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

by

Jack Bloomer

Thesis for the degree of Doctor of Philosophy

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ABSTRACT

FACULTY OF SOCIAL AND HUMAN SCIENCES

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DEOXYGENATED GROUNDWATER UPWELLING IN THE ATLANTIC SALMON INCUBATION ZONE: IMPLICATIONS FOR EMBRYONIC SURVIVAL AND AN OPPORTUNITY FOR ADAPTATION

Jack Bloomer

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 Consequently, wavelet analysis was performed on a range of published and unpublished datasets that monitored oxygen concentration in the incubation zone of Atlantic salmon spawning rivers throughout the UK.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:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
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3. Where I have consulted the published work of others, this is always clearly attributed;
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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* a*l*. 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).

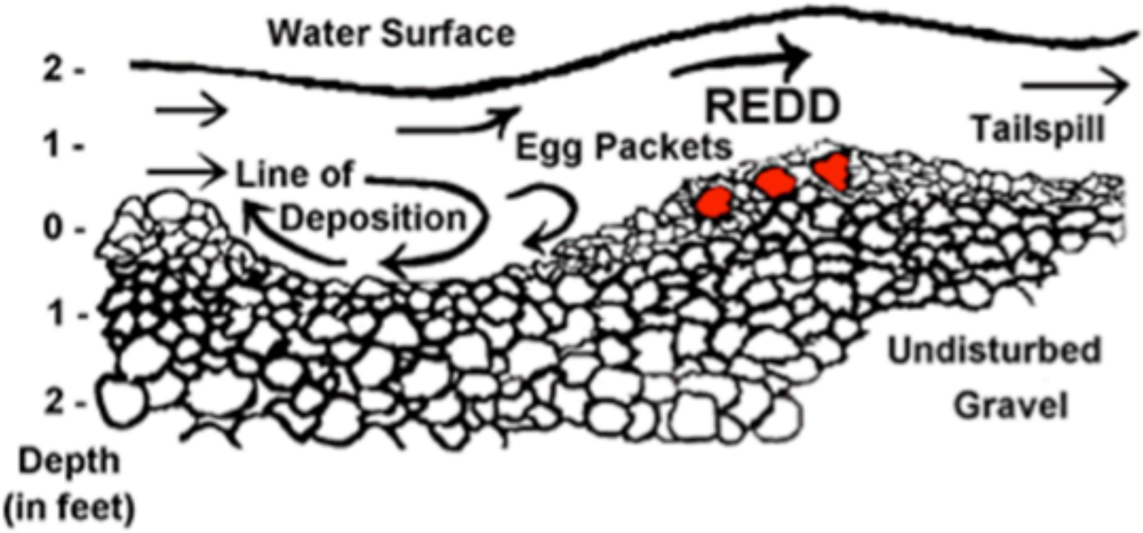

Figure 1.1. Diagram of a typical salmonid redd. Note numerous egg packets at the tail of the redd. Taken from Washington Department of Fish and Wildlife (2015).

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.

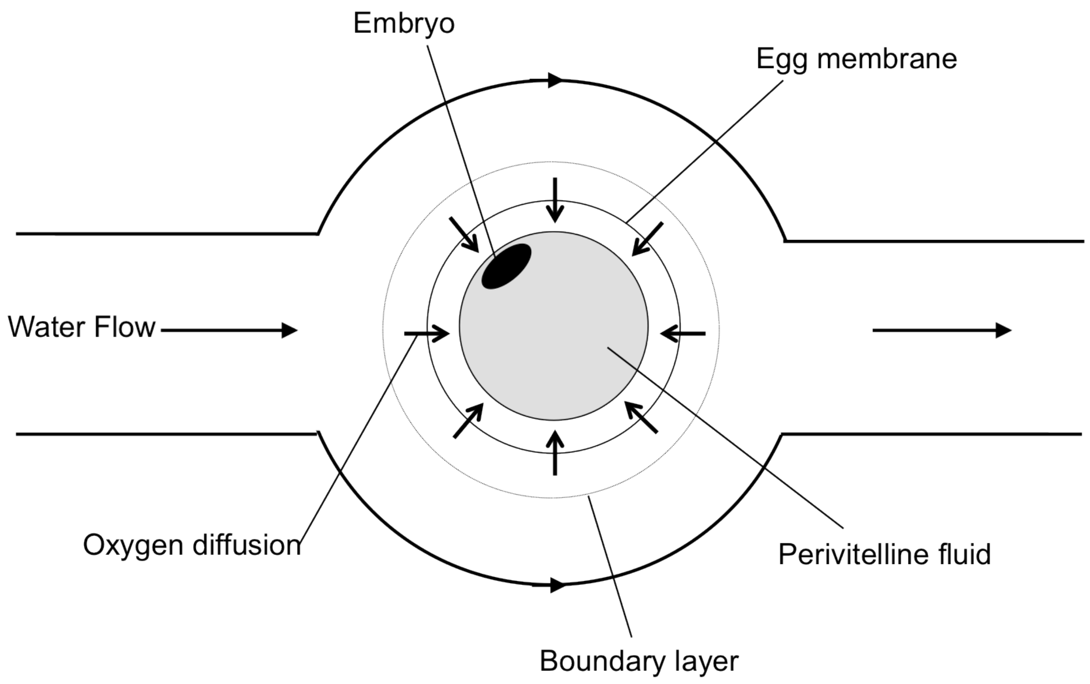

Figure 1.2. Diagram displaying oxygen supply to an individual salmonid egg. Note the boundary layer surrounding the egg. Oxygen supply is determined by the rate of flow and oxygen content of the water.

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 intragravel 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.

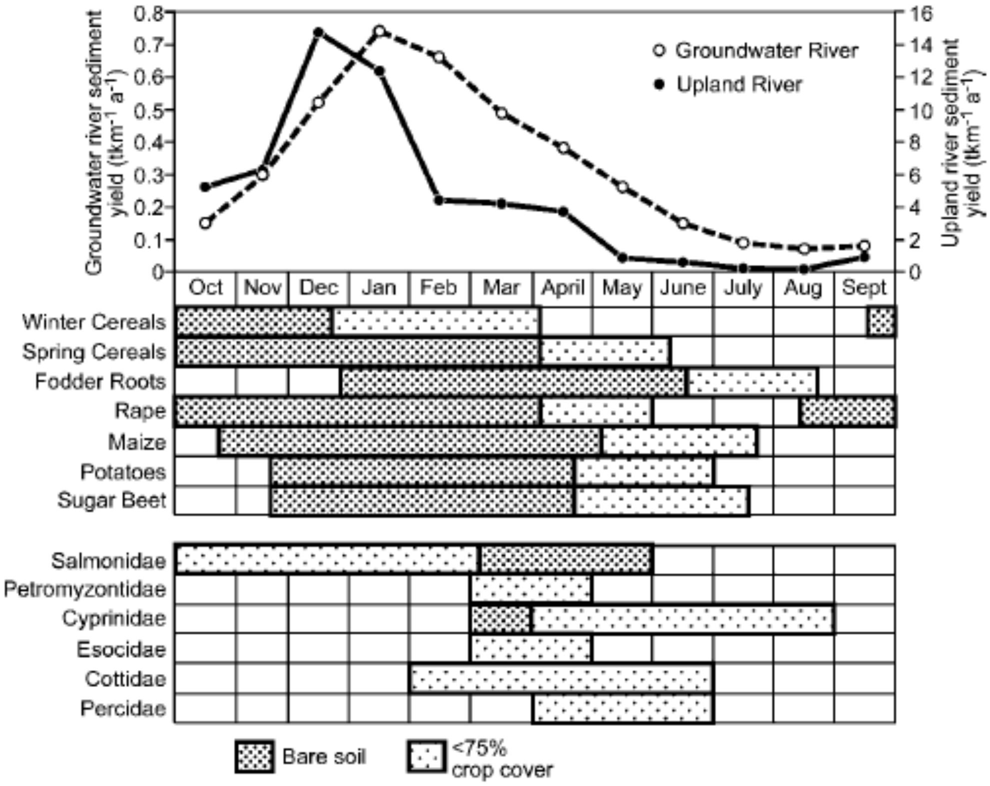

Figure 1.3. Representation of the annual peaks of sediment yield in UK rivers combined with common cropping practices and period where eggs (dotted bars) and hatched embryos (hashed bars) of various fish species are present in rivers. In the winter months, fine sediment load is highest because agricultural 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.

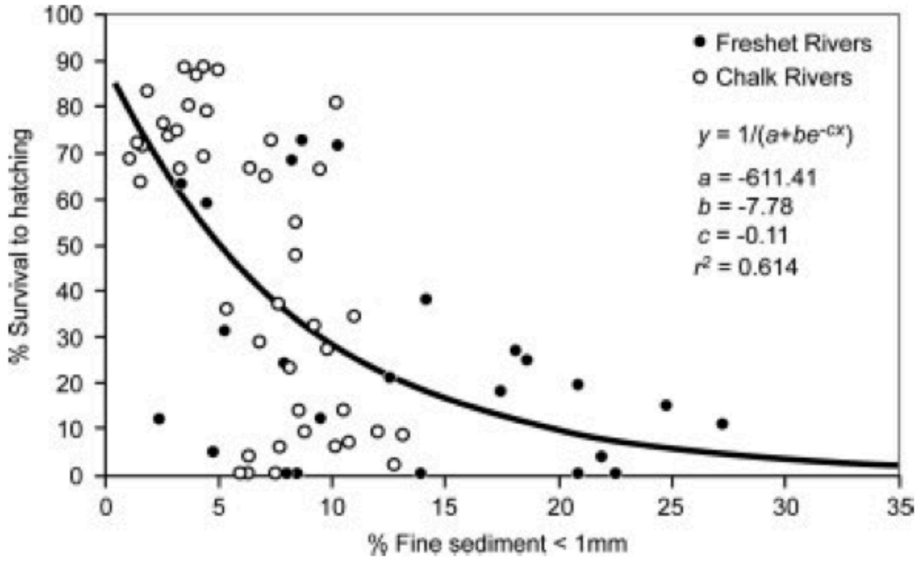

Figure 1.4. Relationship between percentage accumulation of fine sediments and percentage of Atlantic salmon embryos survival to hatch. Taken from Kemp *et al*. (2011) using data from Greig *et al*. (2007), O'Connor & Andrew (1998), Julian & Bergeron (2006).

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.

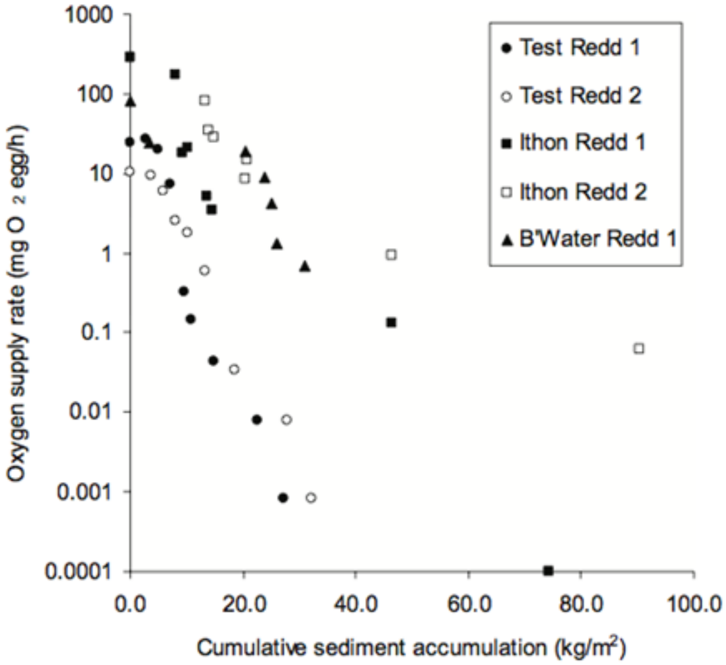

Figure 1.5. Relationship between sediment accumulation and oxygen supply rate at three locations in the UK. Figure taken from Sear *et al*. (2008), data from Greig (2004).

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 (Vaquer-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 redds.

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.

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

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*) redds 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 redds. 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

(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.

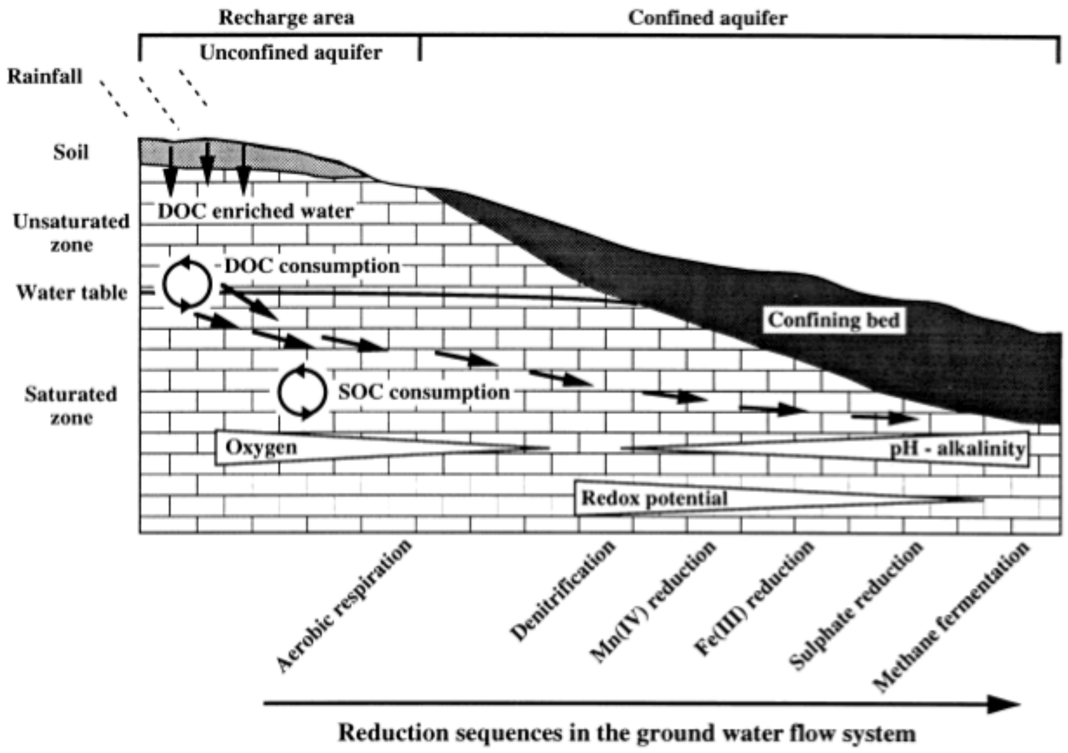

Figure 1.5. Summary of the main physiochemical changes and redox reactions taking place along the flow path of groundwater in a confined aquifer system. DOC = dissolved organic carbon; SOC = sedimentary organic carbon. Taken from Malard & Hervant (1999).

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).

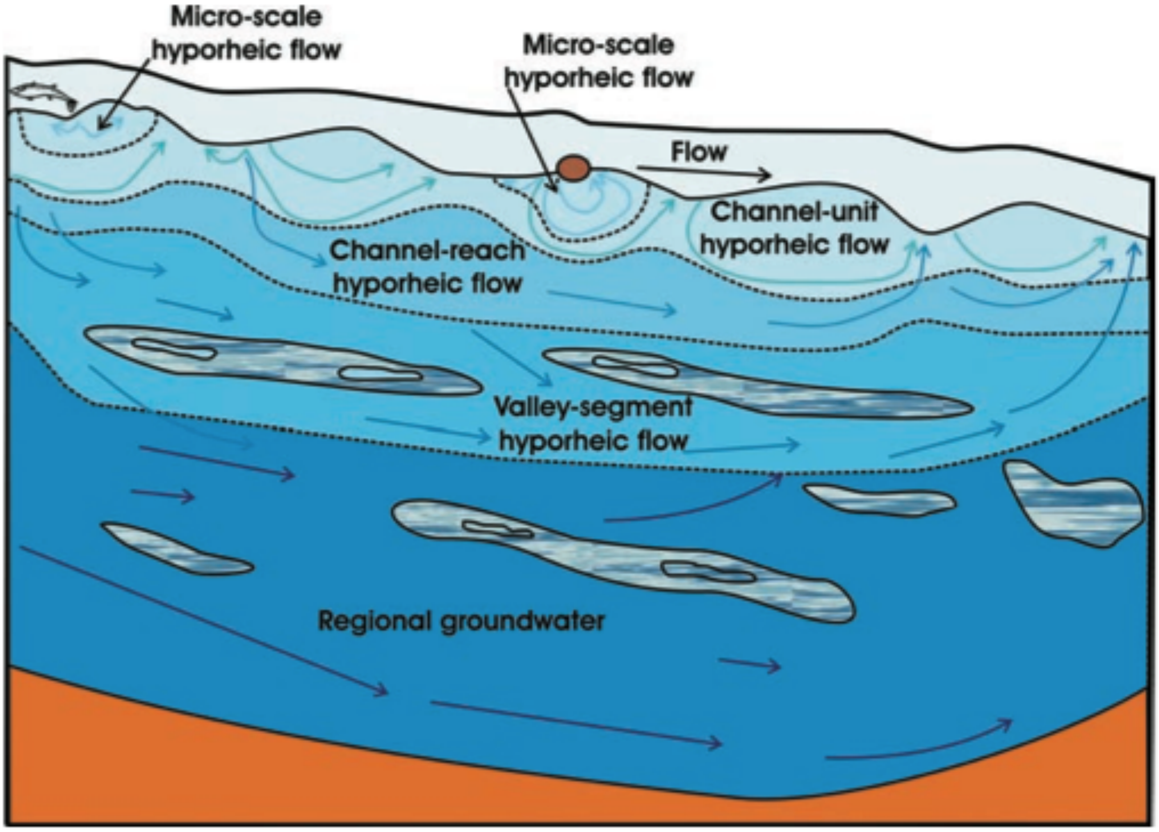

Figure 1.6. Nested diagram of factors influencing groundwater upwelling on a variety of scales. 1: Changes at the valley segment scale e.g. valley confinement, alluvial volume or underlying bedrock; 2: Reach scale exchange in response to changes in alluvial volume, between meander bends etc.; 3: Exchange 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 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 redds (e.g. Greig *et al.* 2007b; Schindler Wildhaber 2012, 2014), which can restrict hyporheic exchange and lead to GW-induced hypoxia in salmonid redds (Greig *et al*. 2007b; Malcolm *et al*. 2010).

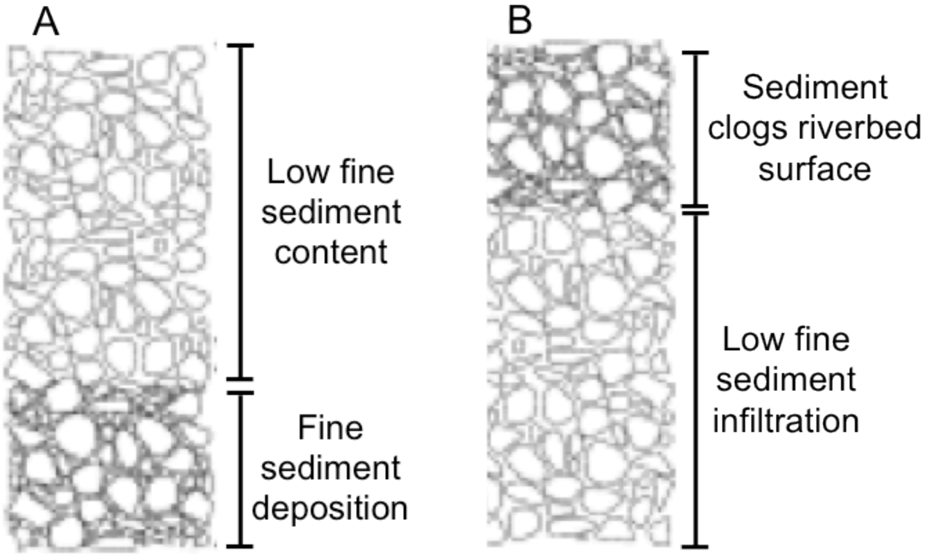

Figure 1.7. Effects of fine sediment deposition on hyporheic exchange. (a) Fine sediments filter through gravels and create an impervious layer that inhibits upwelling. (b) Formation of a sediment seal at the riverbed surface could limit surface water input, resulting in groundwater domination of the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

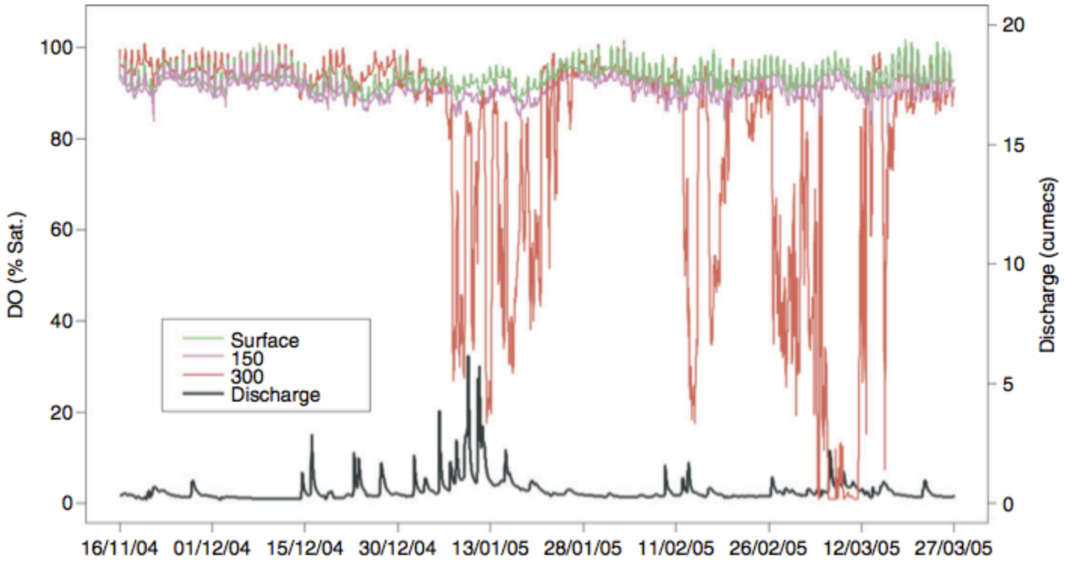

Figure 1.8. Dissolved oxygen concentrations of river water and at 150mm and 300mm depth in the riverbed compared with river discharge levels at the Girnock Burn, Scotland. From Malcolm *et al*. (2006).

