic gradient. This demonstrates the importance of antecedent weather conditions in determining hyporheic water quality and supports the assertion that hypoxia in the incubation zone will be longer and more intense in wetter years (Soulsby et al. 2009). Indeed, prolonged anoxia at the River Rede occurred after unseasonably heavy and extended rainfall (Sear et al. 2014). Long-term
monitoring at a range of sites would help to identify whether extended periods of anoxia is a regular annual occurrence, or only occurs during wetter years.

Data from the Newmills Burn demonstrated that anthropogenic activity can reduce GW – SW connectivity and, consequently, give rise to very poor water quality in the hyporheic zone. This site has been subject to heavy modification and loss of geomorphological complexity that drives hyporheic exchange (e.g. Gooseff et al. 2007; Buffington & Tonina 2009; Malcolm et al. 2010). Furthermore, fine sediment input due to intensive agriculture in the catchment further reduced riverbed roughness that drives hyporheic exchange (Packman et al. 2004). The blanket of fine sediment also created a physical barrier to SW infiltration and caused a GW-dominated, anoxic system (Ryan & Packman 2006). These features limited the effects of hydrological conditions on GW – SW connectivity and led to the loss of seasonal hyporheic exchange variability, as evidenced by continuous dominance of deoxygenated GW.

Alternative causes of oxygen depletion such as breakdown of organic matter (Sear et al. 2008, 2016), algal respiration (Ingendahl et al. 2009) and sediment oxygen demand (Malcolm et al. 2010) could contribute to hyporheic hypoxia. However, this is usually greatest in spring and summer when temperatures and microbial activity are highest (Soulsby et al. 2009; Bowes et al. 2012). Further, when they were monitored, features associated with GW upwelling such as alkalinity, temperature, conductivity and a positive vertical hydraulic gradient frequently co-varied with oxygen concentrations (Table 3.1 and references therein). This supports the assertion that oxygen depletion displayed in the present study occurred as a result of deoxygenated GW upwelling.

Figure 3.7. Conceptual diagram of seasonal changes in groundwater-surface water connectivity and fluctuating hyporheic exchange following high flow events. A (no response to flow event) – following prolonged dry spells, rainfall causes river level and water table to rise but the low water table means that surface water continues to dominate the hyporheic zone; B (hyporheic oxygen sags lag high flow events) (i) – rainfall leads to a slight elevation of the water table but surface water dominates the hyporheic
zone, (ii) rainfall results in discharge and water table elevation, (iii) discharge subsides leading to upwelling; C (positive correlation between discharge and hyporheic oxygen) – (i) groundwater dominates the hyporheic zone, (ii) river volume peaks, resulting in downwelling, (iii) discharge recedes and groundwater dominates again. Adapted from Soulsby et al. (2009).

3.4.3 Hyporheic dissolved oxygen and incubating Atlantic salmon

This chapter demonstrated that GW upwelling can reduce oxygen availability in Atlantic salmon redds at a range of locations and a variety of river types throughout the UK. Laboratory studies have demonstrated that salmonid eggs can survive relatively short periods of low oxygen, particularly at the earliest stages of development (e.g. Alderdice et al. 1958; Silver et al. 1963). However, field studies have demonstrated 0% survival of Atlantic salmon embryos in locations subject to intense GW input (Malcolm et al. 2003, 2010; Greig et al. 2007b). Furthermore, low rates of oxygen delivery during incubation can reduce the post-hatch fitness of juvenile salmonids (Alderdice et al. 1958; Youngson et al. 2004). Therefore, in locations where deoxygenated GW is present, there could be substantial impacts on salmonid populations.

Greater frequency and intensity of oxygen depletion events later in development are important because of physiological changes to the Atlantic salmon embryo. As the embryo develops, its larger body mass requires greater oxygen supply to maintain metabolic function (Hamor & Garside 1976; Gorodilov 1996). As a result, the oxygen deficit for embryos at the later stages of development will be higher than for individuals earlier in development exposed to the same level of hypoxia. Therefore, the fact that embryos are likely to be exposed to the most extreme GW-induced hypoxia at the later stages of development means that the impacts could be particularly severe.

Additional stressors in Atlantic salmon redds could exacerbate the impact of deoxygenated GW upwelling on embryonic development. These stressors can include a high fine sediment load (e.g. Jensen et al. 2009) and the input of toxic materials such as heavy metals and pesticides (Finn 2007). In addition, GW can contain substances such as ammonia (Buss et al. 2004), nitrate (Krause et al. 2009) and a range of anthropogenically-introduced toxins (Stuart et al. 2012) that reduce salmonid egg survival (Kincheloe et al. 1979; Finn 2007). Further, higher temperatures associated with GW (Burkholder et al. 2008; Hannah et al. 2009) mean that embryos in upwelling regions will have a greater oxygen demand (Hamor & Garside 1976), which could enhance their sensitivity to hypoxia. While GW-induced hypoxia is a key determinant of Atlantic salmon incubation quality, it is important to consider the effect of co-stressors that might further limit incubation success.
3.4.4 Management implications

SW input is essential to maintain good water quality in the Atlantic salmon incubation zone; however, this could be reduced by anthropogenic activity. As evidenced by the Newmills Burn data, fine sediment deposition can restrict hyporheic exchange by loss of riverbed roughness and physical blocking of SW downwelling (Packman et al. 2004; Malcolm et al. 2010). Other anthropogenic activities such as dredging and canalisation also reduce riverbed complexity and limit hyporheic exchange (Amoros & Bornette 2002; Hester & Gooseff 2011). Homogenisation of the riverbed is a common anthropogenic impact in river channels. The importance of such activities in terms of GW – SW connectivity and, consequently, hyporheic water quality demonstrates the need to carefully consider their effects in river management strategies.

In agreement with previous work (Cardenas & Wilson 2006; Malcolm et al. 2006; Cardenas & Markowski 2010), this study demonstrated that river discharge influences the supply of well-oxygenated SW to the hyporheic zone. Therefore, artificial alteration of discharge is likely to affect hyporheic exchange. While abstraction of SW for human consumption is widespread, its effects on hyporheic exchange have received relatively little attention and require greater research. River volume is also influenced by flow obstructions such as dams, which control downstream flow rates. Preliminary work in Scotland on a single artificial release from a hydroelectric dam suggested minimal effects on hyporheic exchange (Gibbins et al. 2009). However, the effect of seasonal variation in groundwater and river levels on patterns of hyporheic exchange described here implies that the effect of controlled releases on the intensity of GW upwelling would benefit from further research. Indeed, modified flow from a dam in the Columbia River, USA drives short-term fluctuations in VHG and subsequent variation in hyporheic water quality (Arntzen et al. 2006). On a smaller-scale, weirs (Hester & Doyle 2008) and log-steps (Schindler Wildhaber et al. 2014) can also increase GW upwelling in the hyporheic zone. Therefore, any anthropogenic activities that affect flow regimes are likely to influence hyporheic exchange patterns so should be considered carefully before being implemented in future.

As with all environmental issues, changing climatic patterns are likely to influence the Atlantic salmon incubation zone. Predictions of greater winter precipitation (Kovats et al. 2014; Romero-Lankao et al. 2014) could lead to water table level elevation during incubation (Jackson et al. 2011) and greater GW influence. Furthermore, rising temperatures mean that precipitation in mountainous regions is more likely to fall as rain as opposed to snow (Harrison et al. 2001). This could increase the likelihood that Atlantic salmon eggs will be exposed to deoxygenated GW upwelling. This is because the effect of snowfall on river discharge and the water table is most prominent during the spring snowmelt, because water is stored as snow (Hannaford & Buys
2012). By contrast, the water table of catchments dominated by rainfall usually recharge more rapidly (Scibek et al. 2007), so GW influence will be observed earlier and is more likely to affect Atlantic salmon eggs. Cumulatively, changing climatic patterns are likely to increase the likelihood of low oxygen exposure to Atlantic salmon eggs. While the resolution of climate change issues falls beyond the scope of this paper and river and catchment management, it demonstrates the importance of limiting other anthropogenic activities that could further reduce oxygen supply.

3.5 Conclusions

By collating numerous datasets, this study demonstrates a seasonal pattern in the relationship between GW levels, river discharge and hyporheic dissolved oxygen content. The variable relationship between river discharge and hyporheic exchange has been described before. However, by compiling studies from various locations and times of year, this study demonstrates that the pattern is both widespread and seasonal in nature within the UK. On a broad temporal scale, this seasonality closely follows water table levels with greater GW influence when it is highest. On a shorter temporal scale, hyporheic exchange is influenced by river discharge and fluctuating downward pressure as a result of changing river volume. Consequently, Atlantic salmon eggs are likely to be subjected to sub-optimal rates of oxygen delivery that could influence survival and population strength. Therefore, it is important to carefully consider and plan anthropogenic activities in river catchments to minimise effects on GW – SW interactions.
Chapter 4: Paper 2. The Effects of Episodic Oxygen Depletion on the Survival and Post-Hatch Fitness of Atlantic Salmon Embryos

4.1 Introduction

Despite their ecological and economic importance (e.g. Carss et al. 1990; Environment Agency 2009a), Atlantic salmon are in decline. Many populations throughout Northern Europe have been extirpated (Parrish et al. 1998) and in 2014, the number of mature salmon returning to the United Kingdom to spawn was just 41% of the 1970s average (ICES 2015). The proposed reasons for this decline include overfishing (Parrish et al. 1998), reduced marine survival as a result of changes to climate patterns (Friedland et al. 2000; Otterson et al. 2001) and reduced incubation success due to unfavourable conditions (Chapman 1988). One of the critical drivers of poor incubation success is insufficient oxygen supply (Greig et al. 2007a).

Atlantic salmon deposit eggs in redds which, in ideal conditions, provide embryos with a continuous supply of well-oxygenated water (Greig et al. 2007a). However, a range of natural and anthropogenic factors can reduce oxygen delivery (Sear et al. 2008). For example, sediments can infiltrate salmonid redds, reducing intragravel velocity and therefore delivery of oxygen to the eggs (Greig et al. 2005a; Sear et al. 2008), resulting in high rates of mortality (Kemp et al. 2011). In addition, patterns of hyporheic exchange and GW – SW interactions influence oxygen supply to incubating Atlantic salmon (Malcolm et al. 2003; Sear et al. 2014). GW can be severely oxygen depleted (Malard & Hervant 1999; Zarnetske et al. 2011a, b), and periods of upwelling can trigger episodic periods of hypoxia that could reduce incubation success. The widespread influence of GW in the hyporheic zone of Atlantic salmon spawning rivers (chapter 4) demonstrates that this is an important issue that could influence incubation success throughout their range.

Low oxygen levels during incubation can cause mortality (Malcolm et al. 2003; Greig et al. 2007a) and sublethal reductions of embryo fitness such as altered hatch timing (Youngson et al. 2004; Roussel 2007), increased frequency of deformities (Alderdice et al. 1958) and reduced alevin length and mass at hatch (Youngson et al. 2004; Geist et al. 2006). Indeed, field studies have demonstrated high rates of embryonic mortality (Malcolm et al. 2003, 2010; Greig et al. 2007a) and reductions of post-hatch fitness (Youngson et al. 2004) in locations subject to GW upwelling. While field studies provide critical information regarding incubation success in the wild, their nature makes it difficult to delineate the effect of hypoxia from other stressors that may be
present but are unlikely to be monitored. Furthermore, changing oxygen demand throughout development (Hamor & Garside 1976) means that some life-stages are likely to be more sensitive to hypoxia than others. Difficulties of continuously monitoring embryonic survival in the field due to problems associated with access to study sites makes it difficult to pinpoint these periods of greater sensitivity. Therefore, the ability to induce episodic periods of hypoxia, such as those caused by GW upwelling, in the laboratory would aid this understanding.

Human activities that increase sedimentation and reduce river water volume can influence hyporheic exchange (chapter 4), so understanding the effects of transient periods of oxygen depletion, such as those caused by upwelling, on incubating Atlantic salmon will aid river management. Furthermore, the widespread importance of hyporheic exchange on incubation oxygen regimes (chapter 4) demonstrates the need to better understand their impacts. Therefore, this study investigated the effect of three similar sequences of oxygen depletion events, comparable to those caused by GW upwelling, on Atlantic salmon incubation success. In particular, the effects of these sags on embryonic (1) survival (2) hatch timing and (3) post-hatch biometrics were observed. In addition, the effect of oxygen depletion timing on the magnitude of each response was monitored.

4.2 Methodology

4.2.1 Egg sources

To account for variation among locations, eggs were collected from four hatcheries within the United Kingdom (Fig. 4.1). Except for those of the farmed fish, all eggs and milt were taken from adults naturally returning to their natal stream:

- River Burn hatchery, River Ure, North Yorkshire.
- Kielder hatchery, River South Tyne, Cumbria.
- Kielder Hatchery, River Rede, Northumberland.
- Commercial farm, Argyll and Bute, Scotland.

As the focus of this study was English salmon rivers, the first three sites were selected as they were locations with a sufficient broodstock of wild fish to permit egg samples. In addition, the farmed eggs were selected as they represented eggs taken from an optimum incubation zone. Due to limitations on the number of eggs that could be donated, eggs were obtained from a single fish from each location.
Figure 4.1. Small inset map shows global range of Atlantic salmon in maroon (Taken from FAO 2016). Larger map of mainland United Kingdom shows rivers with native Atlantic salmon populations in blue. Locations from which eggs were sourced are labelled and are in heavier red lines.

Gametes were extracted from adult fish by hand on the morning of 1\textsuperscript{st} December 2014 into clean and dry plastic containers. Eggs were submerged in coelomic fluid to prolong their viability (Bonnet \textit{et al}. 2003). Containers were immediately oxygenated and placed into chilled containers before being transported to the Chilworth Spawning Habitat research facility at the University of Southampton. Transportation of unfertilized gametes results in greater rates of survival than fertilized gametes (Jensen \& Alderdice 1983) and fertilization rates of 95-100\% (Jensen \& Alderdice 1984) have been recorded when the methods described here have been followed.

4.2.2 Research facility and oxygen control

Six thousand eggs (1500 per maternal fish) were fertilised in the facility on the morning of 2\textsuperscript{nd} December 2014 following procedures described by Whitney \textit{et al}. (2013). The eggs of each maternal fish were divided into five groups of 300, each of which was exposed to one of five oxygen treatments described in section 4.2.3. Throughout the experiment, the eggs of each maternal fish were kept separate to ensure genetic differences in incubation behaviour could be
accounted for. However, the eggs of each maternal fish were exposed to identical oxygen regimes, and the data combined during analysis.

In addition, to the 6000 test eggs, a batch of 800 (200 per maternal fish) was held in reserve. Test eggs were left to acclimate to the conditions until daily mortality rates fell below 1%. This occurred after three days, or 22 Degree Days (DDs). During the acclimation period, all eggs within the containers that died were removed and replaced with a live egg from the reserve batch. After 22DDs, those that died were removed but not replaced, as they had not faced the same level of oxygen stress as the test eggs.

Research was conducted in a recirculating tap water fed system described in section 3.2 and figure 4.2. Test eggs were placed into egg chambers made of perspex (25cm x 15cm x 12cm, at a water depth 7cm) lined with artificial grass, a suitable substrate for incubation (Hansen & Møller 1985). All containers had a lid that could be easily removed to minimise disturbance during sampling. Boxes and lids were opaque black to ensure that light damage (Flamarique and Harrower 1999), was minimised. Fine mesh netting was attached around the outflow pipe of each egg box to eliminate the risk of post-hatch alevin being lost.

The bulk flow rate through each egg chamber was maintained at 150cm h\(^{-1}\) ± 3.6% to ensure it was not a limiting factor in the oxygen supply to incubating embryos (Greig et al. 2004).

Oxygen levels were controlled in cylindrical oxygen modification chambers (height 140cm, diameter 50cm; Fig. 4.2) through the addition of nitrogen. When nitrogen dissolves in water, its partial pressure increases, causing the expulsion of dissolved oxygen. Therefore, the amount of nitrogen introduced directly controlled the extent of oxygen depletion. Compressed nitrogen gas was transported through flexible tubing of inner diameter 6mm (RS\(^{®}\)) to acrylic flowmeters (Omega\(^{®}\)). The flowmeters allowed precise control over nitrogen flow, and thereby oxygen levels. Nitrogen gas was transported from the flowmeters to a single fine-bubble air diffuser (Track Lock\(^{®}\)), one of which was placed into each oxygen modification chamber, including the control. Oxygen modification chambers were totally sealed, with the exception of perforations for the water inlets, nitrogen inflow and oxygen probes. This provided greater control over oxygen levels by minimising atmospheric oxygen exchange.

Aandera\(^{®}\) optodes recorded temperatures at 1 minute intervals throughout the experiment and the mean over ten minutes was logged on a Delta-T data logger. Average recorded temperature was 7.62°C (min: 4.94°C; max: 10.32°C). The continuous monitoring of temperature allowed precise calculation of the embryonic developmental stage in DDs (Gorodilov 1996).
Daily water quality checks were taken to measure ammonia, nitrate, nitrite, copper, phosphate and pH levels. Readings were consistently below critical levels recorded for incubating salmonids (Westin 1974; Kincheloe et al.; 1979; Soderberg et al. 1983; Timmons et al. 2002).

**Figure 4.2.** Systematic diagram of recirculating system at the research facility. Arrows indicate direction of water flow. Location of each treatment is labelled. Numbers within egg chambers indicate location of eggs of each maternal fish: 1 = River Ure; 2 = Farmed; 3 = River South Tyne; 4 = River Rede.

### 4.2.3 Oxygen treatments

Five different treatments were established to determine the relative impacts of various oxygen regimes on the survival and post-hatch fitness of Atlantic salmon embryos (Table 4.1; Fig. 4.3A - E). This involved two continuous treatments (Table 4.1; Fig. 4.3A and B) where target oxygen levels were consistent throughout the experiment:

A. Control. Oxygen saturation was maintained close to maximum. Mortality rates in this treatment were used to calculate relative sensitivities to hypoxia of the other treatments;

B. Chronic. After the period of acclimation (22DDs), oxygen levels were maintained at approximately 60%. This enabled comparisons of the response of Atlantic salmon embryos to prolonged mild hypoxia compared to treatments mimicking more extreme, sporadic hypoxia induced by deoxygenated GW.

Three oxygen sag treatments (Table 4.1; Fig. 4.3. C – E) were also developed to imitate episodic periods of oxygen depletion caused by GW upwelling. The sag treatments consisted of a sequence
of ten oxygen depletion events (pulse) lasting 24hrs. Each pulse was separated by a period of oxygen recharge of the same duration. The pulse duration of 24hrs was selected based on analysis of datasets recording the impacts of GW upwelling in Atlantic salmon reds (section 4.3.3). Due to the high degree of variability in oxygen sag duration recorded in natural spawning conditions (table 4.5), a period of 24hrs was selected as this was close to the mean duration of all recorded sags in section 4.3 (29.4hrs) and ensured that, due to logistical constraints, the sequence could be accurately replicated three times. Each sag treatment was consistent in the number of pulses, pulse intensity and pulse duration but differed only by the time that the sequence of pulses began (Table 4.1; Fig. 4.3. C-E). This was altered to mimic seasonal variation of hyporheic exchange patterns (Soulsby et al. 2009). Throughout each sag treatment, pulse intensity gradually increased to simulate the increasing dominance of GW in the incubation zone (section 4.3.2; Table 4.1, Fig. 4.3. C-E). Sag treatment start times are displayed in DDs to enable easier identification of embryonic development periods most sensitive to low oxygen levels. Treatments C-E were the sag treatments and were categorised as follows:

C. Early Sags. Pulse sequence described above commencing at 134DDs;
D. Median Sags. Pulse sequence described above commencing at 229DDs;
E. Late Sags. Pulse sequence described above commencing at 317DDs.

Oxygen levels were continuously monitored in the oxygen modification chambers using Aandera® 4175 optodes at 1 minute intervals and the ten-minute average logged to a Delta-T logger throughout the investigation.

Re-oxygenation occurred between oxygen modification chambers and the egg chambers. This was noted in preliminary investigations and was accounted for by reducing oxygen to levels below the target values. The rate of re-oxygenation was proportional to the amount of oxygen initially removed in the oxygen modification chamber and could be estimated using equation 4.1:

$$E_o = M_o + \left(\frac{C_o - M_o}{100}\times 10\right)$$  \hspace{1cm} (4.1)

where $E_o$ = the estimated oxygen level in the egg chambers; $M_o$ = the oxygen concentration in the oxygen modification chamber; $C_o$ = the oxygen level in the control.

Daily spot checks within egg chambers were conducted using a handheld oxygen probe (YSI® proODO) to ensure oxygen concentrations were close to the target values described in table 4.1 and the estimate calculated using equation 1. Saturation estimates showed strong agreement with recorded values (Fig. 4.4), so validated the use of the above formula for continuous approximation of oxygen conditions experienced by experimental eggs.
Table 4.1. Target oxygen concentrations of all oxygen treatments of the present study. Eggs in continuous treatments (control and chronic) were exposed to stated oxygen concentrations throughout experiment. Sag treatments were exposed to stated oxygen concentrations at specified developmental state of embryos. Start and end times of each pulse are given in degree days to help identify the developmental states at which embryos were most sensitive to low oxygen levels.

<table>
<thead>
<tr>
<th>Continuous Treatments</th>
<th>Sag Treatments</th>
</tr>
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<tbody>
<tr>
<td></td>
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<tr>
<td><strong>Oxygen Saturation (%)</strong></td>
<td><strong>Oxygen Saturation (%)</strong></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td><strong>Chronic</strong></td>
</tr>
<tr>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
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<tr>
<td>4</td>
<td>30</td>
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<td>5</td>
<td>30</td>
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<td>6</td>
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<td>10</td>
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<td>9</td>
<td>10</td>
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<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

4.2.4 Measuring mortality and sublethal effects

Mortality

To compare rates of mortality among treatments, daily checks for dead eggs were conducted. Dead eggs were clearly identified by their opaque white colouration (Fig. 4.5). All dead eggs were immediately removed to prevent the spread of bacterial and fungal infection and the total number in each egg chamber was recorded.

Hatch timing
Chapter 5

Checks for the presence of hatched alevin were also conducted every 24hrs and twice daily during the period of peak hatching (434DDs onwards) to increase data resolution. The number of hatchlings at each sampling time (recorded in DDs) was recorded. All alevin were immediately taken from the system and euthanised using 2-phenoxyethanol solution and preserved in 4% formalin solution (Burke 2011).

**Biometrics**

To sample post hatch biometrics, alevin were removed from the formalin and thoroughly rinsed in deionised water. Initial observations were conducted to detect the presence of developmental abnormalities. These were present in less than 0.4% of sampled individuals and their frequency did not vary among treatments, so were not included in data analysis.

The fork length of a sample of wet alevin from each treatment was measured using a Nikon E100 microscope at 50x magnification. To account for errors, alevin were re-measured and differences were <0.1mm. Alevin were then oven-dried at 60°C for 48hrs (Rombough 1994) and measured for total mass (yolk sac plus body). The body was subsequently detached from the yolk sac and weighed to determine its contribution to total mass. All mass measurements were conducted on a Mettler Toledo AB204-5 balance accurate to 0.1μg.

From the completion of dehydration to measurements of alevin mass, there was the potential for rehydration of the alevin from atmospheric moisture. To determine whether this resulted in a significant increase in mass, a small sample of alevin of each treatment/population group was measured immediately after dehydration and subsequently at 2-hour intervals over a total period of eight hours. Over this time, the total combined mass increased by 0.77%. To ensure that this effect was minimised, all samples were measured within two hours of removal from the dehydrator.