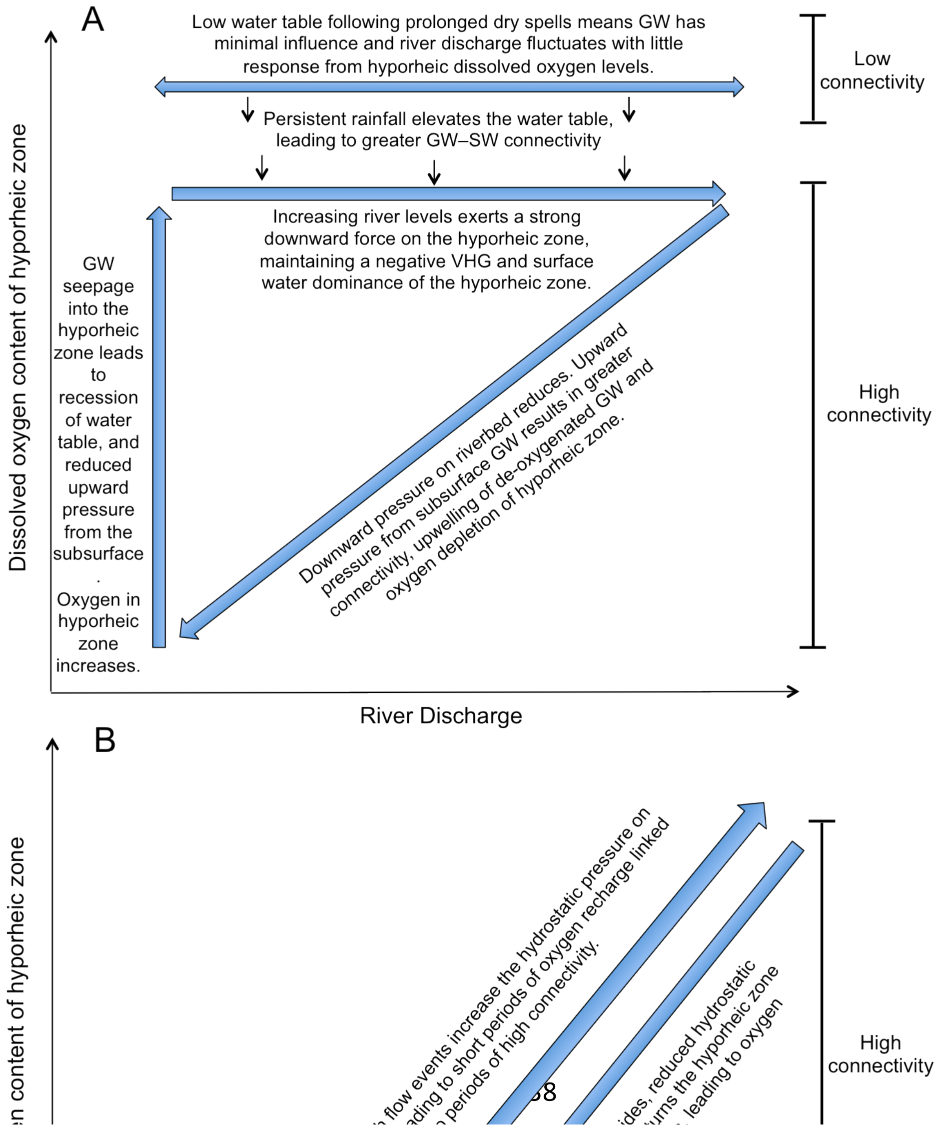

Figure 1.9. Conceptual diagrams of the interactions between river discharge and hyporheic dissolved oxygen concentration based on seasonal changes of groundwater – surface water connectivity. (A) shift from a surface water dominated system due to a low water table to periods of high groundwater – surface 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.

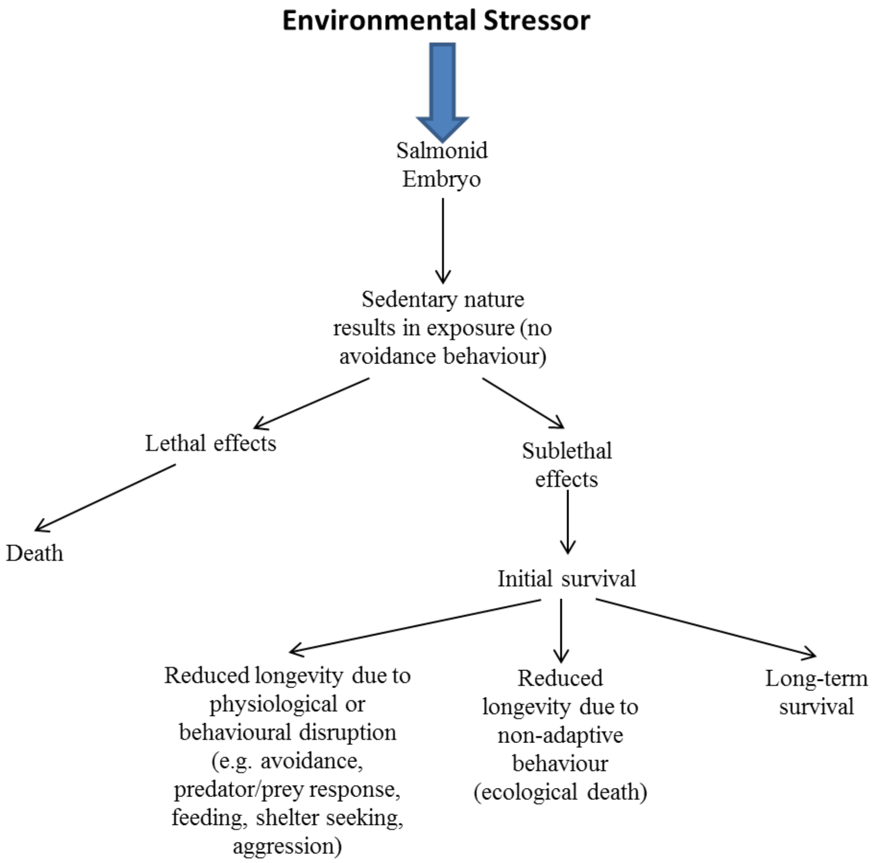

Figure 1.10. Flow chart describing salmonid juvenile response to an environmental stressor during incubation. Adapted from Schreck *et al*. (1997).

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).

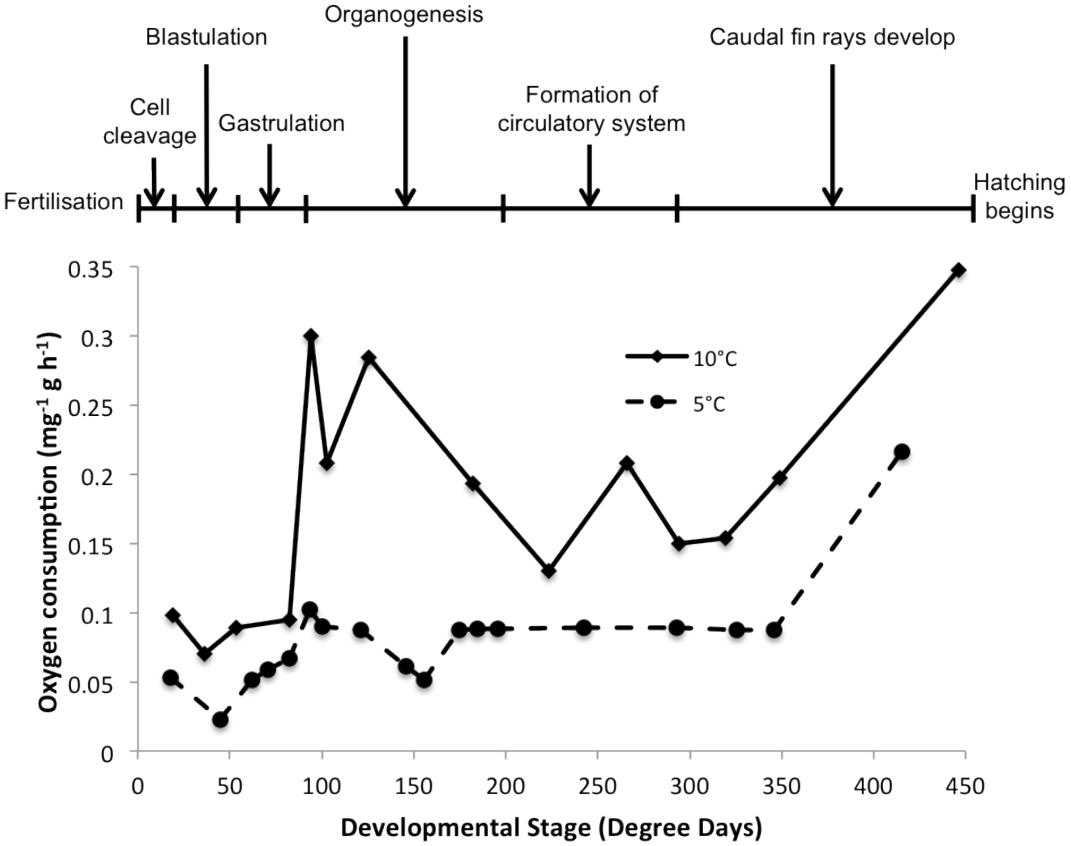

Figure 1.11. Recorded values of oxygen consumption for Atlantic salmon eggs at 5°C and 10°C by developmental stage using data sourced from Hamor & Garside (1976). Key stages of embryonic development are labelled above the graph using data presented in Gorodilov (1996).

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.

Developmental stage	Oxygen requirements ($mg\ O_2\ l^{-1}$)	Source
Eyed	3.1	Hayes *et al*. (1951)
Hatching	7.1	Hayes *et al.* (1951)
Eyed	5	Wicket *et al*. (1954)
Hatch	2.0 - 5.0	Davis (1975)
Throughout development	6.0	Elson (1975) from Stanley & Trial (1995)
Throughout development	5.0	Bjornn & Reiser (1991)
Throughout development	5.0	Gibson (1993)
Throughout development	>7.0	Crisp (1996, 2000)
Throughout development	2.0-8.0	Kondolf (2000)

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, charr (*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 (e.g. Alderdice *et al*. 1958; Harshbarger & Porter 1979; MacKenzie & Moring 1988; Rombough 1988). 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 & Ostaszewska 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

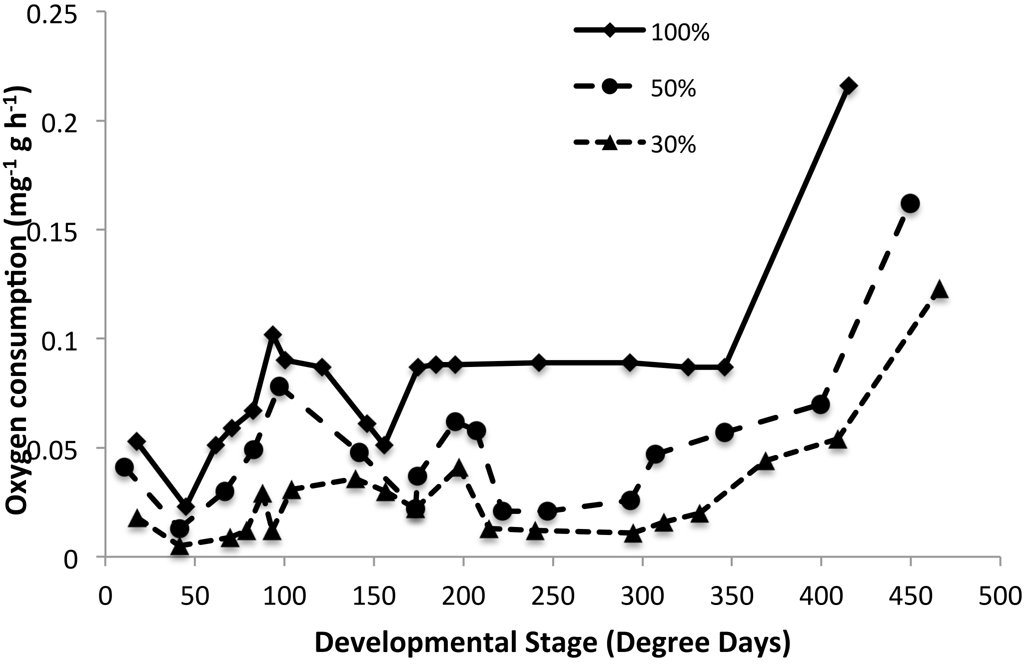

Figure 1.12. Recorded values of oxygen consumption for Atlantic salmon eggs at 5°C at 100%, 50% and 30% oxygen saturation values. Data taken from Hamor & Garside (1976).

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).

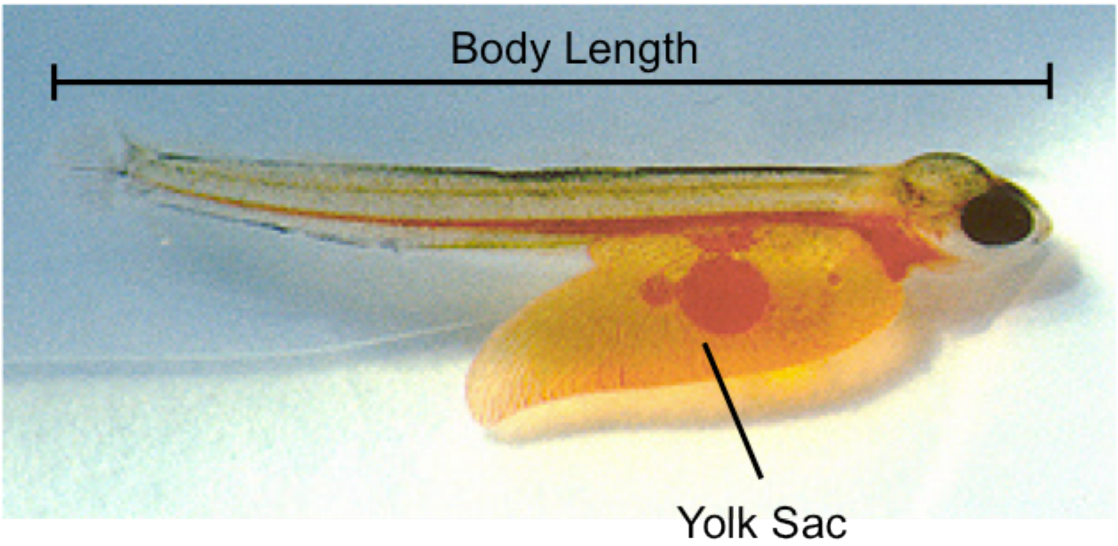

Figure 1.13. Atlantic salmon alevin with key biometric features labelled.

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 competiveness, 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.

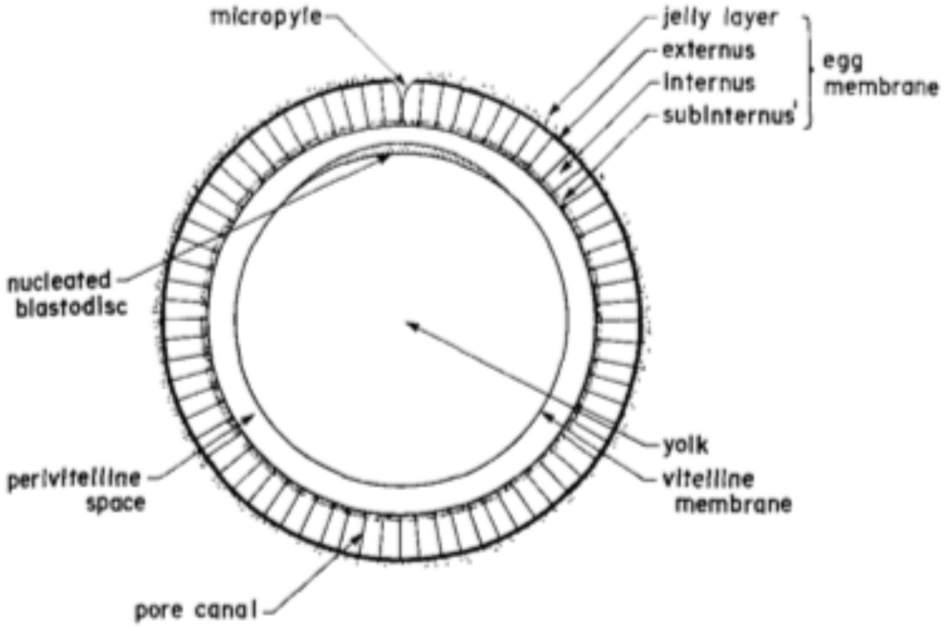

Figure 1.14. Simplified diagram of a fertilised and water-activated salmonid egg. All features, excluding the subinternus, have been observed in all salmonid species. Features are not to scale. From Groot & Alderdice (1985).

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*.

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 3.45×10^5 to 4.80×10^5 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

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).

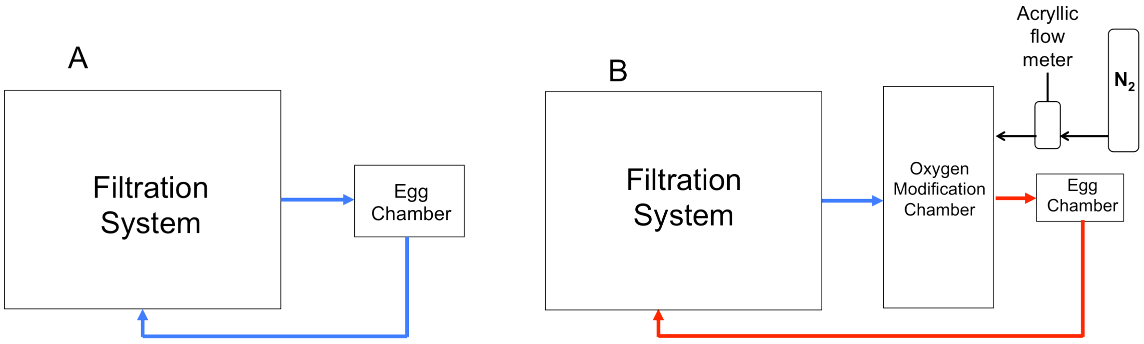

Figure 2.1. Simplified diagram of the initial set-up (A) and the modified set-up (B) including the introduction of the oxygen modification chamber and the nitrogen supply. The set-up described in B was replicated 5 times to enable 5 different oxygen regimes to be

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.

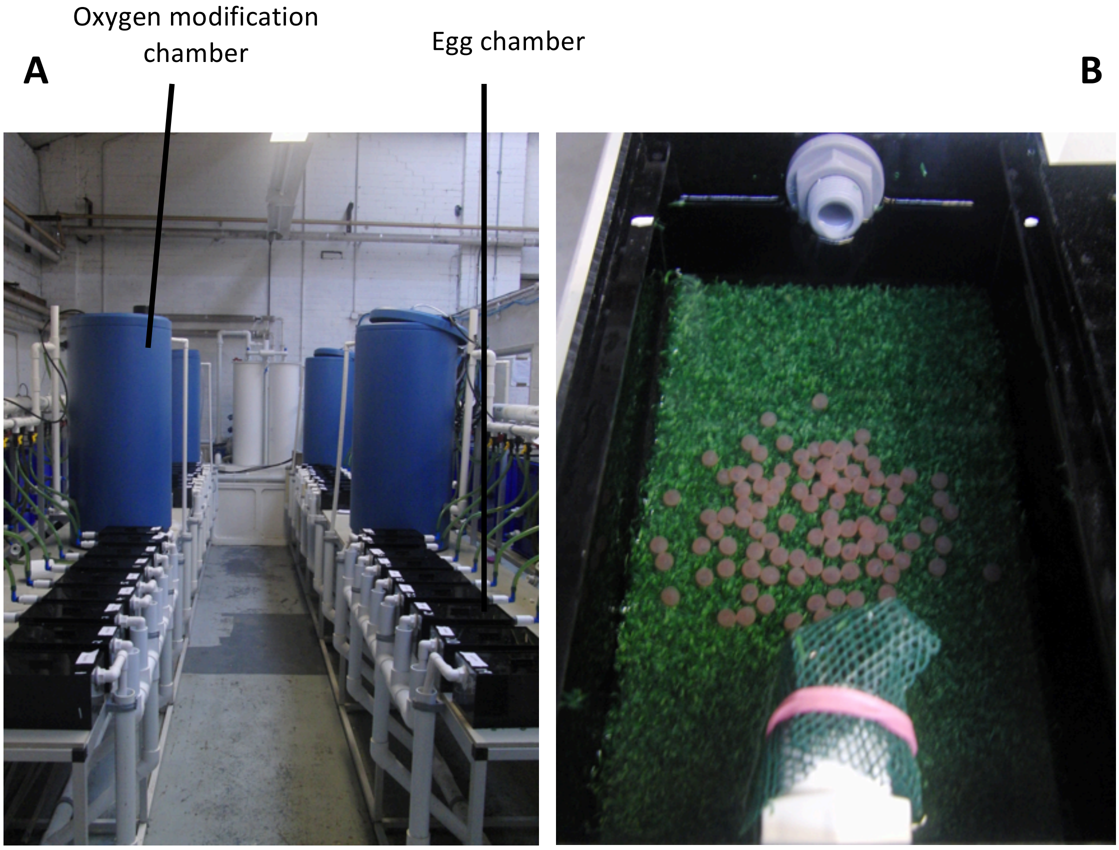

Figure 2.3. Photograph of (A) experimental set-up showing large blue oxygen modification chambers with smaller black perspex egg chambers and (B) egg chambers lined with artificial grass and containing fertilised Atlantic salmon eggs.

Nitrogen was introduced into the oxygen modification chambers (Fig. 2.3) through a ceramic-plate fine-bubble air diffuser (Point Four^{TM}). The rate of nitrogen flow was controlled using acrylic flow meters (Omega®; Fig. 2.1B). A flow rate of approximately 0.06m^3 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).