The developmental state of newly hatched alevin was measured by counting the number of caudal fin rays (CFRs) present (Gorodilov 1996). The first CFR is present when anal and dorsal fin formation begins (approx. 300DDs) and continues until a total of 20-21 CFRs are present in post-hatch alevin (Gorodilov 1996). CFRs are formed at equal developmental intervals (Gorodilov 1996), so they provide a useful indicator of developmental stage (Burke 2011). Number of CFRs present was counted under a Nikon E100 light microscope at 100x magnification (Fig. 4.6).

As alevin were removed from the system and euthanised within 12hrs of hatch, so variable hatch timing meant that raw biometric data could not be used to directly compare the developmental state of alevin across treatments at a specified time. Therefore, alevin developmental state at a
time when all individuals had hatched was estimated using the number of somite pairs present in the embryo as a key indicator of developmental stage (Gorodilov 1996). Somite pairs are formed during the division of axial strips of the mesoderm (Gorodilov 1996). After the first CFR is formed, the rate of somite pair formation is directly proportional to the rate of CFR formation (1 CFR = 8.33 somite pairs). The rate of somite pair formation was estimated using equation 4.2:

$$\lg \tau_t = C + at + bt^2$$  \hspace{1cm} 4.2

where $\tau_t =$ length of time required to form one somite pair at given temperature $t$; $C = 3.0984$; $a = -0.0967$; $t =$ temperature in °C; $b = 0.00207$.

This information was used to estimate the number of CFRs of each individual at a time when all individuals from all treatments had hatched. (526DDs), thereby allowing direct comparisons between alevin of different treatments at a single time.
Figure 4.3. Estimated oxygen regimes Treatment: (A) Control; (B) Chronic; (C) Early sags; (D) Median sags; (E) Late sags. Values calculated using equation 1. Scatter points represent daily spot check values.
Figure 4.4. Comparison of recorded oxygen saturation values in egg chambers with estimated values using formula 4.1. Strong agreement between values ($R^2 = 0.992$, $p<0.001$) validates use of formula 4.1 to estimate oxygen concentration in egg chambers.

Figure 4.5. Eggs within egg chambers. Dead egg slightly right of centre is clearly visible by opaque white colour.
Figure 4.6. Image taken under a Nikon E100 microscope at 100x magnification of the caudal fin of a newly hatched alevin. A caudal fin ray that was used to estimate alevin developmental state is labelled.

4.2.5 Statistical analysis

Mortality

The total number of dead eggs in each egg chamber was counted and used to calculate the mean mortality rate for each treatment. To compare differences among treatments for mortality, one-way ANOVA tests were performed. When statistically significant (p<0.05) differences among treatments were observed, post-hoc Tukey’s tests were conducted to determine between which treatments these differences were observed.

Hatch rates

Upon completion of the investigation, Kaplan-Meier estimators (Kaplan & Meier 1958) were used to compare hatch rates among treatments. This provides a value for the frequency of hatching for each treatment per cumulative 1000DDs (i.e. the cumulative total of the number of DDs that all the eggs of each treatment combined experience. For example, 100 eggs experiencing 10DDs would experience a cumulative total of 1000DDs). This enabled pair-wise comparisons among treatments by producing a ratio that compares the incidence of hatching of two separate treatments.

A value for the hatch rate for each treatment was produced using equation 4.3:

\[ \mu_h = \frac{h}{E} \]  

4.3

where: \( \mu_h \) = hatch rate; \( h \) = number of eggs that hatched; \( E \) = exposure time
Relative risks of hatching (RRs) of two treatments were obtained by taking the hatch rate from the formula above of the two target treatments and dividing them by one another (equation 4.4):

\[ RR = \frac{\mu_{hx}}{\mu_{hy}} \]  

where: \( RR \) = relative risks; \( \mu_{hx} \) = hatch rate of treatment \( x \); \( \mu_{hy} \) = hatch rate of treatment \( y \).

For analysis of hatch rates, mortality was regarded as a random censoring event, independent from hatching. All Kaplan-Meier analysis was conducted on the statistical software Stata IC 12. Hatching incidence and the total time of observation figures was reported from 350DDs. The threshold of 350DDs was based on existing literature that demonstrates no evidence of Atlantic salmon hatching before this stage (Gorodilov 1996), and was supported by the Kaplan Meier hatching curves (Fig. 4.7). Analysis from this point onwards improved the clarity of the data and enabled more detailed comparisons among treatments.

**Alevin biometrics**

A mean value for each of alevin fork length, total mass, proportional body mass, number of CFRs at hatch and number of CFRs at hatch completion for each treatment was calculated. Prior to statistical comparisons, the proportional body mass data were presented in percentage format, so was square root arcsine transformed before statistical analysis.

To compare differences among treatments for all biometrics, one-way ANOVA tests were performed. When statistically significant (\( p<0.05 \)) differences among groups were observed, post-hoc Tukey’s tests were conducted to determine between which treatments these differences were observed. All alevin biometric analysis was conducted on the statistical software SPSS 21.

**Power analysis**

To ensure the sample size was sufficient to provide reliable conclusions, post-hoc power analysis was conducted on the software G*Power. An observed power value of 1.00 was calculated demonstrated the experiment was statistically robust, and the conclusions drawn were valid.

**4.3 Results**

**4.3.1 Mortality**

There were differences among treatments in terms of total mortality (\( F_4, 16 = 9.156, p<0.001 \); Fig. 4.7). These differences were all manifested in comparisons involving the late sags treatment
where mortality rates were higher than all other oxygen regimes (comparison with: control, p = 0.002; chronic, p = 0.009; early sags, p = 0.001; median sags, p = 0.001).

**Figure 4.7.** Mean mortality rates separated by treatment. Error bars indicate standard deviation.

### 4.3.2 Hatch timing

The first egg hatched at 380DDs and the last at 526DDs. Eggs exposed to the early and median sags treatment showed delayed hatch relative to the control (Fig. 4.8), reflected by lower incidence of hatching per 1000DD (Table 4.2, 4.3). Similarly, alevin of the early sag treatment hatched later than those of the chronic treatment. Eggs exposed to the late sags hatched earlier than all other treatments (Table 4.2, 4.3).
Figure 4.8. Kaplan-Meier estimate of the hatching function by treatment (N=6,000). Vertical marks on curves indicate incidence of mortality. Shading indicates confidence intervals. For ease of identification, curves are labelled A-E to match the corresponding treatment. A = control; B = chronic; C = early sags; D = median sags; E = late sags.

Table 4.2. Incidence of hatching and death by treatment (N=6,000).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Total hatched</th>
<th>Total deaths</th>
<th>Time exposed to risk (1,000DD)</th>
<th>Hatching rate from 350DD (per 1000DD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95 % CI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Estimate (Lower Boundary)</td>
</tr>
<tr>
<td>Control</td>
<td>1,200</td>
<td>1,141</td>
<td>59</td>
<td>557.382</td>
<td>8.09</td>
</tr>
<tr>
<td>Chronic</td>
<td>1,200</td>
<td>1,123</td>
<td>77</td>
<td>560.527</td>
<td>7.78</td>
</tr>
<tr>
<td>Early sags</td>
<td>1,200</td>
<td>1,149</td>
<td>51</td>
<td>578.538</td>
<td>7.12</td>
</tr>
<tr>
<td>Median sags</td>
<td>1,200</td>
<td>1,151</td>
<td>49</td>
<td>573.316</td>
<td>7.32</td>
</tr>
<tr>
<td>Late sags</td>
<td>1,200</td>
<td>1,022</td>
<td>178</td>
<td>536.204</td>
<td>8.47</td>
</tr>
</tbody>
</table>

Note. Hatching rate is taken from 350DDs to increase data resolution.
Table 4.3. Comparison of the incidence of hatching among oxygen treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Chronic</th>
<th>Early Sags</th>
<th>Median Sags</th>
<th>Late Sags</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>0.493</td>
<td>0.001</td>
<td>0.017</td>
<td>0.02</td>
</tr>
<tr>
<td>Chronic</td>
<td>0.972</td>
<td>-</td>
<td>0.011</td>
<td>0.091</td>
<td>0.003</td>
</tr>
<tr>
<td>Early Sags</td>
<td>0.874</td>
<td>0.899</td>
<td>-</td>
<td>0.391</td>
<td>0.001</td>
</tr>
<tr>
<td>Median Sags</td>
<td>0.905</td>
<td>0.932</td>
<td>1.036</td>
<td>-</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Late Sags</td>
<td>1.104</td>
<td>1.136</td>
<td>1.264</td>
<td>1.219</td>
<td>-</td>
</tr>
</tbody>
</table>

**Note.** Lower half of matrices indicate the incidence of hatching ratio of the row treatment group over the column treatment group (i.e. a value >1 indicates row treatment has higher incidence of hatch than column treatment). Corresponding p-values produced using a log-rank test of significance are reported in the top half.

4.3.3 Biometrics

Mean alevin fork length differed among treatments (one-way ANOVA - F (4, 908) = 64.191, p<0.001). Alevin of the late sags were approximately 1mm smaller than alevin of the other treatments (Table 4.4) (p<0.001 for all comparisons).

Overall, the total dry body mass of post-hatch alevin was 48.17 ± 8.71mg and there were no differences among treatments. However, mean percentage body mass differed among treatments (F(4, 908) = 139.628, p<0.001). Alevin of the late sags treatment had a lower percentage body mass than those of the other treatments (Table 4.4) (p<0.001 for all comparisons). Furthermore, percentage body mass of the control alevin was greater than the chronic (p<0.001) and median sags (p = 0.019) treatments.

Mean developmental state differed among treatments (F (4, 662) = 312.300, p<0.001). Alevin of the control were more advanced than those of all other treatments (Table 4.4) (p<0.001 for all comparisons) at hatch. By contrast, alevin hatching in the late sags treatment had fewer CFRs at hatch than alevin of all other treatments (p<0.001 for all comparisons).

At the time when all individuals had hatched (526DDs), the estimated number of CFRs present in alevin of all three sag treatments was similar (Table 4.4). However, significantly more CFRs were present for alevin of the control than all other treatments (p<0.001 for all comparisons).
**Table 4.4.** Details of alevin biometrics separated by treatment. Error values indicate standard deviation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Chronic</th>
<th>Early Sags</th>
<th>Median Sags</th>
<th>Late Sags</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length (mm)</strong></td>
<td>17.22 ± 0.83</td>
<td>17.34 ± 0.81</td>
<td>17.19 ± 0.85</td>
<td>17.24 ± 0.81</td>
<td>16.18 ± 1.00</td>
<td>17.05 ± 0.99</td>
</tr>
<tr>
<td><strong>Total dry mass (mg)</strong></td>
<td>48.16 ± 9.02</td>
<td>48.77 ± 8.53</td>
<td>47.00 ± 8.67</td>
<td>47.36 ± 8.77</td>
<td>50.03 ± 8.32</td>
<td>48.17 ± 8.71</td>
</tr>
<tr>
<td><strong>Dry body mass proportion (%)</strong></td>
<td>10.94 ± 2.59</td>
<td>9.58 ± 2.11</td>
<td>10.25 ± 2.41</td>
<td>10.07 ± 2.50</td>
<td>7.50 ± 1.90</td>
<td>9.81 ± 2.58</td>
</tr>
<tr>
<td><strong>Caudal fin rays</strong></td>
<td>14.42 ± 0.90</td>
<td>13.23 ± 1.06</td>
<td>12.87 ± 1.33</td>
<td>12.44 ± 0.77</td>
<td>8.95 ± 0.62</td>
<td>12.80 ± 1.88</td>
</tr>
<tr>
<td><strong>Estimated Caudal Fin Rays at 526DDs</strong></td>
<td>15.17 ± 1.02</td>
<td>13.98 ± 1.14</td>
<td>12.96 ± 1.36</td>
<td>12.64 ± 0.79</td>
<td>12.79 ± 0.94</td>
<td>13.52 ± 1.10</td>
</tr>
</tbody>
</table>

### 4.4 Discussion

The presence of GW in Atlantic salmon redd can result in severe oxygen sags (e.g. Malcolm *et al.* 2006; Soulsby *et al.* 2009; Sear *et al.* 2014). This study showed that similar oxygen sags in the laboratory could reduce embryonic survival, alter alevin hatch timing and affect their post-hatch fitness. Furthermore, the magnitude of these effects varied depending on the timing of the oxygen stress.

#### 4.4.1 Mortality

Overall, mortality rates were low but varied depending on the timing and intensity of oxygen depletion. Eggs exposed to the chronic, early sag and median sag treatments did not have higher rates of mortality than the control. However, in agreement with work on chum salmon (Alderdice *et al.* 1958) and rainbow trout (Rombough 1988), oxygen sags in the latest developmental stages resulted in a substantial rise in mortality. This is because the high embryonic mass at this stage (Gorodilov 1996) leads to an oxygen demand maximum (Hamor & Garside 1976) and greater sensitivity to hypoxia. Throughout their range Atlantic salmon incubate during the wettest period of the year (Malcolm *et al.* 2008). Therefore, the greater water table elevation at this time (McMillan *et al.* 2012) drives a greater frequency and intensity of GW upwelling (Malcolm *et al.* 2006; Soulsby *et al.* 2009; chapter 4). This means the water table level is likely to peak towards
the end of development, leading to maximum GW input, and prolonged and intense oxygen sags, when embryos are most sensitive to hypoxia.

The rate of survival observed in this experiment contrasts with field studies, which indicated deoxygenated GW upwelling can cause up to 100% mortality (Malcolm et al. 2003, 2004, 2010; Greig et al. 2007). Potential reasons for this disparity could be related to (1) differences in oxygen regimes or; (2) fine sediment infiltration leading to a reduction of intragavel velocity in the redd. Field data suggests that GW upwelling can cause more extreme and extended hypoxia than the sequence of episodic oxygen pulses presented here (section 4.3). While studies by Malcolm et al. (2003, 2004) and Greig et al. (2007a) did not record oxygen concentrations below those analysed in this study, they relied on intermittent (≥fortnightly) measurements of hyporheic dissolved oxygen, so could have missed periods of more extreme hypoxia (Malcolm et al. 2006). Furthermore, Malcolm et al. (2010) did record high-resolution data and reported extended periods (>2 weeks) of total anoxia, which was cited as the reason for 100% mortality of Atlantic salmon ova. While the data of the current study implies only relatively mild effects of episodic oxygen depletion patterns, more extreme hypoxia has been observed in spawning rivers and can cause greater rates of mortality (Malcolm et al. 2011).

While GW upwelling is an important stressor that influences oxygen supply to Atlantic salmon ova, fine sediment infiltration into redds can block intragavel pores and reduce intragavel velocity and therefore oxygen supply. Furthermore, a reduction of intragavel velocity reduces removal of toxic waste metabolites such as ammonia, which can cause mortality of Atlantic salmon embryos (Solbe & Shurben 1989). Data from the field sites (Soulsby et al. 2001; Moir et al. 2002; Greig et al. 2007a) suggests that infiltration by fines will have reduced intragavel velocity and exacerbated the effects of deoxygenated GW upwelling. By contrast, the laboratory environment of the present study ensured fine sediment did not influence incubation success. The relative effect of fine sediment infiltration versus GW upwelling is likely to vary among sites, depending on the relative intensity of either variable. However, hypoxia as a result of GW upwelling alone can cause 100% mortality in some situations (Malcolm et al. 2011).

4.4.2 Hatch timing

Oxygen sags during incubation resulted in a substantial shift in the hatch timing of Atlantic salmon embryos. The direction of this shift varied and was determined by the timing of the oxygen sags. Eggs exposed to the early and median sags hatched late and those exposed to the late sags hatched prematurely. Studies on the effects of longer-term chronic oxygen depletion have
observed similar patterns that are considered to be adaptive to maximise immediate survival in suboptimal conditions (Alderdice et al. 1958; Oppen-Berntsen et al. 1990; Youngson et al. 2004).

The direction of the hatch-timing shift varied based on the timing of hypoxia due to physiological changes of the embryo throughout development. Delayed hatch of embryos exposed to oxygen sags in the earlier stages of development is associated with the ability of the embryo to modify oxygen uptake relative to availability (Hamor and Garside 1976). The oxygen consumption and heart rate of incubating teleosts reduce in hypoxic conditions (Hamor & Garside 1976; Czerkies et al. 2002). This leads to a reduction in metabolic rate and a developmental lag that is responsible for the delayed hatch in the present study. The late sags treatment triggered premature hatch because the number of hatching gland cells (HGCs) and amount of hatching enzyme (chorionase) contained within them reaches a maximum just prior to hatching (Luczynski and Ostaszewska 1991). The oxygen stress induced by the late sag treatment, coupled with the maturation of the embryonic hatching apparatus at this time facilitates premature hatching. This mobilises the embryo, enabling escape from unfavourable conditions (Czerkies et al. 2001). The variable nature of these responses indicates the plasticity of the embryonic response to hypoxia and demonstrates the ability to show behavioural adaptation to external stressors.

Embryos exposed to the chronic treatment showed similar hatch rates to the control. The oxygen content of this treatment was consistently close to the upper limits of the critical levels described for Atlantic salmon (Louhi et al. 2008), so it is likely that this was sufficient to ensure the metabolic rate was not restricted.

4.4.3 Biometrics

Alevin of the late sag treatment differed most from the control. They had the shortest body length, the lowest level of yolk-sac absorption and the fewest caudal fin rays. By contrast, alevin of the early and median sags were of similar length to the control, although they did differ in terms of the other measured biometrics.

The finding that hypoxia in earlier stages of development had little effect on alevin length at hatch contrasts with previous work (e.g. Silver et al. 1963; Hamor & Garside 1977; Miller et al. 2008). This is probably due to the fact that extreme hypoxia in these other studies persisted for up to seven days, much longer than the present experiment. In addition, the extended incubation time of the individuals exposed to the early and median sags presumably enabled compensatory growth. Alevin exposed to the late sags were smaller than their conspecifics of all treatments at the time of hatch. It is likely that this is a result of a combination of developmental lag caused by
the earlier pulses of the sag treatment and premature hatch as a result of the stress response to extreme hypoxia described above.

In contrast with other studies, there were no differences among treatments in terms of total mass at hatch. Shumway et al. (1964) found that chronic hypoxia gave rise to alevin of significantly reduced total mass. Further, fieldwork conducted by Youngson et al. (2004) showed that eggs buried deeper in the hyporheos and more strongly exposed to hypoxic GW weighed less. However, Geist et al. (2006) found no differences in total mass between alevin of eggs raised in hypoxic and normoxic conditions. In the study by Shumway et al. (1964), these differences may be related to the more extreme and prolonged nature of oxygen depletion. However, Youngson et al. (2004) only took measurements of oxygen content every two weeks, so it is not possible to definitively draw the same conclusion. The absence of a difference in total mass among treatments in the present study is probably related to lower yolk sac absorption and thereby higher contribution of the yolk sac to total body mass of hypoxic individuals.

In agreement with other studies (Hamor & Garside 1977; Roussel 2007), all hypoxic treatments gave rise to alevin that had a lower body mass as a proportion of total mass. This effect was most pronounced in the late sag treatment. This is attributed to the fact that yolk-feeding fish such as Atlantic salmon distribute energy between growth and metabolism. As oxygen supply is reduced, there is a shift towards less efficient anaerobic processes (Kamler 2008). This means that the rate of conversion of yolk sac tissue into body tissue is reduced.

The use of caudal fin rays as an indicator of alevin development at hatch enabled observation of the extent of developmental retardation and demonstrated that, in all cases, hypoxia during incubation gave rise to underdeveloped alevin. Observation of the raw data suggests that the extent of developmental delay was strongest in the embryos exposed to the late sags treatment. However, using formulae developed by Gorodilov (1996), it was possible to show that the degree of developmental delay was remarkably similar across all sag treatments. This suggests that the primary cause of the difference in developmental state between the alevin of the late sag treatment and the early and median sag treatments was related to variance in hatch timing as opposed to a differential response to hypoxia at different developmental stages.

4.4.4 Implications for survival

This study shows that Atlantic salmon embryos experiencing hypoxia during incubation can have higher rates of mortality, which could influence population strength. While 0% survival as a result of GW upwelling in the field (Malcolm et al. 2003, 2004; Greig et al. 2007) will reduce cohort
strength, even relatively low mortality, such as observed in the present study, could have long-term population impacts. In recent decades the number of Atlantic salmon returning to spawn has declined markedly (ICES 2015). This suggests that, in many locations, post-hatch density will be insufficient to meet the carrying capacity of native streams, leading to under-utilisation of resources (Aprahamian et al. 2003). In these cases, density-independent mortality caused by features of the abiotic environment, such as hypoxia, can have a strong influence on population abundance (Sinclair 1989; Jonsson et al. 1998), leading to further weakening of native populations.

In addition to direct mortality, restricted oxygen supply due to episodic periods of low oxygen can give rise to sublethal effects that could further reduce long-term population strength. Aggregation of hatch timing is an adaptive measure to limit the impacts of predation (Pulliam & Caraco 1984) through appetite satiation or exceeding handling capacity of the predator (Begon & Mortimer 1986). Deviation from the time of peak hatch in either direction observed here dilutes this effect and increases the chances of an individual encountering a predator such as the bullhead (Cottus gobio, Roussel 2007) or burbot (Lota lata, Louhi et al. 2011). In addition, the smaller size and greater yolk sac mass of Atlantic salmon embryos exposed to the late sags treatment reduces their mobility, so inhibits their escape response (Parker 1971; Fresh & Schroder 1987; Sogard 1997). Further, hypoxia during incubation reduces the number and size of white muscle fibres (Matschak et al. 1997), which are essential for rapid acceleration and high swimming speeds (Valente et al. 1999), and thereby escape from predators.

A second critical phase in the juvenile life-stages is the completion of yolk sac absorption and emergence of fry from the gravels. A weakness of this study is that it was not possible to determine whether the hypoxia-induced developmental lag at hatch observed in all the sag treatments would have resulted in delayed emergence. However, other salmonids that hatch later than conspecifics as a result of hypoxic incubation conditions are also likely to emerge from the gravels later (Carlson & Siefert 1974; Roussel 2007). The time of emergence represents a second period of vulnerability in which mortality rates, due to predation from species such as brown trout, can be high (Einum & Fleming 2000). Therefore, late entry into this stage reduces the prey dilution effect. In addition, fry become territorial at this stage, so a developmental lag would mean juveniles emerge at a time of limited habitat availability. If a juvenile is unable to find a territory it can suffer mortality through starvation or predation (Einum & Fleming 2000; Einum & Nislow 2005). The fact that a large proportion of mortality can occur in the fry stage demonstrates the need to study the impacts of GW intrusion into later life-stages than presented here.
Variability among families could result in differences in terms of the consequences of GW upwelling on Atlantic salmon post hatch fitness. Eggs of different families of Chinook salmon (*Oncorhynchus tshawytscha*) have been shown to respond differently to thermal variability (Steel *et al.* 2012) and there is a distinct difference between the eggs of different families in terms of natural survival rates (Johnson *et al.* 2012; Roni *et al.* 2015). This suggests an important genetic influence on incubation success and that oxygen stress could affect eggs of different families unequally.

### 4.5 Conclusion

This study has shown that low oxygen regimes similar to those caused by upwelling GW can reduce survival and post-hatch fitness of Atlantic salmon. In terms of survival, this impact is strongest at the latest stages of development due to the greater oxygen demand at this stage. Similarly, initial observations implied that hypoxia at the latest developmental stages also causes the greatest reductions of post-hatch fitness. However, more detailed analysis showed that one of the main driving factors behind reduced fitness at hatch is deviation in hatch timing. It is likely that underdeveloped alevin hatching at a suboptimal time will have poor rates of survival due to predation. This impact will be enhanced at later life-stages if the developmental delays continue. However, these longer-term impacts require further investigation. The findings of the present study demonstrate the need for a better understanding of the natural and anthropogenic controls on low dissolved oxygen GW upwelling.