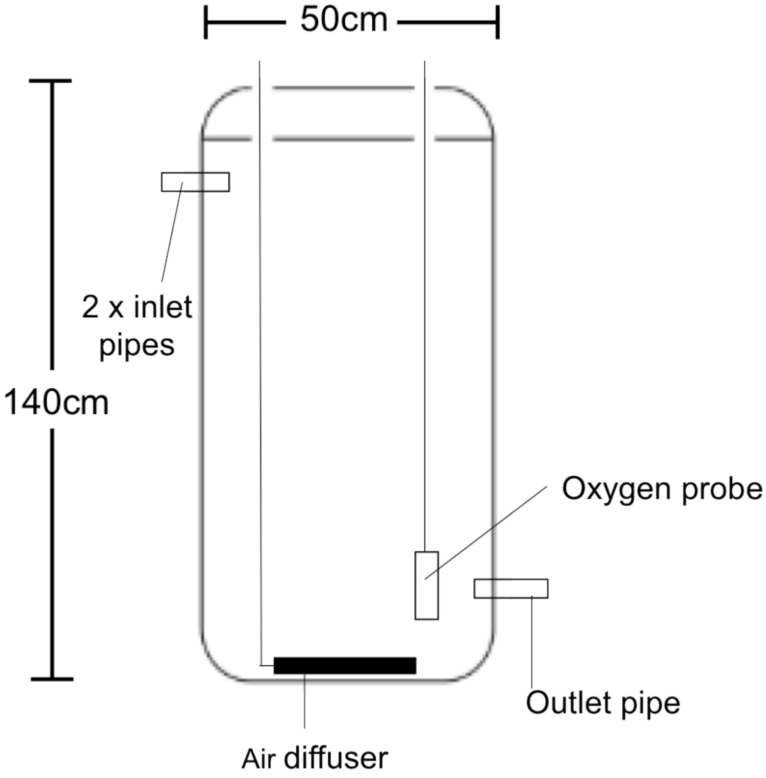

Figure 2.3. Diagram of oxygen modification chamber. Water from the filtration system entered chamber through inlet pipes and flowed to the egg chamber through a gravity-fed system at the outlet pipe.

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).

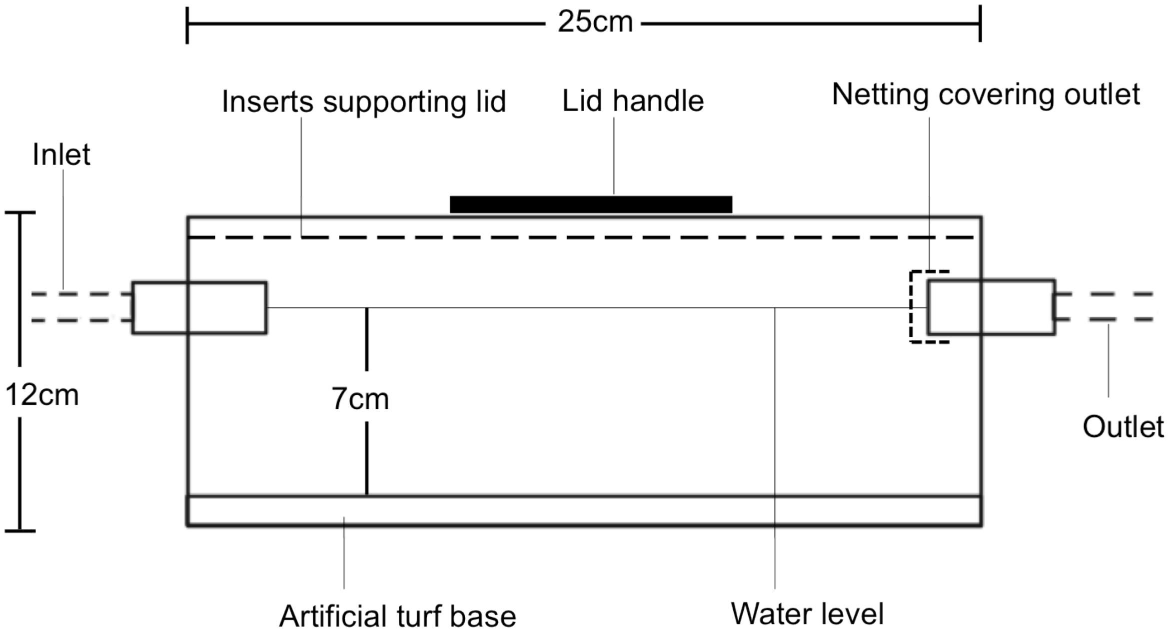

Figure 2.4. Diagram of egg chamber with key features to minimise the introduction of external stressors. Water flow in from the oxygen modification chamber through the inlet pipe and returns to the filtration system through the outlet.

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).

$$J = D\varepsilon_{\tau}\left(\frac{C_o - C_e}{x}\right) \tag{2.1}$$

where *J* = membrane permeability to oxygen (mol cm^{-2} s^{-1}); D = Diffusion coefficient of oxygen in water (cm^{2} s^{-1}); ε_{τ} = 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

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.

$$C_o = \frac{N}{4\pi r^2 k} - C_1 \qquad 2.2$$

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}).

$$C_o = \frac{Nx}{4\pi r^2 D_c} + C_e \qquad 2.3$$

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:

$$D_c = \frac{D\varepsilon_t\delta}{\tau} \qquad 2.4$$

where D = Diffusion coefficient of oxygen in water (cm^2 s^{-1}); ε_τ = membrane porosity (dimensionless); δ = constrictivity; τ = 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.

Chapter 3: Paper 1. De-oxygenated Groundwater Upwelling in the Hyporheic Zone: Implications for Atlantic Salmon Incubation Success

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):

$$O_2 = \frac{14.6p}{1000e^{-0.024T}} \qquad 3.1$$

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.

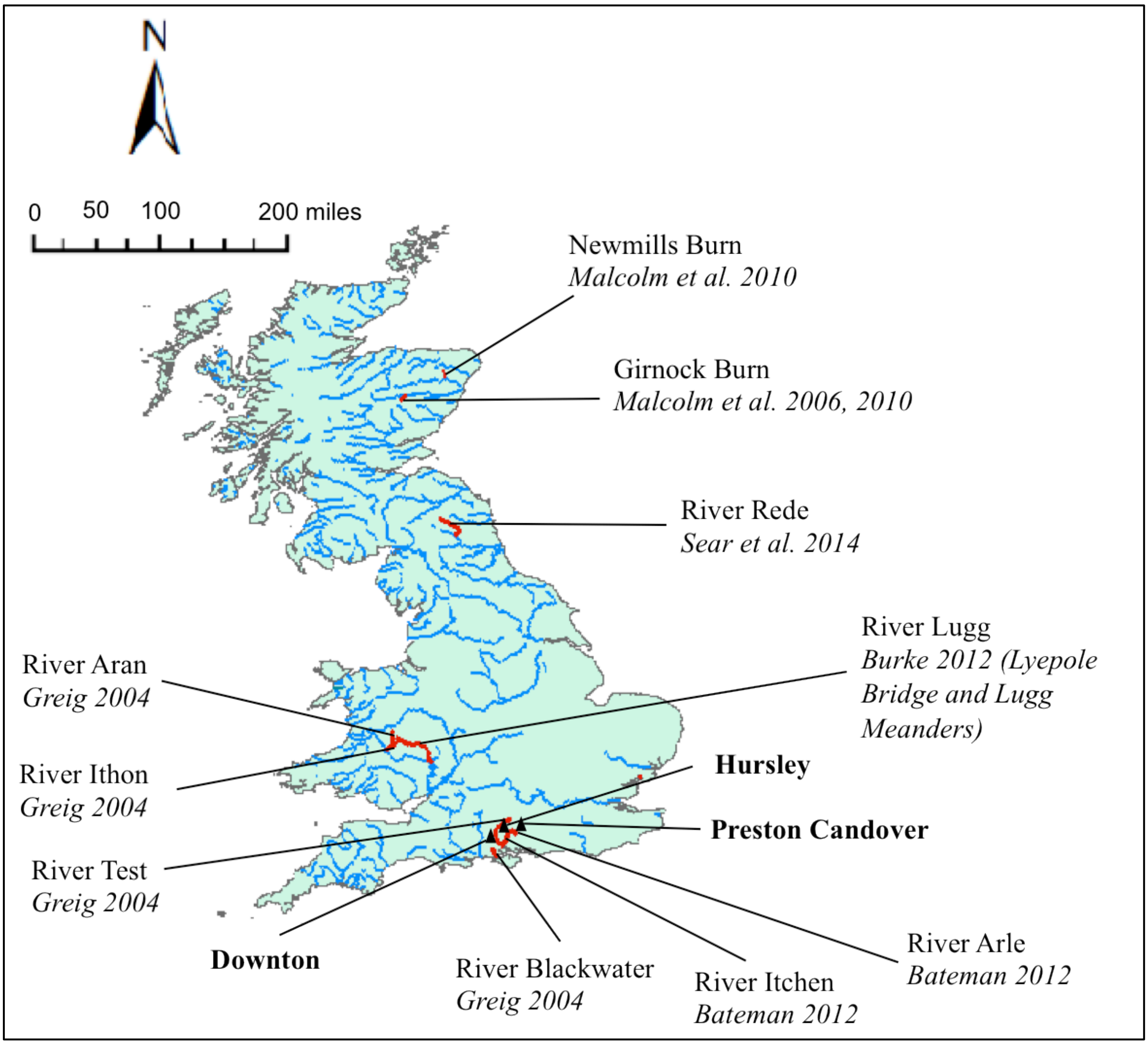

Figure 3.1. Map of rivers hosting Atlantic salmon within the United Kingdom. Rivers from which data on hyporheic dissolved oxygen content and river discharge was used are highlighted in heavier red lines. Triangles indicate location of boreholes used for groundwater data. Rivers are labelled with the 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.

Reference	**River**	**Location**	**Headwater/Trunk Stream**	**Time of Study**	**Hyporheic Depth (cm)**	**Raw data or digitised from publication**	**Temperature Data Source**
Burke 2012	Lugg (Meanders)	Tributary of River Wye, Wales, UK	Trunk	16/02/2008 to 26/04/2008	30	Digitised	Digitised from publication
Burke 2012	Lugg (Lyepole Bridge)	Tributary of River Wye, Wales, UK	Trunk	26/02/2010 to 23/04/2010	30	Digitised	Digitised from publication
Malcolm *et al*. 2006, 2010	Girnock Burn	Caingorms National Park, Scotland	Headwater	16/11/2004 to 27/03/2005	30	Digitised	Digitised from Malcolm *et al*. 2010
Malcolm *et al*. 2010	Newmills Burn	North East Scotland	Headwater	14/11/2006 to 20/02/2007	30	Digitised	Digitised from publication
Bateman 2012	Arle	Tributary of River Itchen, Southern England	Headwater	11/12/2008 to 09/04/2009	20	Raw	Raw data
Greig 2004	Blackwater	Tributary of River Lymington, Southern	Trunk	11/11/2002 to 24/03/2003	25-30	Raw	Raw data
Greig 2004	Test	Southern England	Trunk	14/12/2001 to 15/03/2002	25-30	Raw	Raw data
Greig 2004	Aran	Tributary of River Wye, Wales, UK	Headwater	25/11/2002 to 25/03/2003	25-30	Raw	Raw data
Greig 2004	Ithon	Tributary of River Wye, Wales, UK	Trunk	28/11/2001 to 01/11/2002	25-30	Raw	Raw data
Sear 2014	River Rede	Tributary of River Tyne, Northern England	Headwater	18/02/2011 to 08/06/20111	20	Raw	Raw data

Table 3.2. Catchment characteristics of field sites examined in the present study. Unless otherwise stated, catchment information was obtained directly from studies.

River (Stretch)	Catchment Area (km^2)	GW Dominated/Freshet	Channel Width (m)	Mean Discharge (m^3 s^{-1})	Mean Annual Rainfall (mm)	Dominant local land use	Dominant catchment Geology
Lugg (Meanders)	257.77	Freshet	17.3	3.95	852*	Pasture/Arable	Red sandstone
Lugg (Lyepole Bridge)	231.44	Freshet	12	3.95	852*	Woodland/pasture	Limestone/boulder clay
Girnock Burn	30.3	Freshet	9	0.53	996*	Heather moorland	Granite and Schists
Newmills Burn	25.4	Freshet	1.5 to 2	0.2	800	Arable farming	Psammite and Pelite
Arle	57*	GW Dominated	7.1	1.64	869*‡	Pasture	Chalk
Blackwater	10	GW Dominated	2 to 5	0.92	879*§	Woodland and Heathland	Barton Sands, Barton Clays and Brackleshom
Test	104	GW Dominated	12	11.18	827*	Arable agriculture and grazing land	Chalk
Aran	12	Freshet	5	1.16	1086	Agriculture and heathland	Shale, mudstone, sandstone, limestone
Ithon	32	Freshet	11	11.89	1125*	Livestock farming	Shale, mudstone, sandstone, limestone
River Rede	215	Freshet	14.5	5.93	981*	Pasture/moorland	Carboniferous sandstone

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 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.

Location	**Estimated Time of Spawning (Source)**
River Lugg	6th December (Mawddach hatchery data, Environment Agency)
Girnock Burn	16th November (Webb & McLay 1996)
River Arle	December 26th (Riley & Moore 2000)
River Itchen	December 26th (Riley & Moore 2000)
River Blackwater	December 26th (Riley & Moore 2000)
River Test	December 26th (Riley & Moore 2000)
River Aran	6th December (Mawddach hatchery data, Environment Agency)
River Ithon	6th December (Mawddach hatchery data, Environment Agency)
River Rede	1st December (R. Bond, Personal Communication)

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.

River – Borehole	Correlation coefficient	p-value
Blackwater – Downton	0.880	<0.001
Test – Hursley	0.933	<0.001
Itchen – Hursley	0.958	<0.001
Arle – Preston Candover	0.984	<0.001

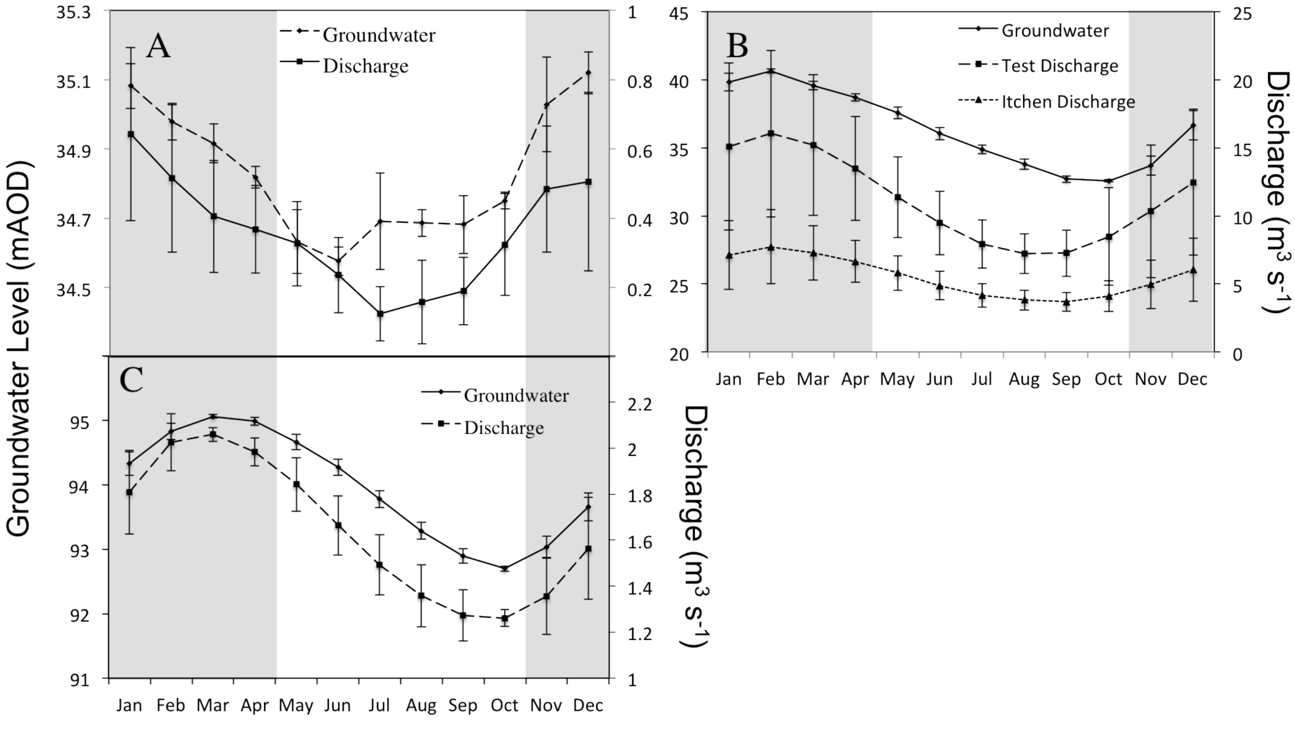

Figure 3.2. Mean monthly river and groundwater levels from 1987-2014 for (A) River Blackwater and Downton borehole; (B) River Test, River Itchen and Hursley borehole; (C) River Arle and Preston Candover borehole. Error bars indicate standard deviation. Shaded grey area indicates

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).

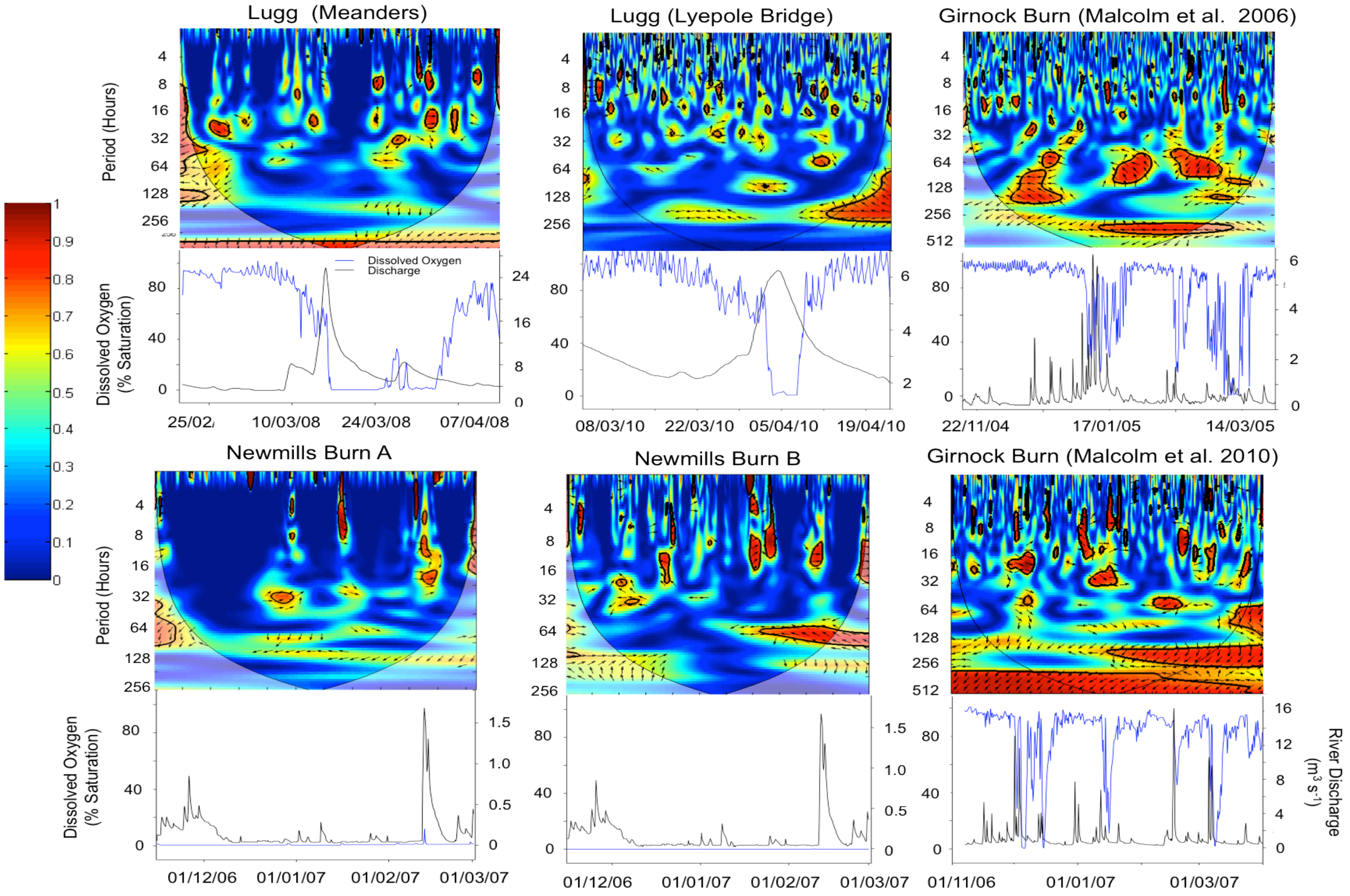

Figure 3.3. Wavelet analysis displaying coherence between river discharge and hyporheic dissolved oxygen levels with raw data presented below. The cone of influence delineates edge effects and is indicated by the translucent shading. Areas outlined by heavy black line indicate coherence at the 95% confidence 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.