5.1 Introduction

Stressors during the incubation stage of salmonid development play a crucial role in shaping individual fitness and population dynamics. Their immobile nature means eggs cannot escape these stressors, so the incubation zone determines the nature of the hazards that they face. Some of the most well-documented causes of salmonid egg mortality are predation (Johnson et al. 2009; Palm et al. 2009), fungal and bacterial colonisation (Bakke & Harris 1998), siltation (Levasseur et al. 2006) and hypoxia (Greig et al. 2007; Malcolm et al. 2011). By burying their eggs in redds, salmonids reduce predation pressure. However, this increases vulnerability to microbial and fungal infection, siltation and hypoxia (Wootten 1990).

Hypoxic stress occurs when oxygen supply to the embryo is insufficient to meet its metabolic demands. Oxygen is supplied to salmonid embryos from a thin film of water surrounding the egg known as the boundary layer (Daykin 1965). The rate at which oxygen diffuses from the boundary layer, across the egg membrane to the perivitelline fluid (PVF) within the egg capsule is determined by the concentration gradient. The gradient, and therefore rate of diffusion, is reduced if the oxygen content in the boundary layer falls due to (1) greater oxygen consumption because of higher temperatures or advanced embryonic development (Hamor & Garside 1976) or (2) poor oxygen supply as a result of low oxygen concentrations and/or inadequate water flow in the redd (Greig et al. 2007). When the rate of oxygen diffusion to the embryo is insufficient to meet its metabolic demands, reductions of survival and post-hatch fitness are frequently observed (e.g. Alderdice et al. 1958; Malcolm et al. 2003; chapter 5).

While external factors influence oxygen supply and demand, the protective membrane that surrounds the embryo can impede diffusion and enhance hypoxic stress (Ciuhandu et al. 2007). Salmonid egg membranes are composed of a dense layer of proteinaceous fibres, interspersed with micropores (Groot & Alderdice 1985). The fraction of the egg surface that is composed of these micropores defines membrane porosity and, along with membrane thickness, controls total permeability (Solomon 1968; Eddy et al. 1990). As a result, greater membrane thickness or reduced porosity could increase the susceptibility of salmonid embryos to low oxygen conditions.
Chapter 6

The importance of porosity and thickness in terms of oxygen supply to embryonic salmonids is analogous to the egg membranes of oviparous organisms throughout the animal kingdom. Variation in micropore density among populations of the Peruvian coot (Fulica americana peruviana) is linked to differences in oxygen supply (Léon-Velarde et al. 1997), and membrane thinning occurs in a range of species experiencing oxygen stress (Cronin & Seymour 2000; Mills et al. 2001; Mueller et al. 2011). In the UK, oxygen supply to Atlantic salmon eggs varies due to differences in water velocity within redds and among river systems (e.g. Grieg et al. 2007), while differences in spawning altitude and latitude influence thermal regimes (Webb & McLay 1996) and, consequently, oxygen demand (Hamor & Garside 1976). Where oxygen supply is lower and/or demand greater, more permeable egg membranes could enhance uptake and compensate for these differences. Population variation in the response of Atlantic salmon eggs to hypoxia has been attributed to differences in the physiological response of the embryo (Côte et al. 2012). However, Côte et al. (2012) did not consider inter-populations differences in oxygen uptake rates, which could be driven by structural variance of the egg membrane.

The relevance of egg structure to embryonic oxygen supply has important management implications for estimating incubation success and developing stock enhancement programmes. The mass transfer model (Daykin 1965) is a popular (e.g. Greig 2004; Sear et al. 2014) deterministic method for predicting salmonid embryo survival based on: (1) dissolved oxygen content in redds and (2) intragravel velocity. The model relies on estimates of membrane porosity and thickness to calculate the required rate of oxygen delivery to the boundary layer to maintain sufficient diffusion to the embryo. If populations vary in terms of membrane porosity and thickness, concurrent variation in oxygen requirements will exist. Furthermore, such population variation could have important implications for stock enhancement initiatives such as in supportive breeding (artificial propagation) programmes that aim to reduce embryonic mortality caused by poor spawning conditions (Pitcher & Neff 2007). These programmes rear eggs of wild-caught fish in a hatchery environment before returning juveniles to native streams. The homogenous nature of the hatchery could result in the loss of population differentiation at the egg-stage and the potential reduction of beneficial traits (Reisinbichler & Rubin 1999; Heath et al. 2003). This could lead to long-term population decline (Bourret et al. 2011). Global weakening of Atlantic salmon populations (ICES 2015) has driven programmes aimed at enhancing their native environments and encouraging stock recovery. However, to ensure these methods are effective, there is a pressing need to understand interactions between Atlantic salmon eggs and their environment.

This study aimed to determine whether Atlantic salmon populations exhibit distinct egg architecture that affects their ability to survive periods of oxygen stress. More specifically,
scanning electron microscopy was used to measure egg size and membrane thickness and porosity to determine: (1) inter-population variability of egg architecture of five Atlantic salmon populations, with a particular focus on permeability to oxygen; (2) the oxygen requirements within the redd to support respiration of each population using the mass transfer model; (3) whether physical differences in membrane structure correlated with temperatures, and thereby oxygen demand, in the spawning environment of each population; and (4) whether visible differences in membrane structure correlated with embryonic tolerance to hypoxia.

5.2 Materials and Methods

5.2.1 Egg sources

Gametes were sourced from five hatcheries hosting Atlantic salmon within the United Kingdom (Fig. 5.1). Eggs were extracted from ten fish per hatchery (Table 5.1). Eggs and milt were extracted by hand into clean and dry plastic containers, ensuring the gametes of each parent fish were kept separate. Eggs were submerged in coelomic fluid to prolong their viability (Bonnet et al. 2003). Egg and milt containers were oxygenated and sealed, then placed into chilled polystyrene boxes and transported to the University of Southampton, Chilworth Spawning Habitat research facility where they were fertilised using procedures described by Whitney et al. (2013).

Gametes of each population were crossed using a full-factorial breeding design (Lynch & Walsh 1998) between four males and ten females, resulting in 40 unique families. Due to space constraints, all families of each population were mixed into a single group within an egg chamber. Consequently, all the eggs of each family were contained within a single chamber per population so, during sampling, the family from which eggs were selected was randomised. Throughout the experiment, the eggs of each population were kept separate.
Table 5.1. Details of hatchery location and typical incubation temperature for the Atlantic salmon eggs of each hatchery with parent number, approximate number of eggs per female used to meet all objectives, and date of stripping and fertilisation for Atlantic salmon eggs of each hatchery.

<table>
<thead>
<tr>
<th>Source River</th>
<th>Hatchery Location</th>
<th>Hatchery location (Lat, Long)</th>
<th>Hatchery altitude (MASL)</th>
<th>Mean incubation temperature (°C)</th>
<th>Females</th>
<th>Eggs per Female</th>
<th>Males</th>
<th>Stripping Date</th>
<th>Fertilisation Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>River Dochart</td>
<td>Perthshire, Scotland</td>
<td>56.41, -3.47</td>
<td>12.42</td>
<td>3.00</td>
<td>10</td>
<td>20</td>
<td>4</td>
<td>10/11/15</td>
<td>11/11/15</td>
</tr>
<tr>
<td>River Tilt</td>
<td>Perthshire, Scotland</td>
<td>56.42, -3.47</td>
<td>12.42</td>
<td>3.00</td>
<td>10</td>
<td>20</td>
<td>4</td>
<td>10/11/15</td>
<td>11/11/15</td>
</tr>
<tr>
<td>Farm</td>
<td>Argyll and Bute, Scotland</td>
<td>55.89, -5.62</td>
<td>6.47</td>
<td>4.00</td>
<td>10</td>
<td>300</td>
<td>4</td>
<td>16/11/15</td>
<td>18/11/15</td>
</tr>
<tr>
<td>River South Tyne</td>
<td>Northumberland, England</td>
<td>55.23, -2.58</td>
<td>210.10</td>
<td>5.12*</td>
<td>10</td>
<td>20</td>
<td>4</td>
<td>02/12/15</td>
<td>03/12/15</td>
</tr>
<tr>
<td>River North Tyne</td>
<td>Northumberland, England</td>
<td>55.23, -2.58</td>
<td>210.10</td>
<td>4.37*</td>
<td>10</td>
<td>20</td>
<td>4</td>
<td>02/12/15</td>
<td>03/12/15</td>
</tr>
</tbody>
</table>

Unless otherwise stated, information is based on the hatchery from which eggs were sourced.

*Temperature data sourced from Environment Agency (2007) for mean river temperature
5.2.2 Population variability of egg architecture

To quantify inter-population variability of membrane architecture, 200 eggs for each population were pooled into a single Perspex chamber (25cm x 15cm x 12cm, water depth 7cm) lined with artificial grass (Hansen & Møller 1985). Oxygen saturated water flowed through the chambers at a bulk rate of 150cm h\(^{-1}\) ± 3.4%. Water temperatures were recorded at 1 minute intervals and the ten minute-mean was logged (using Aandera® Optodes and a Delta-T data logger). Temperature values were used to calculate embryonic developmental state (equation 5.1):

\[ DD = \frac{T}{144} \]  \hspace{1cm} 5.1

**Figure 5.1.** Map of United Kingdom with rivers hosting Atlantic salmon shown in blue. The locations of the egg sources used in this study are labelled and displayed in heavy red.
where \( DD \) = developmental rate of the embryo over each 10-minute reading; 144 = number of readings taken per day; \( T \) = temperature (°C).

The cumulative developmental state was continuously calculated and 50 eggs from each population were sampled at 100DDs and diameter, membranes thickness and porosity were measured. All samples were processed for analysis at 100DDs so that possible changes in egg structure during development (e.g. Cronin & Seymour 2000), did not affect populations unequally.

### 5.2.3 Oxygen requirements to support respiration

To determine the incubation conditions necessary to ensure oxygen delivery to the boundary layer is sufficient to maintain embryonic metabolism, a mass transfer model (Daykin 1965, equation 5.2) was developed. This model calculated threshold oxygen concentration values required to support incubating salmonids based on fixed water velocities. The rate of oxygen diffusion from the boundary layer to the PVF was calculated using membrane porosity and thickness data. Previous application of this model relied on porosity and thickness estimates of Chinook salmon and brown trout presented in Daykin (1965) and Wickett (1975) respectively. In this study, the output of the model using these data (porosity = 0.1; thickness = 0.35μm) was compared with the mean values of porosity and thickness for each Atlantic salmon population calculated previously. All data were compared at 5°C to clarify the importance of membrane structure on oxygen supply requirements:

\[
C_1 = \frac{N_{req} \left( \frac{1}{k} + \frac{x}{D_c} \right)}{4\pi r^2 + C_e} \tag{5.2}
\]

where \( C_1 \) = required oxygen concentration of water within redd to prevent metabolic inhibition; \( N_{req} \) = oxygen consumption of embryo (mg s\(^{-1}\)); \( k \) = mass transfer coefficient (cm\(^2\) s\(^{-1}\)), calculated from formulae in Wickett (1975); \( x \) = membrane thickness (cm), based on mean value for each population; \( D_c \) = membrane diffusivity (cm\(^2\) s\(^{-1}\)), based on mean value for each population; \( r \) = egg radius (cm), based on mean value for each population; \( C_e \) = oxygen concentration within perivitelline fluid of the egg (1.1×10\(^{-3}\) mg cm\(^{-3}\)), which was taken from rainbow trout embryos (Dhiyebi et al. 2013) as values for Atlantic salmon were unavailable. \( D_c \) was calculated using equation 5.3:

\[
D_c = \frac{D_{e,v} \delta}{\tau} \tag{5.3}
\]
where $D =$ Diffusion coefficient of oxygen in water (cm$^2$ s$^{-1}$) at 5°C; $\varepsilon_r =$ membrane porosity (dimensionless); $\delta =$ constrictivity; $\tau =$ tortuosity. Based on Figure 5.2D, constrictivity and tortuosity were assigned a value of 1.

5.2.4 Incubation temperature

Embryonic metabolic rate, and consequently oxygen consumption, is proportional to temperature (Hamor & Garside 1976). Therefore, incubation temperature data were obtained from each source location and used as a proxy for embryonic oxygen demand. Due to an absence of temperature data in Scottish rivers, mean values were collected from the hatchery that incubates the River Tilt and River Dochart eggs (Table 5.1). Similarly, mean temperature data for the farmed eggs were collected from their commercial hatchery (Table 5.1). Winter river temperature data was collected for the River South Tyne and River North Tyne eggs (Table 5.1) from Environment Agency (2007). Temperature data were compared with permeability data for each population to determine covariation between the two variables.

5.2.5 Egg structure and hypoxic tolerance

To determine if structural variation of egg membranes corresponded with differences in embryonic tolerance to hypoxia, 2400 eggs from the farmed population were split into two groups (control and hypoxic) of 1200. Only farmed eggs were used to test for the effect of structural variation on hypoxic sensitivity as this minimised the effect of inter-population differences that might influence tolerance to oxygen stress (e.g. metabolic rate [Biro & Stamps 2010] or physiology [Anttila et al. 2013]). The control group received oxygen saturated (mean: 10.65 ± 1.43 mg l$^{-1}$) water throughout development, while the mean concentration in the hypoxic group was 1.56 ± 0.49 mg l$^{-1}$. Oxygen levels were controlled using the methodology described in chapter 3.2 and 5.2.

Egg chambers were checked every 24h and dead eggs counted and removed. When the number of dead eggs in the hypoxic group exceeded the control, enough live eggs of the control were extracted to ensure the daily number of eggs removed from each group was the same. Therefore, stocking density did not affect groups unequally.

Sample processing and analysis took place three times throughout development when: (1) relative mortality rates (calculated by subtracting mortality rate of the control from the hypoxic group) exceeded 5%, 20% and 50% and; (2) the number of eggs that died in a 24h period was
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sufficient to provide a robust sample size (≥36 eggs). These thresholds were reached at 134DDs, 144DDs and 448DDs, respectively.

To ensure membrane structure did not change when eggs died, a separate group of 500 farmed eggs were incubated under the same conditions as the control group. When eggs in this group died (n=37) they were removed and processed for observation, along with the same number of live eggs. Independent samples t-tests were conducted to determine differences among groups in terms of raw structural features, and no differences were detected (Egg diameter: $t_{72} = 0.209, p = 0.835$; membrane thickness: $t_{72} = 0.200, p = 0.842$; membrane porosity: $t_{72} = 0.032, p = 0.975$). This showed that the structure of eggs does not change within 24h of mortality and validated the methodology described.

5.2.6 Sample processing and analysis

Before analysing membrane structure, the diameter of each egg was measured under a Nikon E100 microscope at 50x magnification using a scaled background and graticule. To account for measurement error, eggs were re-measured and were within <0.1mm of the original values. These values were used to calculate surface area of the egg sphere.

Egg membranes were prepared for observation under the scanning electron microscope (SEM). They were carefully separated from the embryo by puncturing the membrane with a needle to release the internal pressure before peeling it away from the embryo using forceps (Groot & Alderdice 1985). This treatment minimised the destructive nature of the process, and, while minimal damage was observed, any areas demonstrating deformations were not included in analysis. Membranes were subsequently washed in water and fixed in 3% gluteraldehyde solution for one hour, and rinsed with deionised water. The prepared membranes were dehydrated in a sequence of graded ethanol solutions (30%, 50%, 70%, 95%) for 10 minutes each and twice in 100% ethanol for twenty minutes. Finally, they were dehydrated in a Balzers 030 Critical Point Drier. Each membrane was broken into three fragments to observe the inner membrane surface (internus), outer membrane surface (externus) and cross-section (Fig. 5.2D, E). These were affixed to a 12mm carbon tab on an aluminium stub and splutter coated with gold for thirty seconds, before being inspected under the scanning electron microscope (Jeol JCM-6000). Fixation using this method can cause minor shrinkage of biological tissues (Gusnard & Kirschner 1977). However, if this did occur, it was not considered detrimental to the study as all samples experienced the same treatment. This is because shrinkage could be expected to be consistent among eggs, so should not influence comparative analysis.
Membrane thickness was measured at four locations on the cross-sectional fragment and a mean derived to estimate membrane quotient for each egg (equation 5.4):

$$x_p = \frac{x}{\phi}$$

where $x_p$ = membrane quotient; $x$ = mean membrane thickness of individual egg (cm); $\phi$ = mean egg diameter of corresponding population (cm).

The internus was analysed to determine micropore density and diameter because the naturally occurring adhesive film found on the externus (Schmehl & Graham 1987; Fig. 5.2F) obscured images of the micropores. Density was calculated by counting the number of pores in an image of known area, and converting this to the number of pores per mm$^2$. This method assumed pore uniformity across the egg and was not considered detrimental to the experiment as the same method was applied for all membranes. Number of pores per egg was then calculated by multiplying this value by the estimated egg surface area. The diameter of all micropores at the lower left quadrant of an image from two separate locations of each membrane were also measured and used to calculate the mean surface area at the micropore opening (Fig. 5.2B). These values were used to calculate porosity of each egg membrane (equation 5.5).

$$\varepsilon = \frac{A_p N}{A}$$

where $\varepsilon$ = membrane porosity (dimensionless); $A_p$ = mean surface area of micropore opening (cm$^2$); $N$ = number of micropores per egg; $A$ = mean egg surface area of corresponding population (cm$^2$).

Equation 5.6 (Solomon 1968) was used to determine the permeability of membranes to oxygen:

$$J = D \varepsilon \left(\frac{C_0 - C_e}{x}\right)$$

where $J$ = membrane permeability to oxygen (mol cm$^{-2}$ s$^{-1}$); $C_0$ = required oxygen concentration at Atlantic salmon egg surface at 5°C ($2.06\times10^{-7}$ mol cm$^{-3}$) to support maximum respiratory requirements; $C_e$ = oxygen concentration within perivitelline fluid of the eggs ($3.34\times10^{-8}$ mol cm$^{-3}$).

5.2.7 Statistical analysis

To determine differences among populations in terms of membrane thickness, thickness quotient, porosity and permeability to oxygen, one-way ANOVA tests were performed. Readings for the permeability of individual eggs were then correlated with estimated temperatures from their...
native incubation conditions using Spearman’s rank correlation coefficient. Differences in all structural features were also compared among the control, dead and live groups at each mortality threshold using one-way ANOVA tests. When differences among populations or groups were found, Tukey’s post-hoc tests were performed to see where these differences occurred. Membrane thickness quotient and porosity values were presented in proportional format, so were square root arcsine transformed before statistical analysis.

Figure 5.2. Images of membrane structures used in analysis. (A) regular micropore arrangement on membrane internus at 2400x magnification; (B) membrane internus with lower left hand quadrant highlighted to demonstrated section used for measurement of micropore diameter; (C) membrane internus at 9000x magnification; (D) membrane cross-section traversed by pore canals at 1700x magnification; (E) membrane cross-section with thickness dimensions; (F) image of the membrane externus where the adhesive film is present at 900x magnification. An area where the film is missing, revealing the membrane itself can be observed in the centre of the image.
5.3 Results

5.3.1 Population variability of egg architecture

Differences among populations were observed for egg diameter ($F_{4, 176} = 30.14$, $p<0.001$), membrane thickness ($F_{4, 176} = 4.89$, $p<0.001$; Fig. 5.3A), porosity ($F_{4, 176} = 4.046$, $p<0.001$; Fig. 5.3B) and permeability ($F_{4, 176} = 11.941$, $p<0.001$; Fig. 5.3C), but not membrane quotient (Fig. 5.3A). River Tilt eggs had a smaller diameter ($5.91 \pm 0.24$) than all other populations ($p<0.001$) and River Dochart eggs ($6.53 \pm 0.20$mm) were larger than the farmed ($6.36 \pm 0.10$mm, $p=0.034$), River South Tyne ($6.27 \pm 0.33$mm, $p<0.001$) and River North Tyne ($6.24 \pm 0.44$mm, $p<0.001$) populations. The River Tilt membranes were thinner than the River Dochart ($p=0.002$) and River North Tyne ($p=0.045$) eggs. The River South Tyne (0.029) and River North Tyne (0.029) membranes were more porous than the River Dochart (0.021) and Farmed (0.023) eggs (all comparisons: $p<0.001$). The membranes of the River Dochart eggs were less permeable than the River Tilt ($p=0.001$), River South Tyne ($p<0.001$) and River North Tyne ($p<0.001$) populations. In addition, eggs of the River South Tyne fish were more permeable than those of the Farmed ($p<0.001$).

5.3.2 Oxygen requirements to support respiration

There was considerable variability among the studied populations in terms of oxygen requirements as a result of differences in membrane architecture (Fig. 5.4). River South Tyne and River North Tyne eggs had lower oxygen or intragravel velocity requirements than the River Tilt, River Dochart and Farmed eggs. The relative requirements of the River Dochart and River Tilt eggs varied depending on whether oxygen or intragravel velocity was limiting. At high intragravel velocity and low oxygen content, River Dochart eggs had higher requirements than the River Tilt eggs. However, the reverse is true at low intragravel velocity but high oxygen concentrations.

Previous estimates of oxygen supply thresholds calculated using the mass transfer model relied on membrane porosity (0.1) and thickness (35μm) values estimated from Chinook salmon and brown trout, respectively. While thickness data were similar (Fig. 5.3A), porosity values presented here were only 20-30% of the estimates for Chinook salmon. This led to far greater oxygen requirements than previous estimates using the mass transfer model (Fig. 5.4).

5.3.3 Incubation temperature

There was a positive relationship between membrane permeability and the mean incubation temperature experienced by each population ($R^2 = 0.468$, $N=176$, $p<0.001$). The exception
to this trend was for the River Tilt population, which came from the coolest incubation zone but had the third highest recorded membrane permeability.

**Figure 5.3.** Structural features of test eggs (A: membrane thickness and membrane quotient; B: membrane porosity; C: oxygen permeability). Data on the left of the solid line represents mean values to enable comparisons of population variability of egg architecture. Data on the right of the
solid line represents mean values for each group during testing for the effect of egg structure on hypoxic tolerance. Dashed lines separate data for each mortality threshold. Error bars indicate standard deviation.

Figure 5.4. Range of intragravel velocities and oxygen concentrations necessary to support respiratory requirements at 5°C for Atlantic salmon eggs of the five populations investigated in the present study. Mass transfer curve represents estimate of oxygen requirements based on egg structure data presented in Wickett (1975) using diameter 0.6mm, membrane thickness 35μm and porosity 0.1.
5.3.4 Egg structure and hypoxic tolerance

Comparisons of egg diameter (mean: 6.42 ± 0.12mm), membrane thickness or membrane quotient at each mortality threshold revealed no differences among groups (Fig. 5.3A). However, there were differences in membrane porosity among groups at the 5% ($F_{2,97} = 43.54$, $p<0.001$) and 20% ($F_{2,102} = 48.77$, $p<0.001$; Fig. 5.3B), but not the 50% mortality thresholds. At the 5% and 20% thresholds, the membranes of the eggs that died in the hypoxic group were less porous than the control and those that survived hypoxic conditions (all comparisons: $p<0.001$). Membrane permeability to oxygen also differed among groups at the 5% ($F_{2,97} = 30.913$, $p<0.001$; Fig. 5.3C) and 20% mortality thresholds ($F_{2,102} = 36.15$, $p<0.001$; Fig. 5.3B), but not the 50% threshold. The membranes of the eggs that died under the hypoxic conditions at the 5% and 20% mortality threshold were less permeable than the control group and those that survived (all comparisons: $p<0.001$).

5.4 Discussion

Oxygen stress during embryonic development is a critical driver for adaptive responses of populations in a range of species (e.g. Childress & Seibel 1998; Yi et al. 2010). In this study the permeability of Atlantic salmon eggs to oxygen varied among populations and positively
correlated with estimates of oxygen demand for their natal Incubation zones. In addition, this study provides convincing evidence that these structural differences affect hypoxic sensitivity.