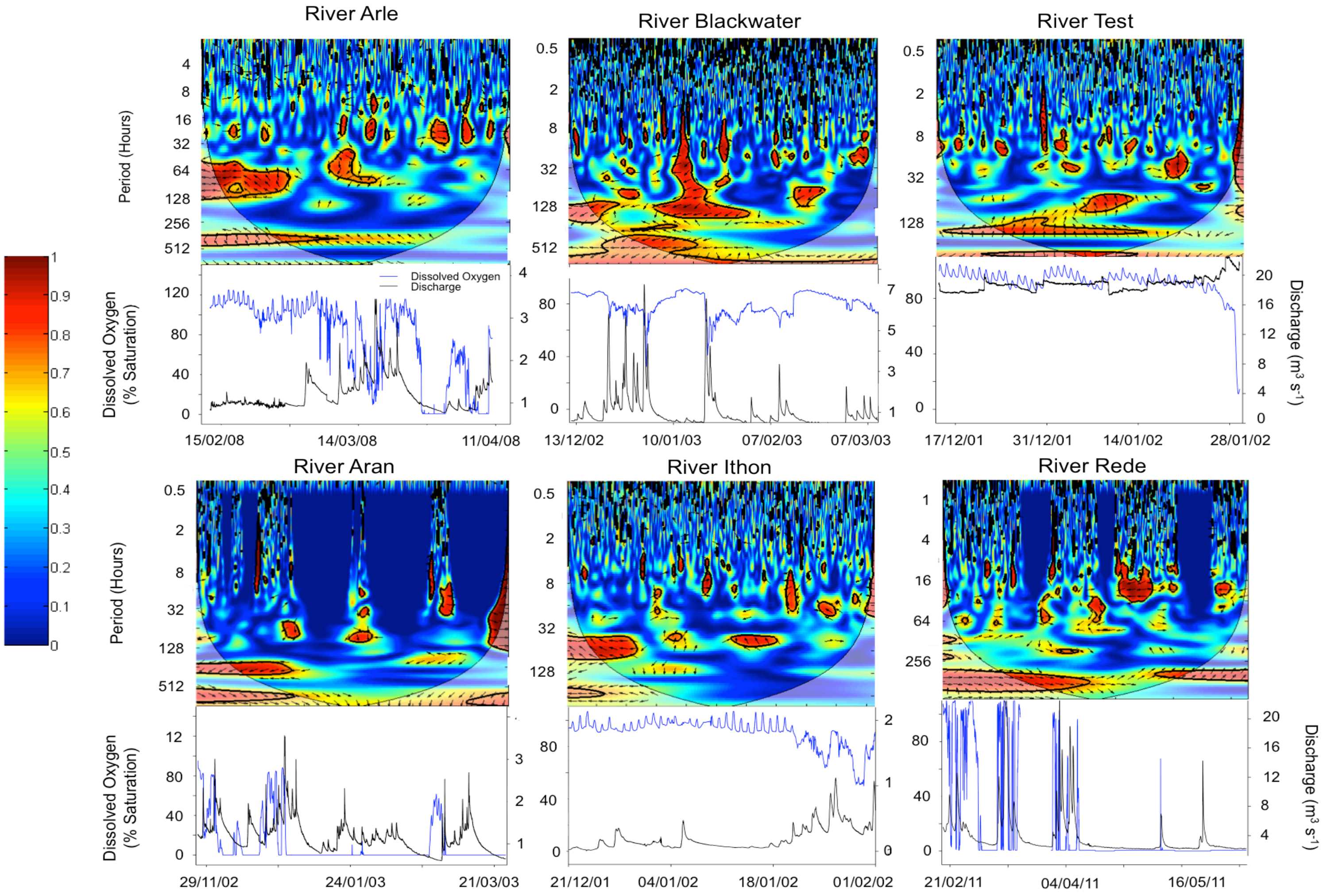

Figure 3.3. Continued

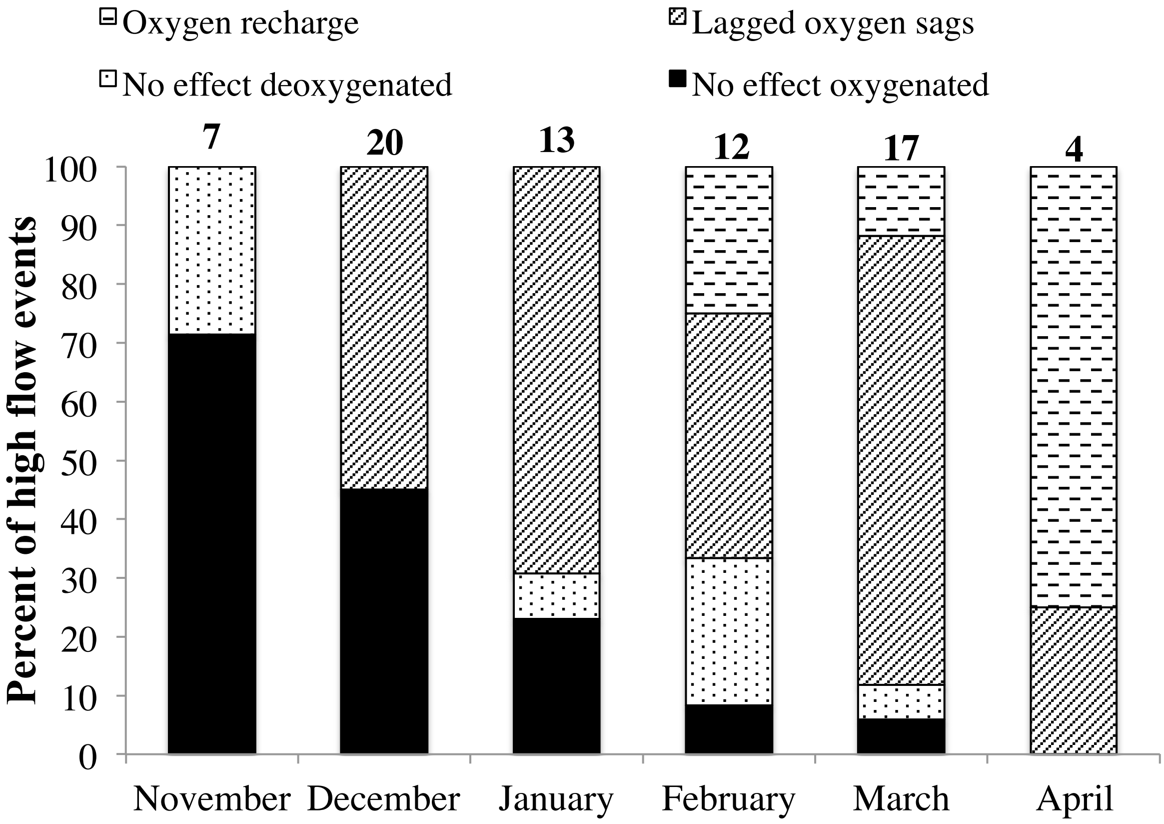

Figure 3.4. Frequency and nature of relationships between river discharge and hyporheic dissolved oxygen content following a high flow event. Data is taken from wavelet analysis displayed in Figure 3.3.Values above bars indicate number of high flow events observed per month.

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.

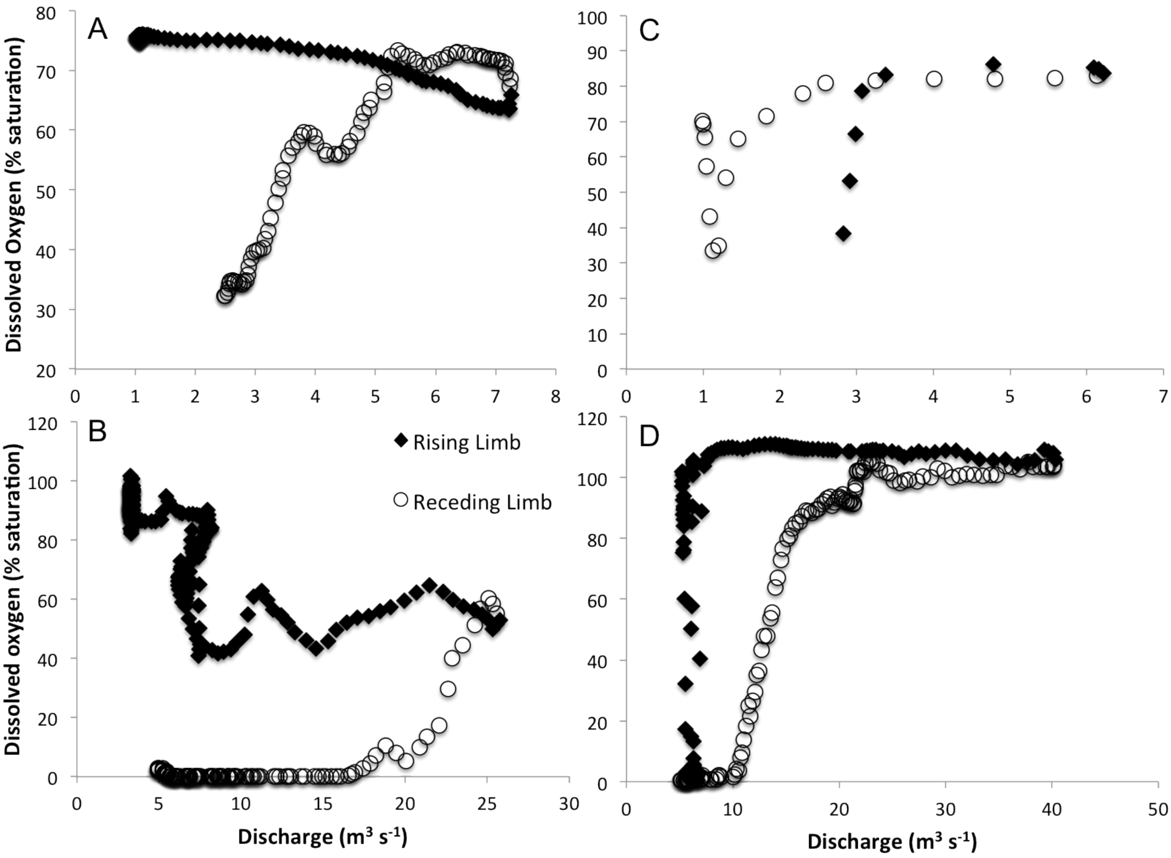

Figure 3.5. The relationship between hyporheic dissolved oxygen content and river discharge levels during peak flow events for (A) River Blackwater from 31/12/2002 to 2/1/2003; (B) River Lugg (Meanders) from 3/3/2008 to 26/3/2008; (C) Girnock Burn (Malcolm 2006) from 9/1/2005 to 11/1/2005; (D) River 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.

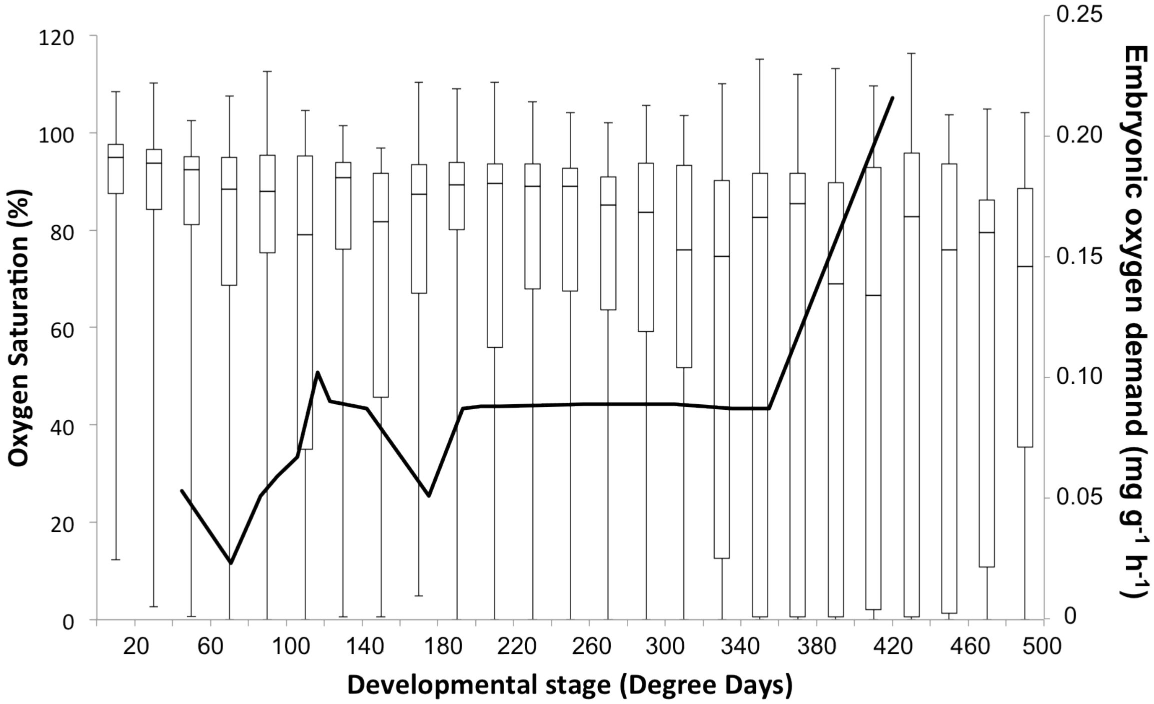

Figure 3.6. Box plot of oxygen content in Atlantic salmon redds during estimated incubation period for all sites combined at intervals of 20 degree days. Heavy line overlaying data indicates oxygen consumption pattern of individual Atlantic salmon eggs throughout development at 5°C in oxygen saturated 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.

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

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; Sousby *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.

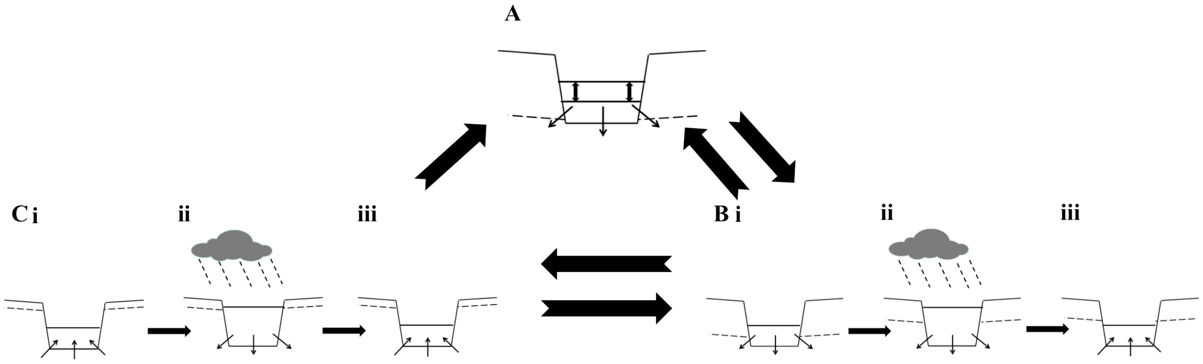

Figure 3.7. Conceptual diagram of seasonal changes in groundwater-surface water connectivity and fluctuating hyporheic exchange following high flow events. A (no response to flow event) – following prolonged dry spells, rainfall causes river level and water table to rise but the low water table means 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.

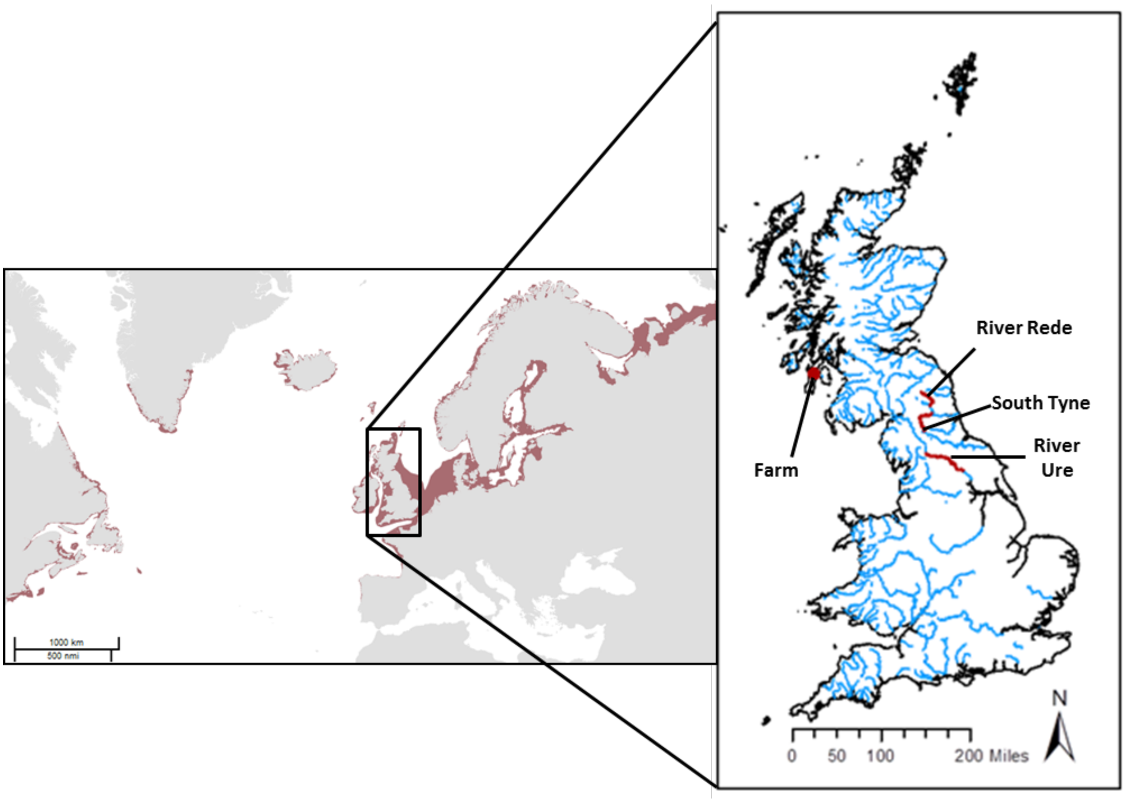

Figure 4.1. Small inset map shows global range of Atlantic salmon in maroon (Taken from FAO 2016). Larger map of mainland United Kingdom shows rivers with native Atlantic salmon populations in blue. Locations from which eggs were sourced are labelled and are in heavier red lines.

Gametes were extracted from adult fish by hand on the morning of 1st December 2014 into clean and dry plastic containers. Eggs were submerged in coelomic fluid to prolong their viability (Bonnet *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 2nd December 2014 following procedures described by Whitney *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).

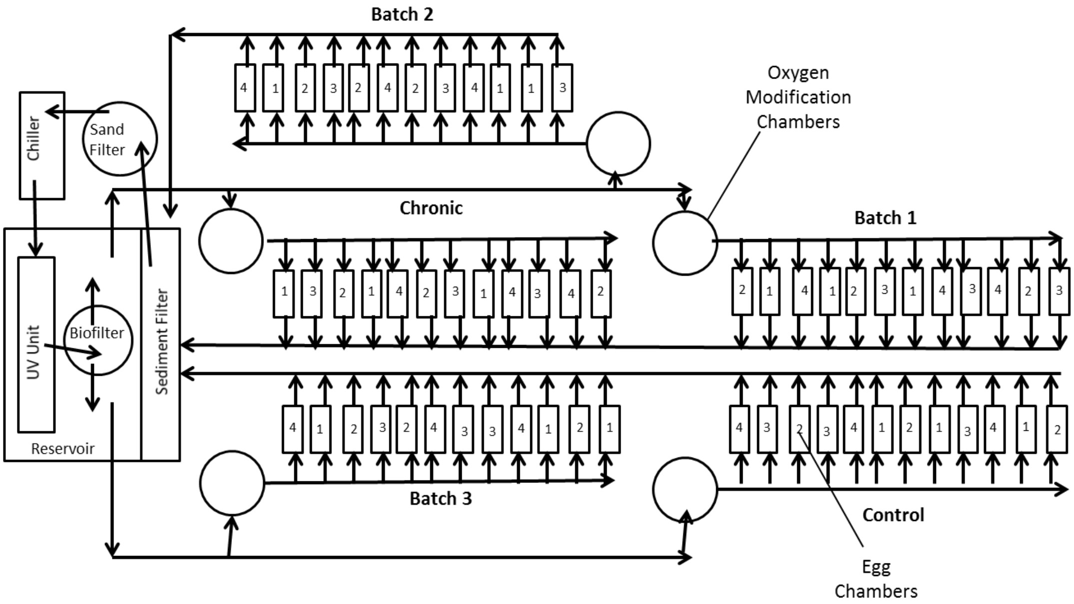

Figure 4.2. Systematic diagram of recirculating system at the research facility. Arrows indicate direction of water flow. Location of each treatment is labelled. Numbers within egg chambers indicate location of eggs of each maternal fish: 1 = River Ure; 2 = Farmed; 3 = River South Tyne; 4 = River Rede.

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 redds (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:

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

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.

Continuous Treatments		**Sag Treatments**							
Control	**Chronic**			**Early Sags**		**Median Sags**		**Late Sags**	
Oxygen Saturation (%)	**Oxygen Saturation (%)**	**Pulse number**	**Oxygen Saturation (%)**	**Start (DD)**	**End (DD)**	**Start (DD)**	**End (DD)**	**Start (DD)**	**End (DD)**
Maximum	**60**	**1**	**50**	134	142	229	236	317	324
		2	**50**	152	161	244	252	331	338
		3	**50**	170	178	259	267	345	352
		4	**30**	185	192	275	284	358	364
		5	**30**	198	204	293	300	371	379
		6	**30**	209	214	309	317	387	395
		7	**10**	221	229	324	331	403	410
		8	**10**	236	243	338	345	418	426
		9	**10**	252	259	351	358	435	441
		10	**10**	267	275	364	371	448	456

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

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:

$$\lg \tau_t = C + at + bt^2 \tag{4.2}$$

where τ_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.