5.4.1 Population variability of egg architecture

Egg size varied substantially among populations and could influence embryonic sensitivity to oxygen stress. Larger eggs, such as those of the River Dochart, are traditionally assumed to give rise to larger alevin with a greater chance of post-hatch survival (Einum & Fleming 1999). While oxygen demand positively correlates with diameter (Krogh 1959), it is not proportional to egg volume (Einum et al. 2002). Indeed, the greater diffusive surface area of larger eggs outweighs the concurrent increase of oxygen demand, and implies that larger eggs are preferential in oxygen stressed environments (Einum et al. 2002). In addition, advective flow to cells increases with diameter (Pahlow et al. 1997), so oxygen supply from the environment will be greater for larger eggs. Despite the benefits of larger eggs in terms of embryonic survival, physiological costs for the maternal fish limit continuous evolution of greater egg size (Einum & Fleming 2000; Kindsvater et al. 2016).

Although differences in membrane thickness among populations were present, membrane quotient data revealed they were primarily a function of egg size. Nevertheless, thicker membranes, such as those of the River Dochart population, reduce permeability by increasing the diffusion distance from the boundary layer to the embryo (Daykin 1965). The mechanical strength of eggs in a range of species increases with membrane thickness (Wolpert 1966; Stehy & Hawkes 1979; De Ketelaere et al. 2002). Therefore, in some instances, membrane thickness could represent a trade-off between the risks of hypoxia and mechanical shock. However, for the populations studied here, the primary determinant of membrane thickness appears to be egg size.

Inter-population variability of membrane porosity drove differences in permeability to oxygen. Greater diffusive area enhances oxygen supply to the embryo from the boundary layer and could be beneficial where oxygen supply is limited or demand is greater. While population diversity in teleost membrane permeability has not been described before, such variability in avian eggs is frequently observed (e.g. Rahn et al. 1977). Indeed, Peruvian coot eggs at higher altitudes, and therefore lower partial pressures, are more permeable to enhance oxygen uptake (Léon-Velarde et al. 1997). The ability of Atlantic salmon eggs to show similar forms of adaptation could enhance survival and suggests the River South Tyne eggs will be most tolerant to oxygen stress.
5.4.2 Oxygen requirements to support respiration

The intragravel velocity and oxygen concentration required to support respiration at consistent temperatures varied among populations, and generally corresponded with values of permeability. However, the relative importance of permeability depends on which factor limits oxygen supply. This is particularly evident when comparing the River Tilt and River Dochart eggs. At high intragravel velocities, River Tilt eggs had lower oxygen requirements than the River Dochart eggs as a result of their greater permeability. However, this was reversed at low intragravel velocities. This is because the greater advective flow to the larger River Dochart eggs (Pahlow et al. 1997) becomes more important at lower velocities. This suggests that, if structural adaptations to low oxygen are present among populations, their nature could vary depending on whether concentration or intragravel velocity limits oxygen supply.

Previous applications of the mass transfer model for Atlantic salmon have relied on membrane porosity estimates that, according to data presented here, were substantially overestimated, leading to underestimation of embryonic oxygen requirements. Wickett (1975) derived a porosity value of 0.1 from a visual estimate of SEM images of a chum salmon egg membrane. This value has subsequently been used in a range of studies that estimated Atlantic salmon embryo survival based on physical conditions within the redd (e.g. Carling 1985; Greig 2004; Sear et al. 2014). By using high-resolution imagery and accurate measurement apparatus, this study demonstrates that this value was overestimated by 71-79%. Therefore, it is suggested that estimates of oxygen supply to salmonid embryos be re-adjusted to represent these considerably lower values. In addition, the importance of inter-population variability should also be considered in future oxygen supply estimates.

5.4.3 Incubation temperature

Greater permeability of eggs from warmer locations could enhance embryonic survival by increasing the rate of solute exchange across the membrane. The higher embryonic metabolic rate at higher temperatures drives greater oxygen demand (Hamor & Garside 1976) that could be balanced by greater permeability. Previously, reduced solubility in warmer conditions led to the assumption that temperature could limit oxygen supply (e.g. Chapelle and Peck 1999; Allen and Castillo 2007). However, recent data imply that greater diffusion rates at higher temperatures compensate for reduced solubility, thereby mitigating the effect (Verberk et al. 2011). Therefore, oxygen stress at higher temperatures is primarily driven by greater oxygen demand, as opposed to reduced supply (Verberk et al. 2011). Greater metabolic rates are also associated with more rapid production of metabolites such as ammonia and carbon dioxide, which can have deleterious
impacts on embryonic survival in high concentrations (Solbe & Shurben 1989; Dhiyebi et al. 2013). Consequently, greater membrane permeability increases the excretion rate of these products and could enhance survival in warmer locations, even where oxygen supply is not limiting.

Theoretically, more permeable membranes could enhance embryonic survival in higher temperatures, but further work is required to demonstrate a causal link between the two variables. Indeed, a range of other factors could also drive selection for greater membrane permeability. For example, GW upwelling can reduce oxygen concentration in Atlantic salmon redds (Malcolm et al. 2010). Furthermore, fine sediment deposition could reduce intragravel velocity, thereby limiting oxygen supply (Greig et al. 2007b) and leading to the build of waste metabolites (Kemp et al. 2011). However, physical determinants of temperature such as spawning latitude and altitude show greater inter-annual consistency among populations than factors such as GW upwelling (Soulsby et al. 2009) or sediment deposition (Owens et al. 1999). Adaptations are more likely to develop when exposed to consistent stressors (Lexer & Fay 2005), so temperature could be a crucial driver of population differentiation of membrane permeability.

5.4.4 Egg structure and hypoxic tolerance

Eggs that survived hypoxia were more permeable than those that died, which implies that membrane architecture is an important determinant of embryonic sensitivity to hypoxia. While previous studies have shown that larger eggs could be better able to survive periods of oxygen stress due to their greater diffusive surface area (Einum et al. 2002), no differences in egg size among groups were present here. In this study, differences in diffusive surface area were primarily driven by membrane porosity as opposed to egg size. Indeed, for all sample groups combined the standard deviation of egg size (4.6%) was substantially lower than that of membrane permeability (24%). This suggests that variability in total diffusive surface area was primarily driven by membrane permeability, and not egg size. If egg size showed greater variability, it could play a stronger role in determining differences in hypoxic sensitivity.

This study provides evidence that membrane permeability influences the sensitivity of incubating Atlantic salmon embryos to hypoxia. This supports the conclusion that inter-population differences in membrane permeability will lead to differing requirements of oxygen supply during incubation. Further, the greater permeability of eggs from warmer environments means they are more likely to survive periods of reduced oxygen supply or greater demand.
5.4.5 Management implications

This study indicates that management techniques aimed at directly supplementing declining stocks through translocation and supportive breeding could have negative implications for long-term population strength. Translocation involves the movement of individuals from one part of their range, usually with a strong population, to another, where numbers are weaker (IUCN 1998). When native individuals breed with non-natives genetic diversity is diluted and can result in the loss of phenotypic differentiation among populations (Eldridge & Naish 2007; Le Cam et al. 2015). With respect to the present study, this could alter egg membrane permeability of distinct populations and influence the survival of embryos exposed to oxygen stress. While supportive breeding does not have the same direct genetic influence as translocation, it reduces selective pressure on the egg stage of the life-cycle. Although low mortality in the hatchery environment means that selection-driven loss of low oxygen adaptation is unlikely (Reisenbichler & Rubin 1999), the process can lead to loss of genetic variability and phenotypes beneficial to the natural habitat (Heath et al. 2003; McClure et al. 2008). Hatchery environments in particular are likely to provide a continuous supply of well-oxygenated water, so differences in membrane permeability are less likely to affect survival. This study adds to the growing body of evidence that supplementary breeding techniques could have long-term negative consequences for salmonid populations (e.g. McClure et al. 2008), so should be used with caution. While strategies such as outplanting of green or eyed-eggs could mitigate this effect, the long-term goal should be incubation zone restoration (Merz et al. 2004) and removal of barriers to fish migration to open up new areas for colonisation (Katopodis 2005).

Climate change induced warming could lead to population straying, which, in a similar way to translocation could dilute the genetic integrity of salmonid populations. While populations naturally stray into non-native rivers to spawn (Jonsson et al. 2003), there is preliminary evidence that the frequency with which this occurs has increased in response to rising water temperatures (Valiente et al. 2010). Data presented here shows that eggs from neighbouring tributaries can vary substantially in terms of structure. If this variation has an adaptive basis, increased population straying and breeding with non-native individuals could lead to genetic dilution, loss of favourable traits and population decline.

5.4.6 Conclusions

For the first time, this study has demonstrated variation among Atlantic salmon populations in terms of membrane permeability. The data presented does not give conclusive proof that this variability is adaptive to oxygen availability. However, it provides convincing evidence that the
most permeable eggs will be best equipped to tolerate external oxygen stress, and have the lowest oxygen delivery requirements. Data on the incubation conditions experienced by the studied populations would enhance these findings substantially.

At the least, these findings demonstrate differentiation among populations in the egg-stage that has not been described before. However, if this differentiation is adaptive, management strategies such as supplementary breeding should be adopted accordingly.
Chapter 6: Conclusions

While brief conclusions were presented in chapters 3 to 5, this section directly addresses the aims and objectives outlined in chapter 1. Furthermore, where relevant, additional findings and considerations that emerged throughout the research are outlined and potential avenues for future research are discussed.

6.1 Aim one: Quantify seasonal patterns of hyporheic exchange at a range of spawning sites, to show how these affect oxygen levels in the Atlantic salmon incubation zone and consider the potential effects on incubation success.

6.1.1 Objective one: Using wavelet analysis to detect seasonal variation in the interactions between river discharge and hyporheic dissolved oxygen content

Covariation between river discharge and hyporheic dissolved oxygen concentration was frequently observed and represented periods of GW – SW connectivity. The level of connectivity varied and was highest in winter and early spring where prolonged rainfall drove water table elevation, leading to greater GW influence in the hyporheic zone. During periods of high connectivity, a hysteretic relationship between river discharge and hyporheic dissolved oxygen was observed, where episodic periods of oxygen depletion followed high flow events. In some cases, continuous rainfall and water table elevation further increased the influence of GW in the hyporheic zone, leading to prolonged low oxygen conditions. The effect of water table elevation on hyporheic exchange demonstrates the importance of antecedent weather conditions on water quality in the salmon incubation zone. This implies that predictions of greater winter rainfall and water table elevation due to climate change could influence the Atlantic salmon incubation zone.

6.1.2 Objective two: Examine data taken throughout Atlantic salmon incubation to determine the effect of deoxygenated groundwater upwelling on the oxygen regimes that embryos experience and its effect on their survival

Frequent periods of oxygen depletion demonstrated that GW upwelling influences water quality in the incubation zone of Atlantic salmon rivers throughout the UK. Indeed, two thirds of studies exhibited intense oxygen sags (<20% saturation) due to GW upwelling, which could severely limit incubation success. Furthermore, GW-induced oxygen sags generally increased in intensity and
duration towards the end of embryonic development. As this period coincides with the timing of greatest embryonic oxygen demand, it could have the most significant impacts on survival.

### 6.1.3 Additional findings: Consequences of groundwater upwelling for models of oxygen supply

One of the most important methods for determining Atlantic salmon incubation quality is the sediment intrusion and dissolved oxygen (SIDO) model, which has been widely implemented for UK (e.g. Pattison et al. 2014; Sear et al. 2014) and North American (e.g. Alonso et al. 1996) salmonid populations. This is a deterministic model that estimates oxygen supply to salmonid eggs based on intragavel velocity, river oxygen concentration and sediment oxygen demand. Consequently, it does not consider the effects of GW upwelling on oxygen supply. Comparison of SIDO modelled data with observed field data demonstrates that, by not considering GW influence, the model can overestimate oxygen supply and embryonic survival (Sear et al. 2014). Furthermore, reduced oxygen content as a result of GW upwelling was a superior predictor of embryonic survival than reduced intragavel velocity due to fine sediment infiltration in some locations (Malcolm et al. 2011). GW upwelling influenced hyporheic dissolved oxygen concentrations at all studied sites, so the SIDO model could frequently overestimate habitat quality. As a result, and in agreement with Malcolm et al. (2011), it is recommended that, where possible, direct measurements of dissolved oxygen concentrations in salmonid reds be used as preferential indicators of incubation quality.