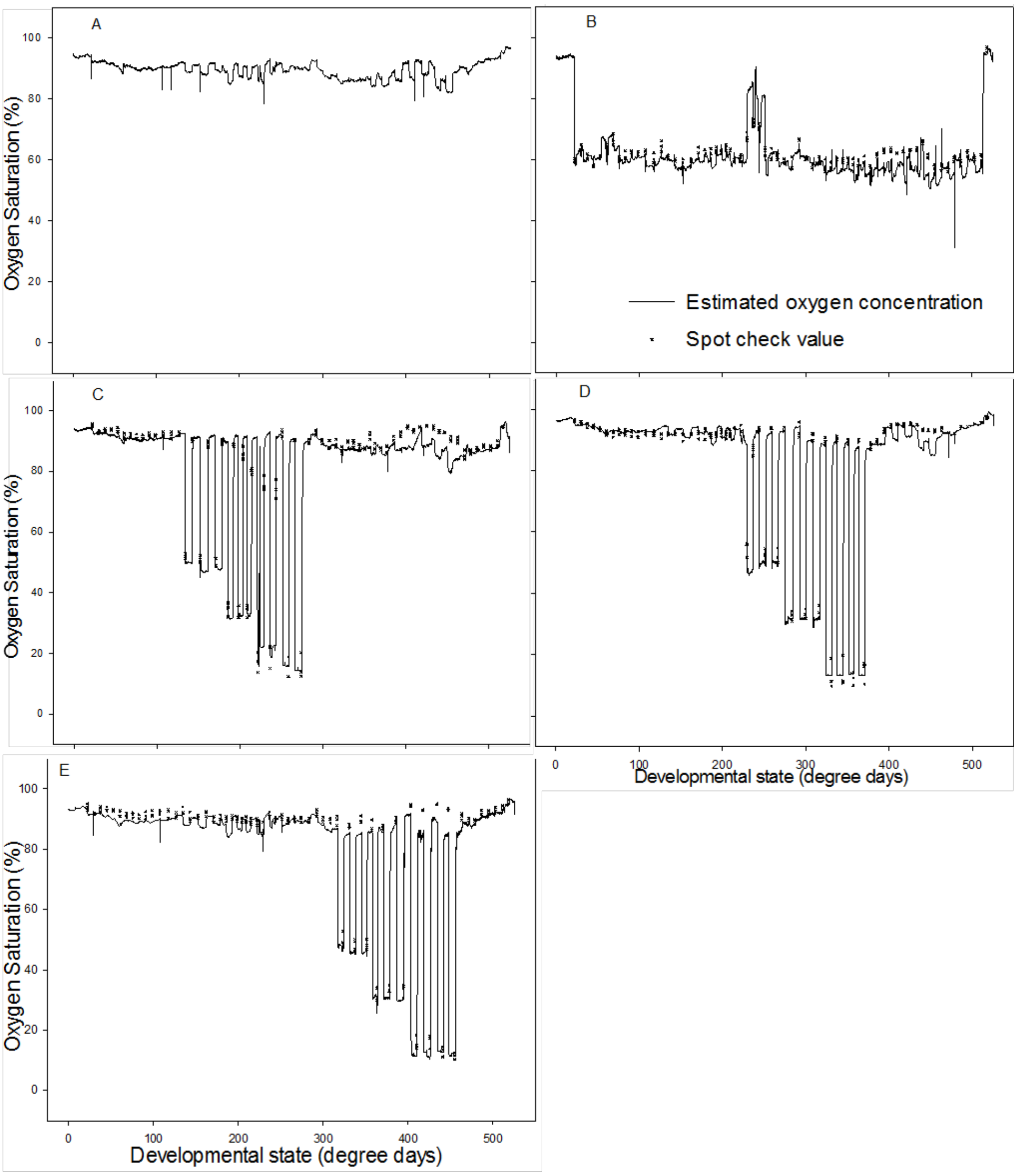

Figure 4.3. Estimated oxygen regimes Treatment: (A) Control; (B) Chronic; (C) Early sags; (D) Median sags; (E) Late sags. Values calculated using equation 1. Scatter points represent daily spot check values.

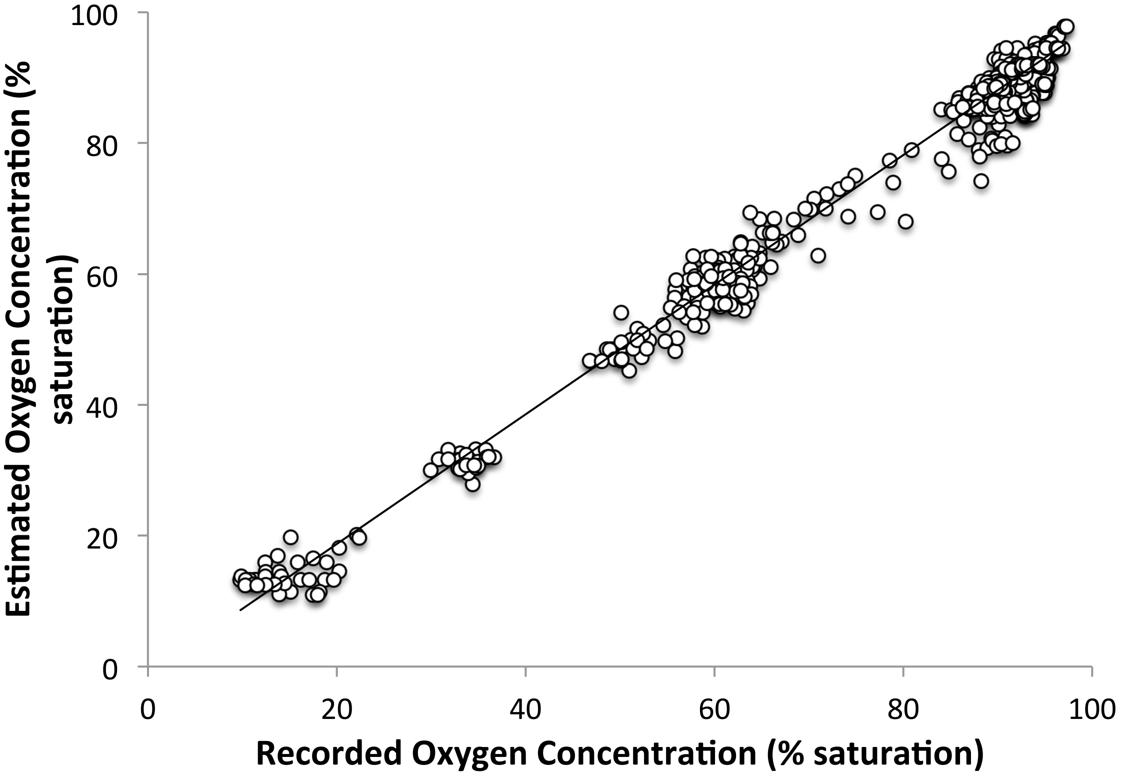

Figure 4.4. Comparison of recorded oxygen saturation values in egg chambers with estimated values using formula 4.1. Strong agreement between values ($R^2 = 0.992$, $p<0.001$) validates use of formula 4.1 to estimate oxygen concentration in egg chambers.

Figure 4.5. Eggs within egg chambers. Dead egg slightly right of centre is clearly visible by opaque white colour.

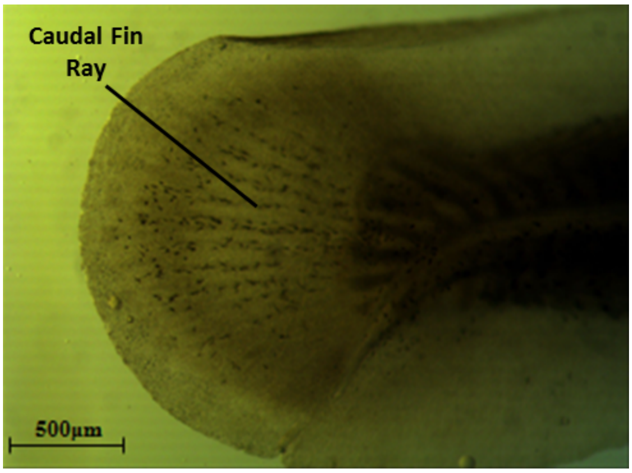

Figure 4.6. Image taken under a Nikon E100 microscope at 100x magnification of the caudal fin of a newly hatched alevin. A caudal fin ray that was used to estimate alevin developmental state is labelled.

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:

$$\mu_h = \frac{h}{E} \tag{4.3}$$

where: μ_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):

$$RR = \frac{\mu_{hx}}{\mu_{hy}} \qquad 4.4$$

where: RR = relative risks; μ_{hx} = hatch rate of treatment x; μ_{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 (F4, 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).

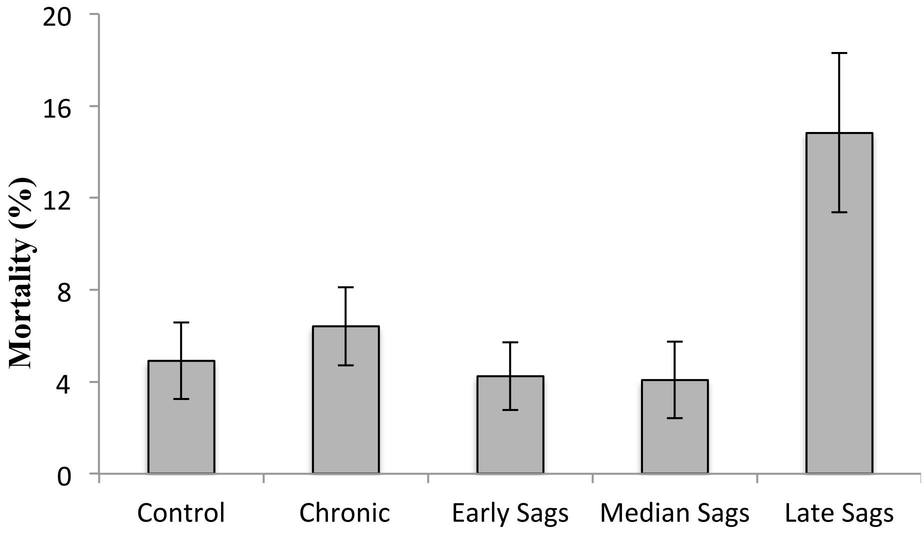

Figure 4.7. Mean mortality rates separated by treatment. Error bars indicate standard deviation.

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).

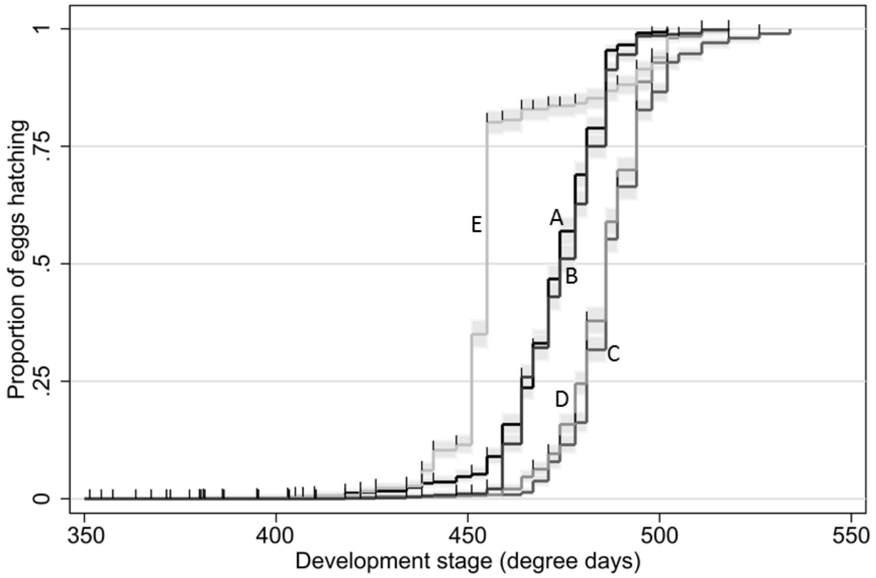

Figure 4.8. Kaplan-Meier estimate of the hatching function by treatment (N=6 000). Vertical marks on curves indicate incidence of mortality. Shading indicates confidence intervals. For ease of identification, curves are labelled A-E to match the corresponding treatment. A = control; B = chronic; C = early sags; D = median sags; E = late sags.

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

Treatment	N	Total hatched	Total deaths	Time exposed to risk (1 000DD)	Hatching rate from 350DD (per 1000DD)		
					Estimate	95 % CI (Lower Boundary)	95 % CI (Upper Boundary)
Control	1,200	1 141	59	557.382	8.09	7.634	8.574
Chronic	1,200	1 123	77	560.527	7.78	7.342	8.253
Early sags	1,200	1 149	51	578.538	7.12	6.717	7.54
Median sags	1,200	1 151	49	573.316	7.32	6.905	7.751
Late sags	1,200	1 022	178	536.204	8.47	7.968	9.008

Note. Hatching rate is taken from 350DDs to increase data resolution.

Table 4.3. Comparison of the incidence of hatching among oxygen treatments.

Treatment	Control	Chronic	Early Sags	Median Sags	Late Sags
Control	-	0.493	0.001	0.017	0.02
Chronic	0.972	-	0.011	0.091	0.003
Early Sags	0.874	0.899	-	0.391	0.001
Median Sags	0.905	0.932	1.036	-	<0.001
Late Sags	1.104	1.136	1.264	1.219	-

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.

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

4.4 Discussion

The presence of GW in Atlantic salmon redds 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 intragravel 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 intragravel pores and reduce intragravel velocity and therefore oxygen supply. Furthermore, a reduction of intragravel 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 intragravel 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 (C*ottus gobio*, Roussel 2007) or burbot (*Lota lota*, 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.

Chapter 5: Paper 3. Variation in the Egg Membrane Structure Among Atlantic Salmon Populations: Implications for Tolerance to Oxygen Stress

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 (Wootton 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.

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.

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

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

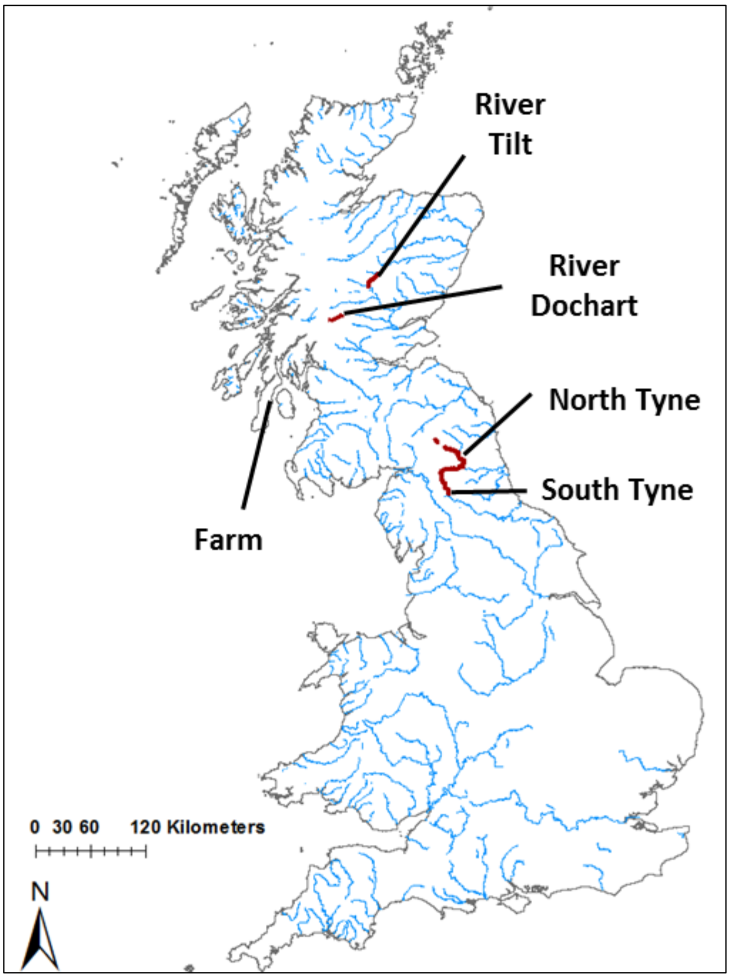

Figure 5.1. Map of United Kingdom with rivers hosting Atlantic salmon shown in blue. The locations of the egg sources used in this study are labelled and displayed in heavy red.

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):

$$DD = \frac{T}{144} \tag{5.1}$$

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:

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

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); $\mathcal{X}$ = 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:

$$D_c = \frac{D\varepsilon_t\delta}{\tau} \qquad 5.3$$

where D = Diffusion coefficient of oxygen in water (cm^2 s^{-1}) at 5°C; ε_τ = membrane porosity (dimensionless); δ = constrictivity; τ = 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 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):

$$x_p = \frac{x}{\varnothing} \tag{5.4}$$

where x_p = membrane quotient; x = mean membrane thickness of individual egg (cm); $\varnothing$ = 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).

$$\varepsilon_\tau = \frac{A_p N}{A} \tag{5.5}$$

where ε_τ = 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:

$$J = D\varepsilon_\tau \left(\frac{C_o - C_e}{x}\right) \tag{5.6}$$

where J = membrane permeability to oxygen (mol cm^{-2} s^{-1}); 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 perivitelline fluid of the eggs (3.34×10^{-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.

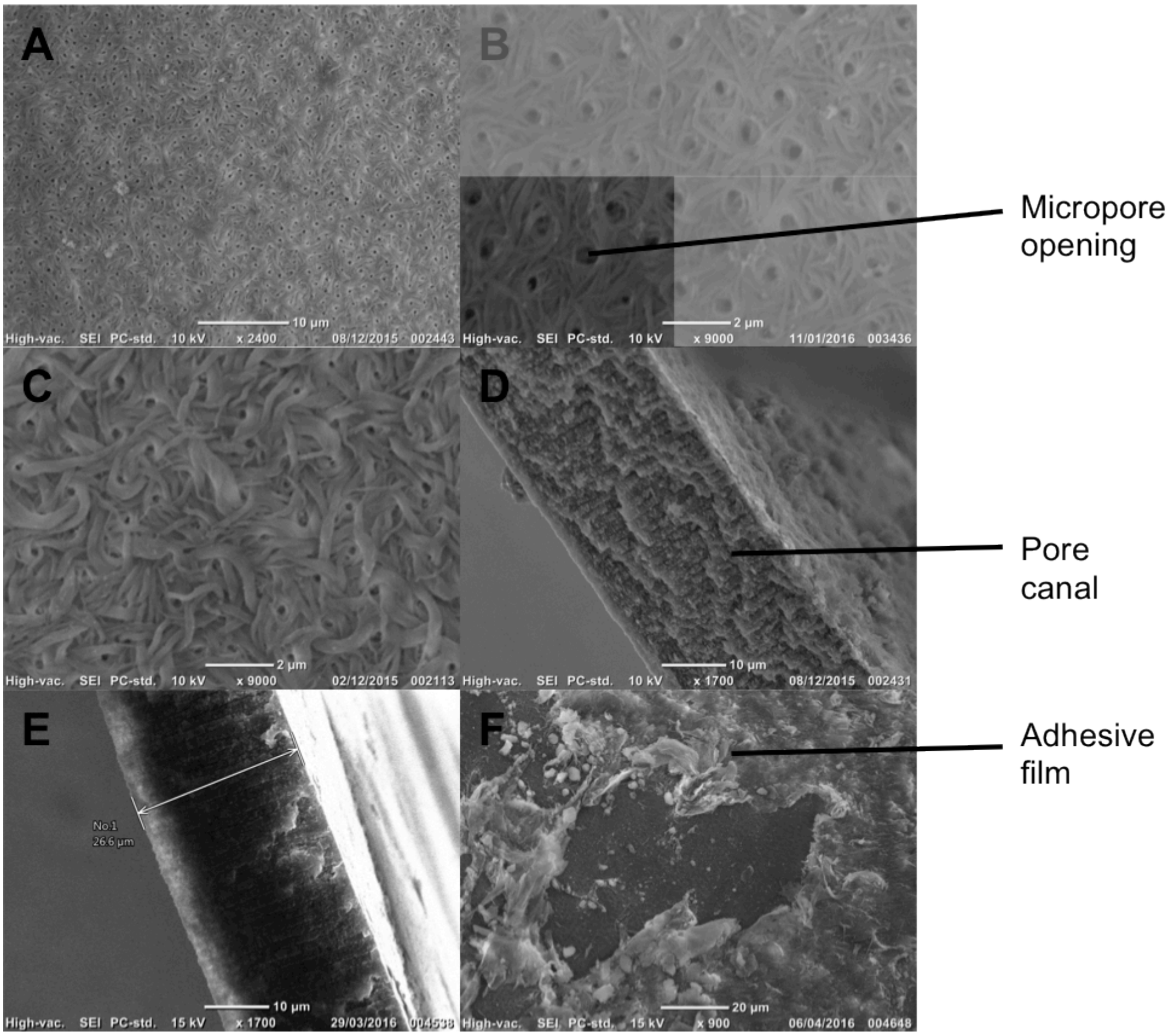

Figure 5.2. Images of membrane structures used in analysis. (A) regular micropore arrangement on membrane internus at 2400x magnification; (B) membrane internus with lower left hand quadrant highlighted to demonstrated section used for measurement of micropore diameter; (C) membrane internus at 9000x 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 ± 0.24) than all other populations (p<0.001) and River Dochart eggs (6.53 ± 0.20mm) were larger than the farmed (6.36 ± 0.10mm, p=0.034), River South Tyne (6.27 ± 0.33mm, p<0.001) and River North Tyne (6.24 ± 0.44mm, 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 (Fig. 5.5, 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.

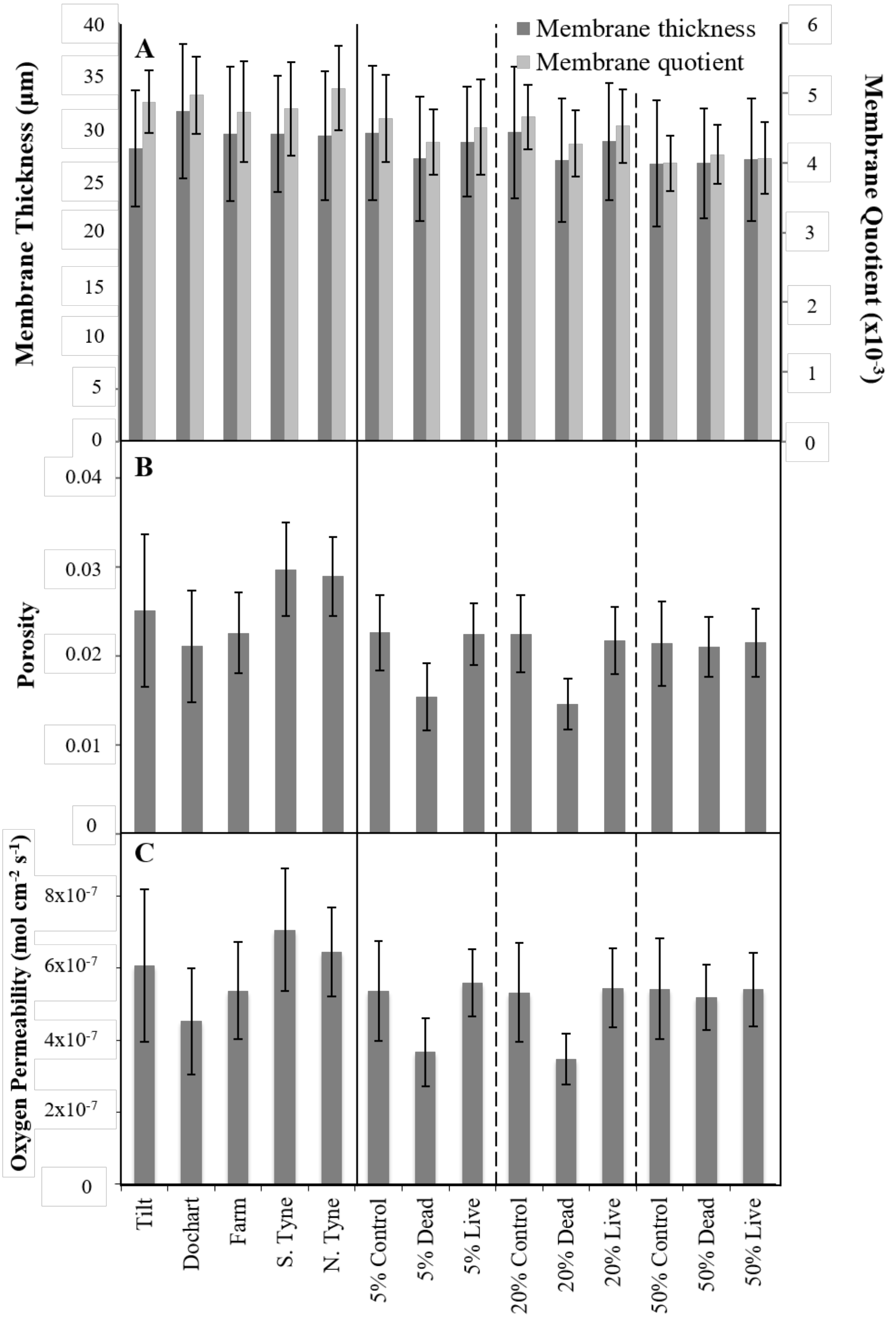

Figure 5.3. Structural features of test eggs (A: membrane thickness and membrane quotient; B: membrane porosity; C: oxygen permeability). Data on the left of the solid line represents mean values to enable comparisons of population variability of egg architecture. Data on the right of the

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.

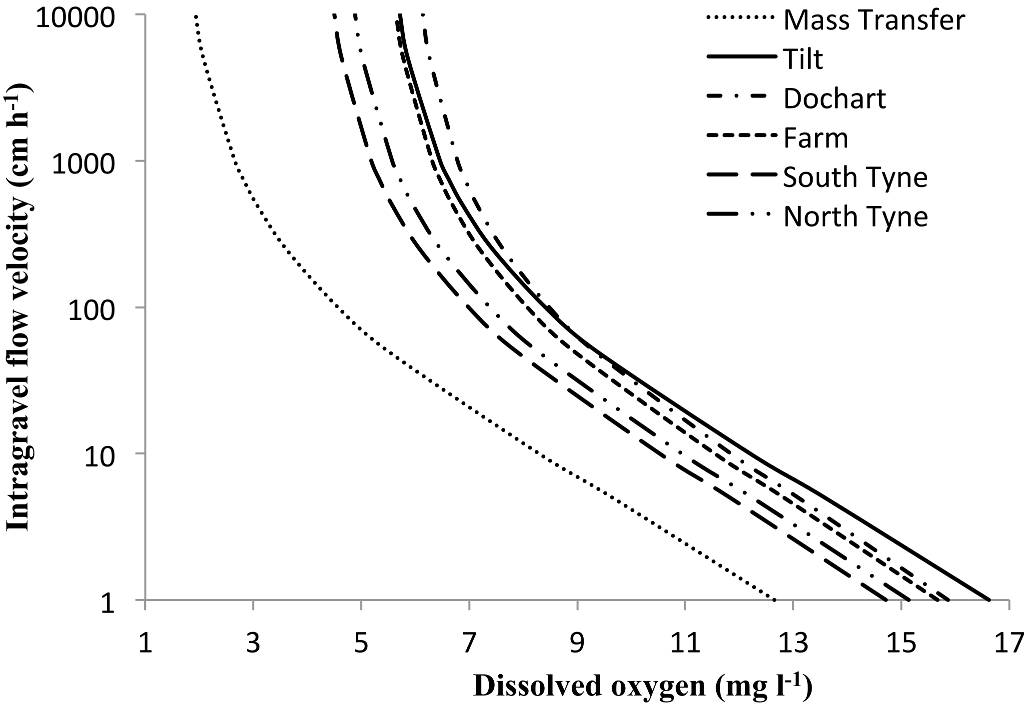

Figure 5.4. Range of intragravel velocities and oxygen concentrations necessary to support respiratory requirements at 5°C for Atlantic salmon eggs of the five populations investigated in the present study. Mass transfer curve represents estimate of oxygen requirements based on egg structure data presented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

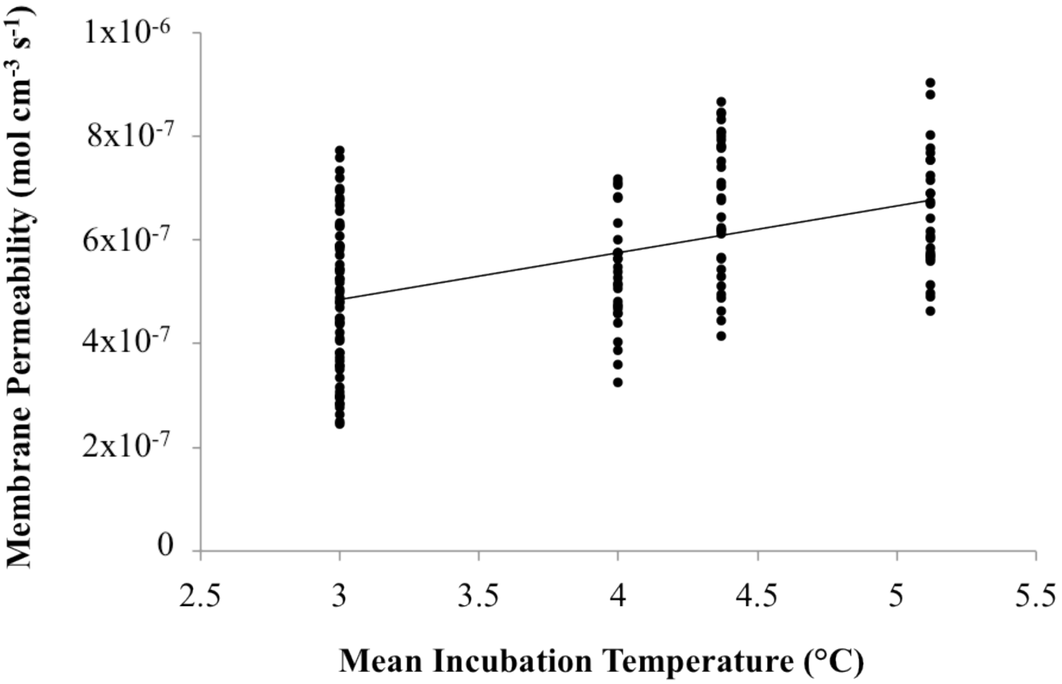

Figure 5.5. The relationship between mean incubation temperature and the membrane permeability of all the eggs sampled ($R^2 = 0.468$, $p<0.001$).

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 intragravel 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 intragravel 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 redds 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 redds 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 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.

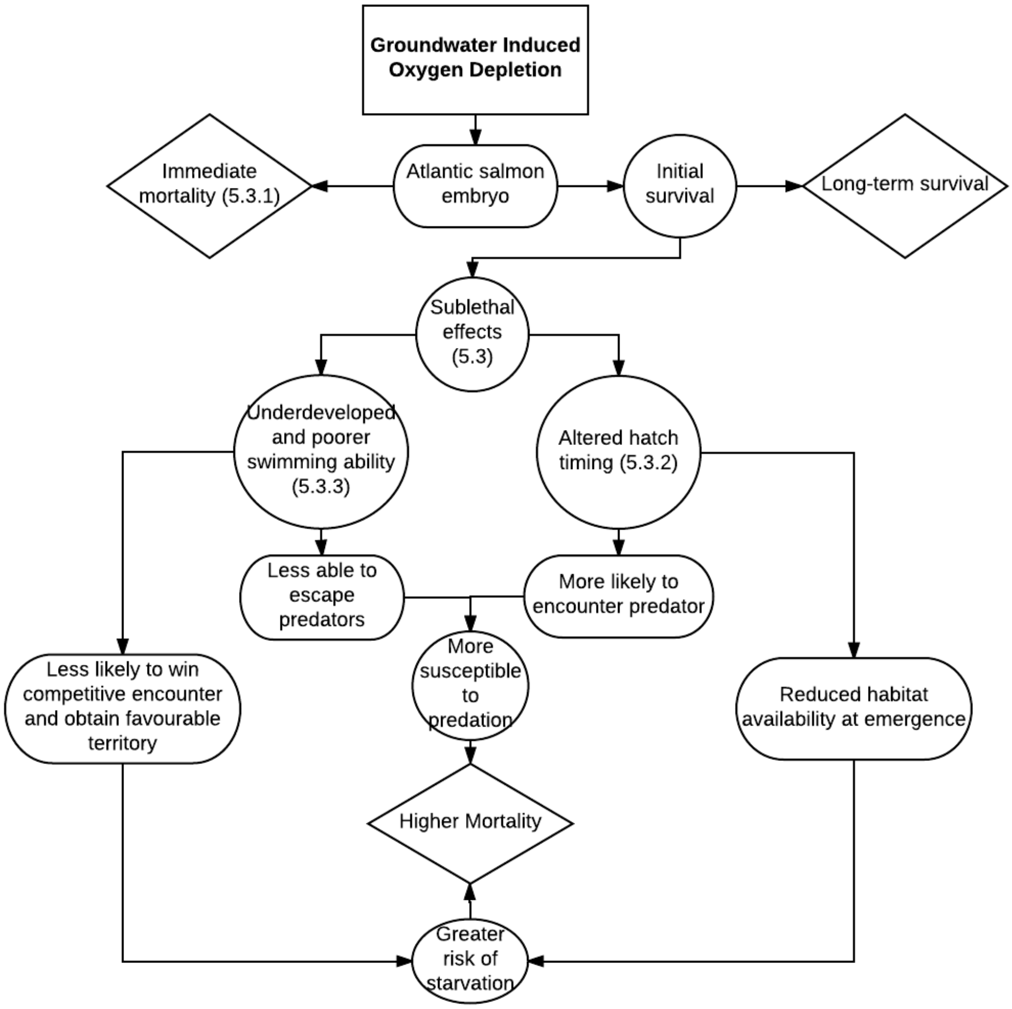

Figure 6.1. Flow chart describing key findings of chapter 5 and potential effects of deoxygenated groundwater upwelling on Atlantic salmon incubation success. Numbers in brackets indicate relevant section where findings are presented.

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.

6.2.2 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.

6.2.3 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.

6.2.4 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).

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).

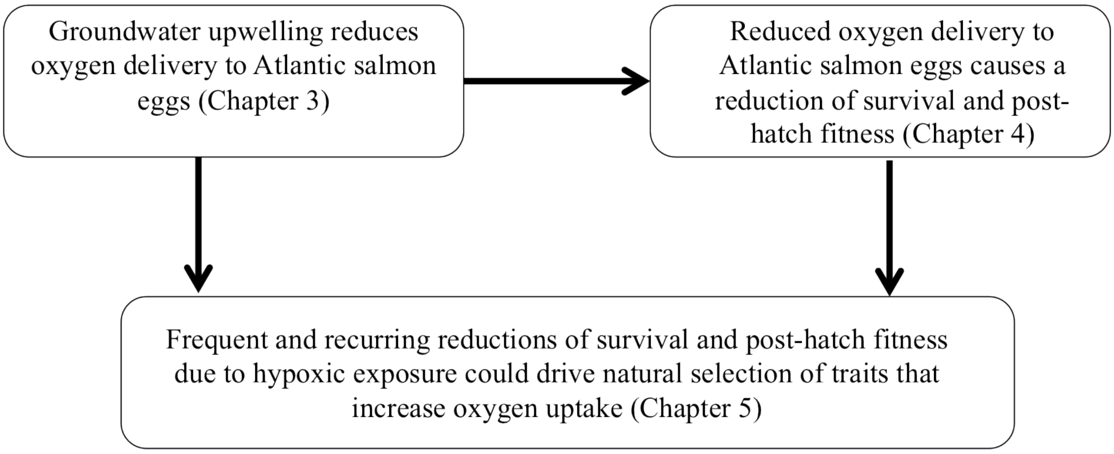

Figure 7.1. Flowchart showing the synthesised outcomes of each research chapter in the present thesis.

List of References

Ackerman, R. A., & Prange, H. D. (1972). Oxygen diffusion across a sea turtle (*Chelonia mydas*) egg shell. *Comp. Biochem. Phys. A* **43**: 905-909. doi: 10.1016/0300-9629(72)90162-4

Alderdice, D. F. & Velsen, F. P. J. (1978). Relation Between Temperature and Incubation Time for Eggs of Chinook Salmon (*Oncorhynchus tshawytscha*). *J. Fish. Res. Board Can.* **35**: 69-75. doi: 10.1139/f78-010

Alderdice, D. F., Wickett, W. P. & Brett, J. R. (1958). Some Effects of Temporary Exposure to Low Dissolved Oxygen Levels on Pacific Salmon Eggs. *J. Fish. Res. Board Can.* **15**: 229-250. doi: 10.1139/f58-013.

Allen, D. A. & Castillo, M. M. (2007). Stream ecology: structure and function of running waters. Springer, Dordrecht, The Netherlands.

Amoros, C., & Bornette, G. (2002). Connectivity and biocomplexity in waterbodies of riverine floodplains. *Freshwater Biology*, **47**: 761-776. doi: 10.1046/j.1365-2427.2002.00905.x

Anderson, J. J. (1992). A vitality-based stochastic model for organism survival. In *Individual-based models and approaches in ecology* (pp. 256-277). Springer US.

Anttila, K., Dhillon, R. S., Boulding, E. G., Farrell, A. P., Glebe, B. D., Elliott, J. A., Walters, W. R. & Schulte, P. M. (2013). Variation in temperature tolerance among families of Atlantic salmon (*Salmo salar*) is associated with hypoxia tolerance, ventricle size and myoglobin level. *J. Exp. Biol.* **216**: 1183-1190. doi: 10.1242/jeb.080556

Aprahamian, M. W., Martin Smith, K., McGinnity, P., McKelvey, S. & Taylor, J. (2003). Restocking of salmonids – opportunites and limitations. *Fish. Res.* **62**: 211-227.

Armitage, P. D., Bowes, M. J. & Vincent, H. M. (2007). Long-term changes in macroinvertebrate communities of a heavy metal polluted stream: the river Nent (Cumbria, UK) after 28 years. *River. Res. Applic.* 23: 997–1015. doi: 10.1002/rra.1022.

Arntzen, E. V., Geist, D. R. & Dresel, P. E. (2006), Effects of fluctuating river flow on groundwater/surface water mixing in the hyporheic zone of a regulated, large cobble bed river. *River Res. Applic.,* **22**: 937–946. doi:10.1002/rra.947

Bakke, T. A., & Harris, P. D. (1998). Diseases and parasites in wild Atlantic salmon (Salmo salar) populations. *Can. J. Fish. Aquat. Sci.* **55**: 247-266. doi: 10.1139/d98-021

Baliunas, S., Frick, P., Sokoloff, D., & Soon, W. (1997). Time scales and trends in the central England temperature data (1659–1990): A wavelet analysis. *Geophys. Res. Lett.* **24**: 1351-1354. doi: 10.1029/97GL01184

Bang, G. (2015). *The Domestic Politics of Global Climate Change: Key Actors in International Climate Cooperation.* Bang, G., Underdal, A. & Andresen, T. (eds.). Edward Elgar Publishing.

Bardonnet, A., Gaudin, P. & Thorpe, J. E. (1993). Diel rhythm of emergence and of first displacement downstream in trout (*Salmo trutta*), Atlantic salmon (*S. salar*) and grayling (*Thymallus thymallus*). *J. Fish Biol.* **43**: 755-762. doi: 10.1111/j.1095-8649.1993.tb01152.x

Barrett, R. D. & Hoekstra, H. E. (2011). Molecular spandrels: tests of adaptation at the genetic level. *Nature Reviews Genetics* **12**: 767-780. doi: 10.1038/nrg3015

Bateman, S. (2012). *Sources and impacts of inorganic and organic fine sediment in salmonid spawning gravels in chalk rivers*. Ph.D thesis, University of Southampton.

Baumann, R., Padeken, S., Haller, E. A., & Brilmayer, T. H. (1983). Effects of hypoxia on oxygen affinity, hemoglobin pattern, and blood volume of early chicken embryos. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **244**: R733-R741.

Baxter, C. V. & Hauer, F. R. (2000). Geomorphology, hyporheic exchange, and selection of spawning habitat by bull trout (*Salvelinus confluentus*). *Can. J. Fish. Aquat. Sci.* **57**: 1470-1481. doi: 10.1139/f00-056

Baxter, C., Hauer, F. R., & Woessner, W. W. (2003). Measuring groundwater–stream water exchange: new techniques for installing minipiezometers and estimating hydraulic conductivity. *T. Am. Fish. Soc.* **132**: 493-502. doi: 10.1577/1548-8659(2003)132<0493:MGWENT>2.0.CO;2

Baxter, J. S. & McPhail, J. D. (1999). The influence of redd site selection, groundwater upwelling, and over-winter incubation temperature on survival of bull trout (*Salvelinus confluentus*) from egg to alevin. *Can. J. Zool.* **77**: 1233-1239. doi: 10.1139/z99-090.

Beacham, T. D. & Murray, C. B. (1985). Effect of Female Size, Egg Size, and Water Temperature on Developmental Biology of Chum Salmon (*Oncorhynchus keta*) from the Nitinat River, British Columbia. *Can. J. Fish. Aquat. Sci.* **42**: 1755-1765. doi: 10.1139/f85-220

Beacham, T. D. & Murray, C. B. (1990). Temperature, Egg Size, and Development of Embryos and Alevins of Five Species of Pacific Salmon: A Comparative Analysis. *T. Am. Fish. Soc.* **119**: 927-945. doi: 10.1577/1548-8659(1990)119<0927:TESADO>2.3.CO;2

Beacham, T. D., Withler, F. C., & Morley, R. B. (1985). Effect of egg size on incubation time and alevin and fry size in chum salmon (*Oncorhynchus keta*) and coho salmon (*Oncorhynchus kisutch*). *Can. J. Zool.* **63**: 847-850. doi: 10.1139/z85-125.

Beall, C. M., Decker, M. J., Brittenham, G. M., Kushner, I., Gebremedhin, A. & Strohl, K. P. (2002). An Ethiopian pattern of human adaptation to high altitude hypoxia. *Proc. Nat. Acad. Sci.* **99**: 17215-17218. doi: 0.1073/pnas.252649199

Begon, M. and Mortimer, M. (1986) *Population Ecology; A Unified Study of Animals and Plants,* 2nd edn., pp. 117-52. Blackwell Scientific Publications, Oxford.

Bell, G. R., Hoskins, G. E. & Bagshaw, J. W. (1969). On the structure and enzymatic degradation of the external membrane of the salmon egg. *Can. J. Zool.* **47**: 146-148. doi: 10.1139/z69-028

Bencala, K. E. (2005). Hyporheic exchange flows *In* Encyclopedia of Hydrological Sciences. Eds. Anderson, M. G. Chapter 113.

Bernardo, J. (1996). The Particular Maternal Effect of Propagule Size, Especially Egg Size: Patterns, Models, Quality of Evidence and Interpretations. *Amer. Zool.* **36**: 216-236. doi: 10.1093/icb/36.2.83

Beschta, R. L. & Jackson, W. L. (1979). The Intrusion of Fine Sediments into a Stable Gravel Bed. *J. Fish. Res. Board Can.* **36**: 204-210. doi: 10.1139/f79-030.

Biggs, J., Williams, P., Whitfield, M., Nicolet, P., Brown, C., Hollis, J., Arnold, D. & Pepper, T. (2007). The freshwater biota of British agricultural landscapes and their sensitivity to pesticides. *Agric. Ecosyst. Environ.* **122**: 137-148. doi: 10.1016/j.agee.2006.11.013.

Biro, P. A., & Stamps, J. A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior?. *Trends Ecol. Evol.* ***25***: 653-659. doi: 10.1016/j.tree.2010.08.003

Bjornn, T., & Reiser, D. W. (1991). Habitat requirements of salmonids in streams. *American Fisheries Society Special Publication* **19** 138.

Blumstock, M., Tetzlaff, D., Dick, J. J., Nuetzmann, G., & Soulsby, C. (2016) Spatial organization of groundwater dynamics and streamflow response from different hydropedological units in a montane catchment. *Hydrol. Process*. **30**: 3735–3753. doi: 10.1002/hyp.10848.

Bonnet, E., Jalabert, B. & Bobe, J. (2003). A 3-day *in vitro* storage of rainbow trout (*Oncorhynchus mykiss*) unfertilised eggs in coelomic fluid at 12°C does not affect developmental success. *Cybium* **27**: 47-51.

Bonnet, E., Fostier, A., & Bobe, J. (2007). Characterization of rainbow trout egg quality: a case study using four different breeding protocols, with emphasis on the incidence of embryonic malformations. *Theriogenology* **67**: 786-794. doi: 10.1016/j.theriogenology.2006.10.008

Bourret, V., Kent, M. P., Primmer, C. R., Vasemägi, A., Karlsson, S., Hindar, K., McGinnity, P., Verspoor, E., Bernatchez, L. & Lien, S. (2013). SNP-array reveals genome-wide patterns of geographical and potential adaptive divergence across the natural range of Atlantic salmon (*Salmo salar*). *Mol. Ecol.* **22**: 532-551. doi: 10.1111/mec.12003

Bowes, M. J., Gozzard, E., Johnson, A. C., Scarlett, P. M., Roberts, C., Read, D. S., ... & Wickham, H. D. (2012). Spatial and temporal changes in chlorophyll-a concentrations in the River Thames basin, UK: are phosphorus concentrations beginning to limit phytoplankton biomass? *Sci. Total Environ.* **426**: 45-55. doi: 10.1016/j.scitotenv.2012.02.056

Brabrand, Å., Koestler, A. G., & Borgstrøm, R. (2002). Lake spawning of brown trout related to groundwater influx. *J. Fish Biol.* **60**: 751-763. doi: 10.1111/j.1095-8649.2002.tb01699.x

Bradford, M. J., Grout, J. A. & Moodle, S. (2001). Ecology of juvenile chinook salmon in a small non-natal stream of the Yukon River drainage and the role of ice conditions on their distribution and survival. *Can. J. Zool.* **79**: 2043-2054. doi: 10.1139/z01-165

Brännäs, E. (1995). First access to territorial space and exposure to strong predation pressure: A conflict in early emerging Atlantic salmon (*Salmo salar* L.) fry. *Evol, Ecol,* **9**: 411. doi:10.1007/BF01237763.