### 6.1.4 Future research

Convincing evidence is presented that deoxygenated GW upwelling is a major determinant of Atlantic salmon incubation quality. However, the extent of this problem varies among locations, seasons and years. As a result, longer-term monitoring at a range of sites is necessary in order to fully characterise seasonal and annual variation in hyporheic exchange patterns. Indeed, monitoring studies lasting for a minimum of five years have been recommended as essential to capture the effect of variable hydrological regimes on hyporheic exchange patterns (Hrachowitz et al. 2009; Soulsby et al. 2009). Knowledge regarding the effect of different hydrological patterns on the prevalence of GW in salmon reds is particularly important due to climate change-driven effects on precipitation and temperature patterns. Enhanced knowledge on this subject could aid fisheries management through predictions of incubation conditions and their effect on embryonic survival and population strength. Furthermore, greater knowledge on features that influence
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Hyporheic exchange could be incorporated into models such as SIDO to provide improved techniques for modelling incubation success.

6.2 Aim two: Identify the effect of episodic periods of low oxygen, such as that caused by deoxygenated groundwater upwelling, on Atlantic salmon incubation success

This study demonstrated that episodic periods of low oxygen, similar to those induced by GW upwelling, reduce the survival and post-hatch fitness of Atlantic salmon embryos. These effects have the potential to influence the long-term strength of individuals and populations (Fig. 6.1), so GW should be considered as an important determinant of incubation quality.
Figure 6.1. Flow chart describing key findings of chapter 5 and potential effects of deoxygenated groundwater upwelling on Atlantic salmon incubation success. Numbers in brackets indicate relevant section where findings are presented.

6.2.1 **Objective one:** To determine the survival of Atlantic salmon eggs exposed to episodic periods of low oxygen at different periods of their development.

The mortality rate of Atlantic salmon eggs exposed to episodic periods of low oxygen was greatest at the latest stages of development due to higher embryonic oxygen demand associated with greater body mass. While mortality rates were greater when embryos experienced low oxygen in the later stages of development, they were still substantially lower than values observed in the field. This is probably related to the greater intensity and duration of oxygen GW-induced oxygen sags recorded in the field (chapter 4) compared to those investigated here, or additional stressors such as fine sediment input.
Objective two: To determine how hatch timing of Atlantic salmon varies when exposed to episodic periods of low oxygen at different stages of development.

Low oxygen during incubation resulted in a hatch-timing shift of Atlantic salmon embryos, the direction of which was determined by the developmental stage at which they experienced hypoxia. Low oxygen at earlier developmental stages resulted in delayed hatch, which was representative of reduced oxygen consumption and metabolic suppression during exposure. However, hypoxia at the latest stages of development triggered premature hatch, which mobilised the embryo, enabling it to escape the zone of hypoxia. This multi-directional hatch-timing shift demonstrates that the response of the embryo to hypoxia is determined by its physiological state. While the response of the embryo to hypoxia during incubation increases the likelihood that it will survive until hatch, altered hatch timing could increase the risk of mortality in later life-stages.

Objective three: To determine how the post-hatch fitness of Atlantic salmon varies when exposed to episodic periods of low oxygen at different stages of development.

Individuals that experienced episodic low oxygen were smaller and underdeveloped and likely to have a reduced swimming ability. Alevin hatched in an underdeveloped state because of inefficient anaerobic metabolism that take place during exposure to hypoxia. Observations of underdevelopment due to hypoxia were exacerbated in the alevin exposed to hypoxia at the latest stages of development because they hatched prematurely. These effects could result in greater susceptibility to predation and reduced competitiveness with conspecifics. Furthermore, developmental lag could result in late entry to the fry stage and inability to obtain favourable habitats.

Future research: Effects of different levels of hypoxic exposure

The data presented here demonstrates the effect of a particular sequence of episodic low oxygen events on Atlantic salmon embryo survival. However, variable hyporheic exchange patterns mean exposure to GW-induced hypoxia is likely to vary in timing, duration and intensity annually. This provides a wide scope for future investigation. Currently, there is relatively little work on the effect of exposure duration on salmonid survival. Periods of GW dominance vary substantially in terms of duration, so this is something that would particularly benefit from future research. Other features to consider include the duration of recovery between periods of oxygen depletion or the effect of more intense periods of chronic oxygen depletion than 60% saturation tested here.
6.2.5 Future research: Synergistic effects of other stressors

Hypoxia is a well-studied incubation stressor, however a range of other factors can limit embryonic survival and are frequently present in Atlantic salmon redds. Of particular relevance to this study, would be the combined effects of low oxygen with other potential stressors frequently found in GW such as high nitrate and ammonium content. Furthermore, the warmer temperatures associated with GW dominated regions of the riverbed could increase embryonic oxygen demand and, therefore, sensitivity to low oxygen conditions. Eggs spawning in natural conditions are unlikely to experience a single stressor in isolation throughout their incubation, so there is a clear need to test how these stressors interact.

6.2.6 Future research: Long-term survival

The sublethal effects observed in this study have been shown to persist to the point of emergence (e.g. Roussel 2007), but the extent to which they affect later life-stages is currently unknown. Early life-experiences such as occupation of territory in the fry stage can influence access to food, growth rates and migration timing (Brännäs 1995). As underdeveloped and late-emerging fry are less likely to obtain favourable territories (Cutts et al. 1999), the impacts of poor oxygen supply in the incubation phase could persist into later life-stages and affect life-history patterns such as the timing of migration. However, evidence from other teleosts suggests they could exhibit periods of compensatory growth after hatching to offset the developmental lag triggered by low oxygen supply during incubation such as that seen in other teleosts (Foss & Imsland 2002). Therefore, an experimental design that enables individuals to be tracked to the point of migration, or even beyond, would enhance understanding of the long-term impacts of low oxygen supply during incubation.

6.3 Aim three: Identify variation in the egg structure of Atlantic salmon eggs and whether this corresponds to differences in tolerance to low oxygen.

6.3.1 Objective one: To determine differences in egg membrane structure among populations of Atlantic salmon, with a particular focus on permeability to oxygen

Substantial differences among populations in terms of egg size, membrane thickness and membrane porosity and permeability were observed. Variation among distinct populations
implies a genetic basis for membrane structural features, which could have important management implications.

6.3.2 Objective two: To determine oxygen requirements of Atlantic salmon eggs to meet their oxygen requirements based on population differences of membrane structure

The structural features described in objective one gave rise to differences among populations in terms of oxygen supply requirements based on the mass transfer theory. The River South Tyne and River North Tyne eggs were most permeable to oxygen, so had the lowest requirements in terms of oxygen delivery rates. However, permeability is not the sole determinant of oxygen supply requirements. Indeed, the larger eggs of the River Dochart population had lower oxygen requirements than the more permeable River Tilt eggs at low intragravel velocities. This is because larger eggs induce greater advective flow and their egg packets have greater permeability, so compensated somewhat for slow flow rates. This implies that, should structural adaptation of eggs to hypoxia exist, its nature could vary depending on whether intragravel velocity or oxygen concentration limits supply.

Updated values of membrane structure presented in chapter 6 demonstrate that previous values used in the application of the mass transfer theory were inaccurate. In particular, membrane porosity was substantially overestimated. This meant that previous applications of the mass transfer theory could have underestimated oxygen supply requirements.

6.3.3 Objective three: To determine whether membrane structural differences are linked to incubation conditions of the natural spawning conditions of each population

There was a significant correlation between membrane permeability and typical incubation temperatures of each population. As temperature drives oxygen demand, greater permeability in warmer locations could be beneficial as it enhances oxygen uptake and could compensate for increased demand. Furthermore, high permeability enhances the excretion of waste metabolites, the production of which increases with temperature. So, even where oxygen is not limiting, greater membrane permeability could be beneficial in warmer locations.
6.3.4 Objective four: To determine whether visible differences in membrane structure correlate with differences in the sensitivity of eggs to low oxygen levels.

Eggs that survived low oxygen conditions had significantly greater permeability than the eggs that died. This provides convincing evidence that egg membrane structure plays an important role in the sensitivity of embryos to hypoxia. Furthermore, it suggests that River South Tyne and River North Tyne eggs will be more tolerant to low oxygen supply or periods of greater demand than those from the farm or River Tilt and River Dochart.

6.3.5 Additional findings: Implications for sediment-induced oxygen stress

Physical blocking of micropore canals in the egg membrane by clay particles has been cited as a reason for sediment-induced oxygen depletion and mortality of Atlantic salmon embryos (Greig et al. 2005b). This was based on previous estimates taken from chum salmon that the diameter of micropores is 500 to 1500nm (Bell et al. 1969). However, data presented here demonstrates a mean micropore diameter of 267nm (range 182 – 357nm). While fine sediments do occur within this size fraction, this represents a substantially smaller proportion of clay particles than previously estimated. Consequently, this mode of sediment-induced hypoxia could have been overestimated and requires further investigation.

6.3.6 Future research: Identification of adaptation

The structural variation of egg membranes among populations and its effect on embryonic survival identified in chapter 6 opens a new avenue for research. Perhaps of most pressing need is to conclusively identify whether structural differences among populations represents natural variation, or adaptation to incubation conditions. Knowledge could be enhanced through:

1. Collection of data on the spawning conditions of tested populations, particularly with regards to rates of oxygen supply. This would enhance understanding of links between membrane structure and incubation conditions;
2. Common garden experiments to support the theory that structural variation among populations drives hypoxic tolerance. This would involve simultaneously rearing eggs of each population in an identical hypoxic environment and measuring rates of survival and post-hatch fitness, using similar methods described by Côte et al. (2012).
Chapter 6

6.3.7 Effect of supportive breeding on egg structure

Supportive breeding as a cause of genetic dilution and loss of adaptive traits requires further examination. Ideally this would involve testing egg membrane structure of a single population before and after the introduction of a supportive breeding programme. However, this is challenging because of an absence of data on egg architecture of Atlantic salmon throughout its range. Alternatively, testing of egg structure in a river system likely to begin a supportive breeding programme in the near future, followed by continuous sampling for a prolonged period after the introduction of the programme, using similar methodology described by Heath et al. (2003), could provide critical data. While this would require long-term commitment, it could enhance knowledge of fisheries management practices substantially.

Chapter 7: Synthesis of findings

This thesis is composed of three distinct results chapters that, in isolation, advance understanding of the causes and effects of oxygen stress in the Atlantic salmon incubation zone. Here, the
conclusions outlined in chapter six are synthesised to demonstrate how they form a single, coherent body of work.

Chapter three demonstrated that GW upwelling can cause episodic periods of hypoxia in the Atlantic salmon incubation zone, furthermore the frequency, duration and intensity of such hypoxia generally increased at the later stages of embryonic development. Chapter four showed that such periods of episodic hypoxia could reduce the survival and post-hatch fitness of Atlantic salmon embryos. In addition, embryos that experience hypoxia at the later stages of development were more likely to suffer mortality and severe reductions of post-hatch fitness. Therefore, the increasing intensity of deoxygenated GW upwelling events at the later stages of development could have particularly severe effect. Finally, the potential long-term effect of reduced incubation success as a result of insufficient oxygen supply driven by GW upwelling could be the development of increased egg membrane permeability that compensates for reduced oxygen supply by increasing oxygen uptake, as described in chapter five (Fig. 7.1).

In conclusion, this thesis demonstrates a cause of low oxygen supply in the incubation zone (GW upwelling, chapter 3) and potential short-term (reduced survival and post-hatch fitness, chapter 4) and long-term (adaptation of increased egg membrane permeability, chapter 5) effects of such stress (Fig. 7.1).

**Figure 7.1.** Flowchart showing the synthesised outcomes of each research chapter in the present thesis.
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