Braun, D. C., Patterson, D. A., & Reynolds, J. D. (2013). Maternal and environmental influences on egg size and juvenile life-history traits in Pacific salmon. *Ecol. Evol.* **3**: 1727-1740. doi: 10.1002/ece3.555

British Geological Survey. (2016). *Groundwater data and information.*Available: http://www.bgs.ac.uk/research/groundwater/datainfo/dataInformation.html. Last accessed 31st October 2016

Brunke, M. & Gonser, T. (1997). The ecological significance of exchange processes between rivers and groundwater. *Freshwater Biol.* **37**: 1-33. doi: 10.1046/j.1365-2427.1997.00143.x

Buffington, J. M. & Tonina, D. (2009). Hyporheic Exchange in Mountain Rivers II: Effects of Channel Morphology on Mechanics, Scales, and Rates of Exchange. *Geography compass* **3**: 1038-1062. doi: 10.1111/j.1749-8198.2009.00225.x.

Burke, N. (2011). *Physical controls on salmon spawning habitat quality and embryo fitness: An integrated analysis*. Ph.D thesis, University of Southampton.

Burkhalter, D. E. & Kaya, C. M. (1977). Effects of Prolonged Exposure to Ammonia on Fertilized Eggs and Sac Fry of Rainbow Trout (*Salmo gairdneri*). *T. Am. Fish. Soc.* **106**: 470-475. doi: 10.1577/1548-8659(1977)106<470:EOPETA>2.0.CO;2

Burkholder, B. K., Grant, G. E., Haggerty, R., Khangaonkar, T. & Wampler, P. J. (2008). Influence of hyporheic flow and geomorphology on temperature of a large, gravel-bed river, Clackamas River, Oregon, USA. *Hydrol. Process.* **22**: 941-953. doi: 10.1002/hyp.6984

Burow, K. R., Nolan, B. T., Rupert, M. G. & Dubrovsky, N. M., (2010). Nitrate in groundwater of the United States, 1991− 2003. *Environ. Sci. Technol.* **44**: 4988-4997. doi: 10.1021/es100546y.

Burt, T. P., Pinay, G., Matheson, F. E., Haycock, N. E., Butturini, A., Clement, J. C., Danielscu, S. D., Dowrick, D. J., Hefting, M. M., Hillbricht-Ilkowska, A. & Maitre, V. (2002). Water table fluctuations in the riparian zone: comparative results from a pan-European experiment. *J. Hydrol.* **265**: 129-148. doi: 0.1016/S0022-1694(02)00102-6

Buss, S. R., Herbert, A. W., Morgan, P., Thornton, S. F. & Smith, J. W. N. (2004). A review of ammonium attenuation in soil and groundwater. *Q. J. Eng. Geol. Hydrogeol.* **37**: 347-359. doi: 10.1144/1470-9236/04-005.

Butler, I. B., Schoonen, M. A., & Rickard, D. T. (1994). Removal of dissolved oxygen from water: a comparison of four common techniques. *Talanta*, **41**: 211-215. doi: 10.1016/0039-9140(94)80110-X

Butler, J. R. A., Radford, A., Riddington, G. & Laughton, R. (2009). Evaluating an ecosystem service provided by Atlantic salmon, sea trout and other fish species in the River Spey, Scotland: The economic impact of recreational rod fisheries. *Fish. Res.* **96**: 259-266. doi:10.1016/j.fishres.2008.12.006.

Calderwood, W. L. (1901). Water temperature in relation to the early annual migration of salmon from the sea to rivers in Scotland. *Twentieth Annual Report of Fishery Board for Scotland, Part II, The Salmon Fisheries, Paper,2*, p.111.

Cardenas, M.B. & Wilson, J. L. (2006). The influence of ambient groundwater discharge on exchange zones induced by current-bedform interactions. *J. Hydrol.* **331**: 103–109. doi: 10.1016/j.jhydrol.2006.05.012.

Cardenas, M. B., & Markowski, M. S. (2010). Geoelectrical imaging of hyporheic exchange and mixing of river water and groundwater in a large regulated river. *Environ. Sci. Technol.* **45**: 1407-1411. doi: 10.1021/es103438a

Carey, S. K., Tetzlaff, D., Buttle, J., Laudon, H., McDonnell, J., McGuirre, K., Seibert, J., Soulsby, C. & Shanley, J. (2013). Use of color maps and wavelet coherence to discern seasonal and interannual climate influences on streamflow variability in northern catchments. *Water Resour. Res*. **49**: 6194-6207. doi: 10.1002/wrcr.20469.

Carling, P.A. (1985) *Oxygen flux through salmonid spawning gravels.* Ambleside, UK, Freshwater Biological Association, 34pp.(WIT/73/25).

Carlson, A. R., & Siefert, R. E. (1974). Effects of reduced oxygen on the embryos and larvae of lake trout (*Salvelinus namaycush*) and largemouth bass (*Micropterus salmoides*). *J. Fish. Board Can.* **31**: 1393-1396. doi: 10.1139/f74-165

Carss, D. N., Kruuk, H. & Conroy, J. W. H. (1990). Predation on adult Atlantic *Salmo salar* L. by otters *Lutra lutra* in the River Dee system, Aberdeenshire, Scotland. *J. Fish. Biol*. **37**: 935-944. doi: 10.1111/j.1095-8649.1990.tb03597.x.

Casas-Mulet, R., Saltveit, S. J., & Alfredsen, K. (2015). The survival of Atlantic salmon (*Salmo salar*) eggs during dewatering in a river subjected to hydropeaking. *River Res. Applic.* **31**: 433-446. doi: 10.1002/rra.2827

Cederholm, C. J., Johnson, D. H., Bilby, R. E., Dominguez, L. G., Garrett, A. M., Graeber, W. H., Greda, E. L., Kunze, M. D., Marcot, B. G., Palmisano, J. F., Plotnikoff, R. W., Pearcy, W. G., Simenstad, C. A., & Trotter, P. C. (2000). Pacific Salmon and Wildlife - Ecological Contexts, Relationships, and Implications for Management. Special Edition Technical Report, Prepared for D. H. Johnson and T. A. O'Neil (Managing directors), Wildlife-Habitat Relationships in Oregon and Washington. WDFW, Olympia, Washington.

Chadwick, E. M. P. (1982). Stock-Recruitment Relationship for Atlantic Salmon (*Salmo salar*) in Newfoundland Rivers. *Can. J. Fish. Aquat. Sci.* **39**: 1496-1501. doi: 10.1139/f82-201

Chapelle, G., & Peck. L. S. (1999). Polar gigantism dictated by oxygen availability. *Nature* **399**: 114–115. doi: 10.1038/20099

Chapman, D. W. (1988). Critical review of variables used to define effects of fines in redds or large salmonids. *T. Am. Fish. Soc.* **117**: 1-21. doi: 10.1577/1548-8659.

Charles, F., Sanchez, J., & Gontard, N. (2006). Absorption kinetics of oxygen and carbon dioxide scavengers as part of active modified atmosphere packaging. *Food Eng.* **72**: 1-7. doi: 10.1016/j.jfoodeng.2004.11.006

Chawler, R. C. & Pourhashemi, A. (2004). A Mass Transfer Experiment Using Deoxygenation and Aeration of Water. *Proc. Am. Soc. Eng. Educ.* 2425-2436

Chevalier, B. E., & Carson, C. (1985). *Modeling the Transfer of Oxygen Between the Stream and Stream Substrate: With Application to the Survival Rates of Salmonid Embryos*. Colorado State University, Department of Agricultural and Chemical Engineering.

Childress, J. J. & Seibel, B. A. (1998). Life at stable low oxygen levels: adaptations of animals to Oceanic oxygen minimum layers. *J. Exp. Biol.* **201**: 1223-1232.

Ciuhandu, C. S., Stevens, E. D. & Wright, P. A. (2005). The effect of oxygen on the growth of *Oncorhynchus mykiss* embryos with and without a chorion. *J. Fish Biol.* **67**: 1544-1551. doi: 10.1111/j.1095-8649.2005.00856.x

Ciuhandu, C. S., Wright, P. A., Goldberg, J. I., & Stevens, E. D. (2007). Parameters influencing the dissolved oxygen in the boundary layer of rainbow trout (*Oncorhynchus mykiss*) embryos and larvae. *J. Exp. Biol.* **210**: 1435-1445. doi: 10.1242/jeb.02754

Clarke, A., & Fraser, K. P. P. (2004). Why does metabolism scale with temperature? *Funct. Ecol.* **18**: 243-251. doi: 10.1111/j.0269-8463.2004.00841.x

Collins, G. M., Clark, T. D. & Carton, A. G. (2015). Physiological plasticity *v.* inter-population variability: understanding drivers of hypoxia tolerance in a tropical estuarine fish. *Mar. Freshwater Res.* **67**: doi: 10.1071/MF15046.

Côte, J., Roussel, J.-M., Le Cam, S., Bal, G. & Evanno, G. (2012), Population differences in response to hypoxic stress in Atlantic salmon. *J. Evol. Biol.* **25**: 2596–2606. doi:10.1111/jeb.12007

Crisp, D. T. (1988). Prediction, from temperature, of eyeing, hatching and ‘swim-up’ times for salmonid embryos. *Freshwater Biol.* **19**: 41-48. doi: 10.1111/j.1365-2427.1988.tb00325.x

Crisp, D. T. & Carling, P. A. (1989). Observations on siting, dimensions and structure of salmonid redds. *J. Fish Biol.* **34**: 119-134. doi: 10.1111/j.1095-8649.1989.tb02962.

Crisp, D. T. (1996). Environmental requirements of common riverine European salmonid fish species in fresh water with particular reference to physical and chemical aspects. *Hydrobiologia* **323**: 201-221. doi: 10.1007/BF00007847

Crisp, D.T. (ed) (2000) Life Cycles and General Biology, in Trout and Salmon: Ecology, Conservation and Rehabilitation, Blackwell Science Ltd, Oxford, UK. doi: 10.1002/9780470999776.ch2

Crispell, J. K. & Endreny, T. A. (2009). Hyporheic exchange flow around constructed in-channel structures and implications for restoration design. *Hydrol. Process.* **23**: 1158-1168. doi: 10.1002/hyp.7230

Crispo, E., & Chapman, L. J. (2008). Population genetic structure across dissolved oxygen regimes in an African cichlid fish. *Mol. Ecol.* **17**: 2134-2148. doi: 10.1111/j.1365-294X.2008.03729.x

Crispo, E., & Chapman, L. J. (2009). Temporal variation in population genetic structure of a riverine African cichlid fish. *J. Hered.* doi: 10.1093/jhered/esp078

Crispo, E. & Chapman, L. J. (2010), Geographic variation in phenotypic plasticity in response to dissolved oxygen in an African cichlid fish. *J. Evol. Biol.* **23**: 2091–2103. doi:10.1111/j.1420-9101.2010.02069.x

Cronin, E. R. & Seymour, R. S. (2000). Respiration of the eggs of the giant cuttlefish *Sepia apama*. *Mar. Biol.* **136**: 863-870. doi: 10.1007/s002270000274

Cunjak, R. A., Prowse, T. D. & Parrish, D. L. (1998). Atlantic salmon (*Salmo salar*) in winter: “the season of parr discontent”? *Can. J. Fish. Aquat. Sci.* **55**: 161–180. doi: 10.1139/d98-008

Curry, R. A., Noakes, D. L. G., & Morgan, G. E. (1995). Groundwater and the incubation and emergence of brook trout (*Salvelinus fontinalis*). *Can. J. Fish. Aquat. Sci.* **52**: 1741–1749. doi: 10.1139/f95-766

Cussler, E. L. (1997). *Diffusion: Mass Transfer in Fluid Systems* (2nd ed.). New York: Cambridge University Press.

Cutts, C. J., Brembs, B., Metcalfe, N. B. & Taylor, A. C. (1999). Prior residence, territory quality and life-history strategies in juvenile Atlantic salmon (*Salmo salar* L.). *J. Fish. Biol.* **55**: 784-794. doi: 10.1111/j.1095-8649.1999.tb00717.

Czerkies, P., Brzuzan, P., Kordalski, K., Luczynski, M. (2001). Critical partial pressures of oxygen causing precocious hatching in *Coregonus lavaretus* and *C. albula* embryos. *Aquaculture* **196**: 151-158. doi: 10.1016/S0044-8486(00)00545-7

Czerkies, P., Kordalski, K., Golas, T., Krysinski, D., & Luczynski, M. (2002). Oxygen requirements of whitefish and vendace (Coregoninae) embryos at final stages of their development. *Aquaculture 211*: 375-385. doi: 10.1016/S0044-8486(02)00049-2

Danielopol, D. L. & Niederreiter, R. (1987). A sampling device for groundwater organisms and oxygen measurement in multi-level monitoring wells. *Stygologia* **3**: 252-263.

Danner, G. R. (2008). Salmonid embryo development and pathology. In *American Fisheries Society Symposium* (Vol. 65).

Dauble, D. D., & Watson, D. G. (1990). *Spawning and abundance of fall chinook salmon (Oncorhynchus tshawytscha) in the Hanford Reach of the Columbia River, 1948--1988* (No. PNL-7289). Pacific Northwest Lab., Richland, WA (USA).

Davenport, J., Lønning, S., & Kjørsvik, E. (1986). Some mechanical and morphological properties of the chorions of marine teleost eggs. *J. Fish Biol.* **29**: 289-301. doi: 10.1111/j.1095-8649.1986.tb04946.x

Davis, J. C. (1975). Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: a review. *J. Fish. Board Can.* **32**: 2295-2332. doi: 10.1139/f75-268.

Davy-Bowker, J., Sweeting, W., Wright, N., Clarke, R. T. & Arnott, S. (2006). The distribution of benthic and hyporheic macroinvertebrates from the heads and tails of riffles. *Hydrobiologia* **563**: 109-123. doi: 10.1007/s10750-005-1482-9.

Daykin, P. N. (1965). Application of mass transfer theory to problem of respiration of fish eggs. *J. Fish Res. Board Can.* **22:** 159–171.

De Ketelaere, B., Govaerts, T., Coucke, P., Dewil, E., Visscher, J., Decuypere E. & De Baerdemaeker, J. (2002) Measuring the eggshell strength of 6 different genetic strains of laying hens: Techniques and comparisons. *British Poultry Science* **43**: 238-244, doi: 10.1080/00071660120121454

Depledge, M. H. (1987). Enhanced copper toxicity resulting from environmental stress factor synergies. *Comp. Biochem. Physiol. C Comp. Pharmacol.* **87**: 15-19. doi: 10.1016/0742-8413(87)90172-1.

DeVries, P. (1997). Riverine salmonid egg burial depths: review of published data and implications for scour studies. *Can. J. Fish. Aquat. Sci.* **54**: 1685-1698. doi: 10.1139/f97-090.

Dhiyebi, H. A., O'Donnell, M. J. & Wright, P. A. (2013). Water chemistry in the microenvironment of the rainbow trout *Oncorynchus mykiss* embryos is affected by development, the egg capsule and crowding. *J. Fish Biol.* **82**: 444-457. doi: 10.1111/j.1095-8649.2012.03491.x.

Dillon, M. E., Wang, G., & Huey, R. B. (2010). Global metabolic impacts of recent climate warming. *Nature* **467**: 704-706. doi: 10.1038/nature09407

Downing, K. M., & Merkens, J. C. (1957). The influence of temperature on the survival of several species of fish in low tensions of dissolved oxygen. *Annals of Applied Biology* **45**: 261-267. doi: 10.1111/j.1744-7348.1957.tb00465.x

Eddy, F. B., Ward, M. R., Talbot, C., & Primmett, D. (1990). Ionic movements across the chorion in newly shed salmon eggs (*Salmo salar* L.). *J. Comp. Physiol. B* **159**: 771-776. doi: 10.1007/BF00691723. doi: 10.1007/BF00691723

Einum, S. & Fleming, I. A. (1997). Genetic divergence and interactions in the wild among native, farmed and hybrid Atlantic salmon. *J. Fish Biol.* **50**: 634-651. doi: 0.1111/j.1095-8649.1997.tb01955.x.

Einum, S., & Fleming, I. A. (1999). Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality. *Proc. R. Soc. Lond. B.* **266**: 2095-2100. doi: 10.1098/rspb.1999.0893.

Einum, S. & Fleming, I. A. (2000a). Selection against late emergence and small offspring in atlantic salmon (*salmo salar*). *Evolution* **54**: 628-639. doi: 10.1111/j.0014-3820.2000.tb00064.x

Einum, S., & Fleming, I. A. (2000b). Highly fecund mothers sacrifice offspring survival to maximize fitness. *Nature* **405**: 565-567. doi: 10.1038/35014600.

Einum, S., Hendry, A. P. & Fleming, I. A. (2002). Egg-size evolution in aquatic environments: does oxygen availability constrain size? *Proc. R. Soc. Lond. B.* **269**: 2325-2330. doi: 10.1098/rspb.2002.2150.

Einum, S. & Nislow, K. H. (2005). Local-scale density-dependent survival of mobile organisms in continuous habitats: an experimental test using Atlantic salmon. *Oecologia* **143**: 203-210. doi: 10.1007/s00442-004-1793-y.

Eldridge, W. H. & Naish, K. A. (2007). Long-term effects of translocation and release numbers on fine-scale population structure among coho salmon (*Oncorhynchus kisutch*). *Mol. Ecol.* **16**: 2407-2421. doi: 10.1111/j.1365-294X.2007.03271.x.

Ellis, A. E. (1999). Immunity to bacteria in fish. *Fish Shellfish Immun.* **9**: 291-308. doi: 10.1006/fsim.1998.0192

Elson, P. F. (1975). Atlantic salmon rivers, smolt production and optimal spawning: An overview of natural production. International Atlantic salmon foundation special publication series 6:96-119.

Environment Agency (2007). Climate change impacts and water temperature. Science Report: SC060017/SR.

Environment Agency (2009a). Economic evaluation of inland fisheries: Managers report from science project SC050026/SR2. Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/291109/scho0109bpgi-e-e.pdf.

Environment Agency (2009b). The Hyporheic Handbook: A handbook on the groundwater – surface water interface and hyporheic zone for environment managers. Integrated catchment science programme. Science Report: SC050070.

Ferreira, A. L., Loureiro, S., & Soares, A. M. (2008). Toxicity prediction of binary combinations of cadmium, carbendazim and low dissolved oxygen on Daphnia magna. *Aquat. Toxicol.* **89**: 28-39. doi: 10.1016/j.aquatox.2008.05.012

Finn, R. N. (2007). The physiology and toxicology of salmonid eggs and larvae in relation to water quality criteria. *Aquat. Toxicol.* **81**: 337-354. doi: 10.1016/j.aquatox.2006.12.021.

Firpo, G., Angeli, E., Repetto, L. & Valbusa, U. (2015). Permeability thickness dependence of polydimethylsiloxane (PDMS) membranes. *J. Memb. Sci.* **481**: 1-8. doi: 10.1016/j.memsci.2014.12.043

Flamarique, I. N. & Harrower, W. L. (1999). Mortality of Sockeye Salmon Raised Under Light Backgrounds of Different Spectral Composition. *Environ. Biol. Fishes* **55**: 279-293. doi: 10.1023/A:1007528603387/

Fleming, I. A. & Gross, M. R. (1990). Latitudinal clines: a trade-off between egg number and size in pacific salmon. *Ecology* **71**: 1-11. doi: 10.2307/1940241.

Foote, C. J. & Brown, G. S. (1998). Ecological relationship between freshwater sculpins (genus Cottus) and beachspawning sockeye salmon (Oncorhynchus nerka) in Iliamna Lake, Alaska. *Can. J. Fish. Aquat. Sci.*: **55**: 1524–1533. doi: 10.1139/f98-034

Foss, A. & Imsland, A.K. (2002) Compensatory growth in the spotted wolfish *Anarhichas minor* (Olafsen) after a period of limited oxygen supply*. Aquaculture Research* **33**: 1097-1101. doi: 10.1046/j.1365-2109.2002.00768.x

Foster, S. S. D. & Chilton, P. J. (2003). Groundwater: the processes and global significance of aquifer degradation. *Phil. Trans. R. Soc. B.* **358**: 1957-1972. doi: 10.1098/rstb.2003.1380

Fraser, D. J., Weir, L. K., Bernatchez, L., Hansen, M. M. & Taylor, E. B. (2011). Extent and scale of local adaptation in salmonid fishes: review and meta-analysis. *Heredity* **106**: 404-420. doi: 0.1038/hdy.2010.167.

Fraysse, B., Mons, R., & Garric, J. (2006). Development of a zebrafish 4-day embryo-larval bioassay to assess toxicity of chemicals. *Ecotoxicology and environmental safety* **63** 253-267. doi: 10.1016/j.ecoenv.2004.10.015.

Freeze, R. A. & Cherry, J. A. (1979). *Groundwater* (No. 629.1 F7).

Fresh, K. L., & Schroder, S. L. (1987). Influence of the abundance, size, and yolk reserves of juvenile chum salmon (*Oncorhynchus keta*) on predation by freshwater fishes in a small coastal stream. *Can. J. Fish. Aquat. Sci.* **44**: 236-243.doi: 10.1139/f87-033

Friedland, K. D., Hansen, L. P., Dunkley, D. A., MacLean, J. C. (2000). Linkage between ocean climate, post-smolt growth, and survival of Atlantic salmon (*salmo salar* L.) in the North Sea area. *ICES Journal of Marine Science* **57**: 419-429.

Fuiman, L. A. & Werner, R. G. (2002). Fishery science: the unique contribution of early life stage. *UK. Blackwell Science Ltd., 326p.*

Gaino, E., Sartori, M. & Rebora, M. (2001). Chorionic fine structure of eggs from some species of *Proboscidoplocia* (Ephemeroptera, Ephemeroidea). Italian J. Zool. **68**: 1-8. doi: 10.1080/11250000109356376.

García de Leaniz, C., Fraser, N. & Huntingford, F. A. (2000). Variability in performance in wild Atlantic salmon, *Salmo salar* L., fry from a single redd. *Fisheries Manag. Ecol.* **7**: 489-502. doi: 10.1046/j.1365-2400.2000.00223.x

Garcia de Leaniz, C., Fleming, I. A., Einum, S., Verspoor, E., Jordan, W. C., Consuegra, S., Aubin-Horth, N., Lajus, D., Letcher, B. H., Youngson, A. F., Webb, J. H., Vøllestad, L. A., Villanueva, B., Ferguson, A. & Quinn, T. P. (2007). A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. *Biol. Rev.* **82**: 173–211. doi: 10.1111/j.1469-185X.2006.00004.x

Geist, D. R., & Dauble, D. D. (1998). Redd site selection and spawning habitat use by fall chinook salmon: the importance of geomorphic features in large rivers. *Environ. Manage.* **22**: 655-669. doi: 10.1007/s002679900137

Geist, D. R. (2000). Hyporheic discharge of river water into fall chinook salmon (*Oncorhynchus tshawytscha*) spawning areas in the Hanford Reach, Columbia River. *Can. J. Fish. Aquat. Sci.* **57**: 1647-1656. doi: 10.1139/f00-102

Geist, D. R., Abernathy, C. S., Hand, K. D., Cullinan, V. I., Chandler, J. A. & Groves, P. A. (2006). Survival, development, and growth of fall chinook salmon embryos, alevins, and fry exposed to variable thermal and dissolved oxygen regimes. *T. Am. Fish. Soc.* **135**: 1462-1477. doi: 10.1577/T05-294.1.

Gusnard, D. & Kirschner, R. H. (1977). Cell and organelle shrinkage during preparation for scanning electron microscopy: effects of fixation, dehydration and critical point drying. *Journal of Microscopy* **110**: 51–57. doi:10.1111/j.1365-2818.1977.tb00012.x

Gibbins, C., Soulsby, C., & Malcolm, I. (2009, April). Impact of artificial freshet releases on channel hydraulics and the hyporheic zone of a gravel bed river. In *EGU General Assembly Conference Abstracts* (Vol. 11, p. 10800).

Gibbins, C. N., Grant, J., Malcolm, I. A., & Soulsby, C. (2016). Influence of groundwater chemistry on hyporheic invertebrate assemblages is revealed by fine-scale sampling. *Fundam. Appl. Limnol.* **187**: 207-221. doi: 10.1127/fal/2016/0787

Gibson, R. J. (1993). The Atlantic salmon in fresh water: spawning, rearing and production. *Rev. Fish Biol. Fisher.* **3**: 39-73. doi: 10.1007/BF00043297.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., & Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science* **293**: 2248-2251. doi: 10.1126/science.1061967

Goncalves, I. B., Ahnesjö, I., & Kvarnemo, C. (2015). The evolutionary puzzle of egg size, oxygenation and parental care in aquatic environments. In *Proc. R. Soc. B* (Vol. 282, No. 1813, p. 20150690). doi: 10.1098/rspb.2015.0690

Gooseff, M. N., Anderson, J. K., Wondzell, S. M., LaNier, J. & Haggerty, R. (2006), A modelling study of hyporheic exchange pattern and the sequence, size, and spacing of stream bedforms in mountain stream networks, Oregon, USA. *Hydrol. Process.* **20**: 2443–2457. doi:10.1002/hyp.6349

Gooseff, M. N., Hall Jr., R. O. & Tank, J. L. (2007) Relating transient storage to channel complexity in streams of varying land use in Jackson Hole, Wyoming. *Water Resour. Res.* **43**, W01417, doi:10.1029/2005WR004626.

Gorodilov, Y. N. (1996). Description of the early ontogeny of the Atlantic salmon, *Salmo salar*, with a novel system of interval (state) identification. *Environ. Biol. Fish.* **47**: 109-127. doi: 10.1007/BF00005034.

Gracey, A. Y., Troll, J. V., & Somero, G. N. (2001). Hypoxia-induced gene expression profiling in the euryoxic fish *Gillichthys mirabilis*. *Proc. Nat. Acad. Sci.* **98**: 1993-1998. doi: 10.1073/pnas.98.4.1993

Greig, S. M. (2004). *An assessment of factors influencing the ability of U.K. spawning gravels to support the respiratory requirements of Atlantic salmon (Salmo salar) embryos*. Ph.D thesis. University of Southampton, UK.

Greig, S. M., Sear, D. A. & Carling, P. A. (2005a). The impact of fine sediment accumulation on the survival of salmon progeny: Implications for sediment management. *Sci. Total Environ.* **344**: 241-258. doi: 10.1016/j.scitotenv.2005.02.010.

Greig, S. M., Sear, D. A., Smallman, D. J. & Carling, P. A. (2005b). Impact of clay particles on the cutaneous exchange of oxygen across the chorion of Atlantic Salmon eggs. *J. Fish Biol*. **66**: 1681-1691. doi: 10.1111/j.0022-1112.2005.00715.x

Greig, S. M., Sear, D. A. & Carling, P. A. (2007a). A review of factors influencing the availability of dissolved oxygen to incubating salmonid embryos. *Hydrol. Proc.* **21**: 323-334. doi: 10.1002/hyp.6188.

Greig, S., Sear, D. & Carling, P. A. (2007b). A field-based assessment of oxygen supply to incubating Atlantic salmon (Salmo salar) embryos. *Hydrological Processes* **21***:* 3087-3100.

Grinsted, A., Moore, J. C. Jevrejeva, S. (2004). Application of the cross wavelet transform and wavelet coherence to geophysical time series *Nonlinear Processes Geophys.*, **11**: 561–566, doi:10.5194/npg-11-561-2004

Groot, E. P. & Alderdice, D. F. (1985). Fine structure of the external egg membrane of Pacific salmon and steelhead trout. *Can. J. Zool*. **63**: 552-566. doi: 10.1139/z85-082.

Gruber, K. & Wieser, W. J. (1983) Energetics of development of the alpine charr,*salvelinus alpinus*, in relation to temperature and oxygen. *J. Comp Physiol B* **149**: 485-493. doi:10.1007/BF00690007.

Hackney, C., Darby, S. E., & Leyland, J. (2013). Modelling the response of soft cliffs to climate change: A statistical, process-response model using accumulated excess energy. *Geomorphology*, *187*, 108-121. doi: 10.1016/j.geomorph.2013.01.005

Hamor, T. & Garside, E. T. (1976). Developmental rates of embryos of Atlantic salmon, *Salmo salar* L., in response to various levels of temperature, dissolved oxygen, and water exchange. *Can. J. Zoolog.* **54**: 1912-1917. doi: 10.1139/z76-221.

Hamor, T., & Garside, E. T. (1977). Size relations and yolk utilization in embryonated ova and alevins of Atlantic salmon *Salmo salar* L. in various combinations of temperature and dissolved oxygen. *Can. J. Zool.* **55**: 1892-1898. doi: 10.1139/z77-242

Hanazato, T., & Dodson, S. I. (1995). Synergistic effects of low oxygen concentration, predator kairomone, and a pesticide on the cladoceran Daphnia pulex. *Limnol. Oceanogr.* **40**: 700-709. doi: 10.4319/lo.1995.40.4.0700

Hannaford, J., & Buys, G. (2012). Trends in seasonal river flow regimes in the UK. *J. Hydrol.* **475:** 158-174. doi: 10.1016/j.jhydrol.2012.09.044

Hannah, D. M., Malcolm, I. A., & Bradley, C. (2009). Seasonal hyporheic temperature dynamics over riffle bedforms. *Hydrol. Process.* **23**: 2178-2194. doi: 10.1002/hyp.7256

Hanrahan, T. P. (2008). Effects of river discharge on hyporheic exchange flows in salmon spawning areas of a large gravel-bed river. *Hydrol. Process*. **22**: 127–141. doi:10.1002/hyp.6605

Hansen, E. A. (1975). Some Effects of Groundwater on Brown Trout Redds. *T. Am. Fish Soc.* **104**: 100-110. doi: 10.1577/1548-8659(1975)104<100:SEOGOB>2.0.CO;2

Hansen, T. J. & Møller, D. (1985). Yolk Absorption, Yolk Sac Constrictions, Mortality, and Growth During First Feeding of Atlantic Salmon (*Salmo salar*) Incubated on Astro-turf. *Can. J. Fish. Aquat, Sci.* **42**: 1073-1078. doi: 10.1139/f85-133.

Harrison, S. J., Winterbottom, S., & Johnson, R. (2001). *Climate change and changing snowfall patterns in Scotland*. Edinburgh: Scottish Executive Central Research Unit.

Harrison, J., Frazier, M. R., Henry, J. R., Kaiser, A., Klok, C. J., & Rascón, B. (2006). Responses of terrestrial insects to hypoxia or hyperoxia. *Respir. Physiol. Neurobiol.* **154**: 4-17. doi: 10.1016/j.resp.2006.02.008

Harshbarger T.J. & Porter P.E. (1979) Survival of brown trout eggs: two planting techniques compared. *The Progressive Fish-Culturist* **41**: 206–209. doi: 10.1577/1548-8659(1979)41[206:SOBTE]2.0.CO;2

Harvey, J. W. & Bencala, K. E. (1993). The effect of streambed topography on surface-subsurface water exchange in mountain catchments. *Water Resour. Res.* **29**: 89–98. doi: 10.1029/92WR01960

Hatcher, K., (1980). Sediment Component of Oxygen Demand in Streams. Institute of Natural Resources, University of Georgia, Athens, Georgia.

Hauer, C., Unfer, G., Tritthart, M. & Habersack, H. (2011), Effects of stream channel morphology, transport processes and effective discharge on salmonid spawning habitats. *Earth Surf. Process. Landforms*, **36**: 672–685. doi:10.1002/esp.2087

Haycock, N. E. & Burt, T. P. (1993). Role of floodplain sediments in reducing the nitrate concentration of subsurface run-off: A case study in the Cotswolds, UK. *Hydrol. Process*. **7**: 287–295. doi:10.1002/hyp.3360070306

Hayes, F. R., Wilmot, I. B. & Livingstone, D. A. (1951). The oxygen consumption of the salmon egg in relation to development and activity. *J. Exp. Zool.* **116**: 377–395. doi:10.1002/jez.1401160302.

Heath, D. D., Heath, J. W., Bryden, C. A., Johnson, R. M. & Fox, C. W. (2003). Rapid Evolution of Egg Size in Captive Salmon. *Science* **299**: 1738-1740. doi: 10.1126/science.1079707.

Heggenes, J., Bremset, G., & Brabrand, Å. (2010). Groundwater, critical habitats, and behaviour of Atlantic salmon, brown trout and Arctic char in streams. *NINA Rapport 654: 28 pp. Norsk institutt for naturforskning, Trondheim., 654*.

Heinimaa S. & Heinimaa P. (2004) Effect of the female size on egg quality and fecundity of the wild Atlantic salmon in the sub-arctic River Teno. *Boreal Environment Research* **9**: 55–62.

Heming, T. A. (1982). Effects of temperature on utilization of yolk by chinook salmon (*Oncorhynchus tshawytscha*) eggs and alevins. *Can. J. Fish. Aquat. Sci.* **39***:* 184-190. doi: 10.1139/f82-021.

Hendricks, S. P. & White, D. S. (1995). Seasonal biogeochemical patterns in surface water, subsurface hyporheic, and riparian ground water in a temperate stream ecosystem. *Archiv für Hydrobiologie* **134**: 459-490.

Hester, E. T. & M. W. Doyle (2008), In-stream geomorphic structures as drivers of hyporheic exchange, *Water Resour. Res*. **44**, W03417, doi:10.1029/2006WR005810.

Hester, E. T. & Gooseff, M. N. (2011). Hyporheic Restoration in Streams and Rivers, in Stream Restoration in Dynamic Fluvial Systems (eds A. Simon, S. J. Bennett and J. M. Castro), American Geophysical Union, Washington, D. C.. doi: 10.1029/2010GM000966

Heugens, E. H., Hendriks, A. J., Dekker, T., Straalen, N. M. V., & Admiraal, W. (2001). A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. *Crit. Rev. Toxicol.* ***31***: 247-284. doi: 10.1080/20014091111695.

Hie, Y. & Liu, C. (2011). Optimizing the design of in situ sediment oxygen demand measurement chambers. *Int. J. Sed. Res.* **26**: 222-229. doi: 10.1016/S1001-6279(11)60088-7

Hochachka, P. W. (1997). Oxygen—A Key Regulatory Metabolite in Metabolic Defense Against Hypoxia. *Am. Zool.* **37**: 595-603. doi: 10.1093/icb/37.6.595.

Holling, C. S. (1959). The natural control of animal populations. *J. Anim. Ecol*, **18**: 1-35.

Holmstrup, M., Bindesbøl, A. M., Oostingh, G. J., Duschl, A., Scheil, V., Köhler, H. R., Loureiro, S., Soares, A. M., Ferreira, A. L., Kienle, C. & Gerhardt, A. (2010). Interactions between effects of environmental chemicals and natural stressors: a review. *Sci. Total Environ.* **408**: 3746-3762. doi: 10.1016/j.scitotenv.2009.10.067

Hrachowitz, M., Soulsby, C., Tetzlaff, D., Dawson, J. J. C., Dunn, S. M., Malcolm, I. A. (2009). Using long-term data sets to understand transit times in contrasting headwater catchments. *J. Hydrol.* **367**: 237-248. doi:10.1016/j.jhydrol.2009.01.001.

Huber, E., Huggenberger, P., Epting, J. & Schindler Wildhaber, Y. (2013). Spatiotemporal scales of river-groundwater interaction: a multidimensional hydrogeological investigation approach. *Grundwasser* **18**: 159-172. doi: http://dx.doi.org/10.1007/s00767-013-0220-x.

Huusko, A., Greenberg, L., Stickler, M., Linnansaari, T., Nykänen, M., Vehanen, T., Koljonen, S., Louhi, P. & Alfredsen, K. (2007). Life in the ice lane: the winter ecology of stream salmonids. *River Res. Applic.* **23**: 469-491. doi: 10.1002/rra.999

ICES (2015) Report of the working group on north Atlantic salmon (WGNAS). Moncton, Canada.

Ingendahl, D., Borchardt, D., Saenger, N., & Reichert, P. (2009). Vertical hydraulic exchange and the contribution of hyporheic community respiration to whole ecosystem respiration in the River Lahn (Germany). *Aquat. Sci.* **71**: 399-410. doi: 10.1007/s00027-009-0116-0

IUCN (International Union for Conservation of Nature). (1998). Guidelines for re-introductions. IUCN, Gland, Switzerland.

Jackson, C. R., Meister, R. & Prudhomme, C. (2011). Modelling the effects of climate change and its uncertainty on UK chalk groundwater resources from an ensemble of global climate model predictions. *J. Hydrol*. **399**: 12-28. doi: 10.1016/j.jhydrol.2010.12.028.

Jensen, J. O. T. & Alderdice, D. F. (1983). Changes in mechanical shock sensitivity of coho salmon (*Oncorhynchus kisutch*) eggs during incubation. *Aquaculture* **32**: 303-312.

Jensen, J. O. T. & Alderice, D. F. (1984). Effect of temperature on short-term storage of eggs and sperm of chum salmon (Oncorhynchus keta). *Aquaculture* **37**: 251-265.

Jensen, J. O. T. & Alderice, D. F. (1989). Comparison of mechanical shock sensitivity of eggs of five Pacific salmon (*Oncorhynchus*) species and steelhead trout (*Salmo gairdneri*). *Aquaculture* **78**: 163-181. doi: 10.1016/0044-8486(89)90030-6.

Jensen, D. W., Steel, E. A., Fullerton, A. H., & Pess, G. R. (2009). Impact of fine sediment on egg-to-fry survival of Pacific salmon: a meta-analysis of published studies. *Rev. Fish. Sci.* **17**: 348-359. doi:

Jezierska, B., Ługowska, K. & Witeska, M. (2009). The effects of heavy metals on embryonic development of fish (a review). *Fish physiol. biochem.* **35**: 625-640. doi:10.1007/s10695-008-9284-4.

Johnson, J. H., Nack, C. C., & Chalupnicki, M. A. (2009). Predation by fallfish (*Semotilus corporalis*) on Pacific salmon eggs in the Salmon River, New York. *J. Great Lakes Res.* **35**: 630-633. doi: 10.1016/j.jglr.2009.08.001

Johnson, C.L., Roni, P., & Pess, G.R. (2012). Parental effect as a primary factor limiting egg-to-fry survival of spring Chinook salmon in the Upper Yakima River Basin. *Trans. Am. Fish. Soc.* **141**: 1295–1309. doi:10.1080/00028487.2012. 690815.

Johnston, I. A., Strugnell, G., McCracken, M. L., & Johnstone, R. (1999). Muscle growth and development in normal-sex-ratio and all-female diploid and triploid Atlantic salmon. *J. Exp. Biol.* **202**: 1991-2016.

Jonsson, B., Jonsson, N., & Hansen, L. P. (2003). Atlantic salmon straying from the River Imsa. *J. Fish Biol.* **62**: 641-657. doi: 10.1046/j.1095-8649.2003.00053.x

Jonsson, B., & Jonsson, N. (2016). Trans-generational maternal effect: temperature influences egg size of the offspring in Atlantic salmon *Salmo salar*. *J. Fish Biol.* **89**: 1482-1487. doi: 10.1111/jfb.13040

Joyce, W., Ozolina, K., Mauduit, F., Ollivier, H., Claireaux, G., & Shiels, H. A. (2016). Individual variation in whole-animal hypoxia tolerance is associated with cardiac hypoxia tolerance in a marine teleost. *Biology letters* **12**: 20150708. doi: 10.1098/rsbl.2015.0708

Julien, H. P. & Bergeron. N. E. (2006). Effect of fine sediment infiltration during the incubation period on Atlantic salmon (*Salmo salar*) embryo survival. *Hydrobiologia* **563**: 61–71. doi: 10.1007/s10750-005-1035-2.

Kagawa, Y., Miyahara, H., Ota, Y., & Tsuneda, S. (2016). System for measuring oxygen consumption rates of mammalian cells in static culture under hypoxic conditions. *Biotech. Prog.* **32**: 189-197. doi: 10.1002/btpr.2202

Kamler, E. (2008). Resource allocation in yolk-feeding fish. *Rev. Fish Biol. Fisher.* **18**: 143-200. doi: 10.1007/s11160-007-9070-x

Kang, S., & Lin, H. (2007). Wavelet analysis of hydrological and water quality signals in an agricultural watershed. *J. Hydrol.* **338**: 1-14. doi: 10.1016/j.jhydrol.2007.01.047

Kaplan, E. L. & Meier, P. (1958). Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* **53**: 457-481. doi:

Kasahara, T. & Hill, A. R. (2007). Lateral Hyporheic Zone Chemistry in an Artificially Constructed Gravel Bar and a Re-Meandered Stream Channel, Southern Ontario, Canada. *JAWRA J. Am. Water Resour. Assoc.* 43: 1257–1269. doi:10.1111/j.1752-1688.2007.00108.x

Kasahara, T., & Wondzell, S. M. (2003). Geomorphic controls on hyporheic exchange flow in mountain streams. *Water Resour. Res.* **39**: 1005, doi:10.1029/2002WR001386.

Käser, D. H., Binley, A., Heathwaite, A. L. & Krause, S. (2009). Spatio-temporal variations of hyporheic flow in a riffle-step-pool sequence. *Hydrol. Process.* **23**: 2138-2149. doi: 10.1002/hyp.7317.

Katopodis, C. (2005). Developing a toolkit for fish passage, ecological flow management and fish habitat works. *J. Hydraul. Res.* **43**: 451-467. doi: 10.1080/00221680509500144

Kayar, S. R., Snyder, G. K., Birchard, G. F., & Black, C. P. (1981). Oxygen permeability of the shell and membranes of chicken eggs during development. *Resp. Physiol.* **46**: 209-221.

Kazakov, R. V. (1981). The effect of the size of Atlantic salmon, *Salmo salar* L., eggs on embryos and alevins. *J. Fish Biol.* **19**: 353-360. doi: 10.1111/j.1095-8649.1981.tb05838.x

Keener, V. W., Feyereisen, G. W., Lall, U., Jones, J. W., Bosch, D. D., & Lowrance, R. (2010). El-Niño/Southern Oscillation (ENSO) influences on monthly NO 3 load and concentration, stream flow and precipitation in the Little River Watershed, Tifton, Georgia (GA). *J. Hydrol.* **381**: 352-363. doi: 10.1016/j.jhydrol.2009.12.008.

Kemp, P., Sear, D., Collins, A., Naden, P. & Jones, I. (2011). The impacts of fine sediment on riverine fish. *Hydrol. Process.* **25**: 1800-1821. doi: 10.1002/hyp.7940.

Ketola, H. G., Bowser, P. R., Wooster, G. A., Wedge, L. R. & Hurst, S. S. (1999). Effects of Thiamine on Reproduction of Atlantic Salmon and a New Hypothesis for Their Extirpation in Lake Ontario. *T. Am. Fish. Soc.* **129**: 607-612. doi: 10.1577/1548-8659(2000)129<0607:EOTORO>2.0.CO;2

Kincheloe, J. W., Wedemeyer, G. A. & Koch D. C. (1979). Tolerance of developing salmonid eggs and fry to nitrate exposure. *Bull. Environ. Contam. Toxicol*. **23**: 575–578.

Kindsvater, H. K., Braun, D. C., Otto, S. P., & Reynolds, J. D. (2016). Costs of reproduction can explain the correlated evolution of semelparity and egg size: theory and a test with salmon. *Ecology letters* **19**: 687-696. doi: 10.1111/ele.12607.

Klok, C. J., Sinclair, B. J., & Chown, S. L. (2004). Upper thermal tolerance and oxygen limitation in terrestrial arthropods. *J. Exp. Biol.* **207**: 2361-2370. doi: 10.1242/jeb.01023

Kondolf, G. M. & Wolman, M. G. (1993). The sizes of salmonid spawning gravels. *Water. Resour. Res.* **29**: 2275-2285.

Kondolf, G. M. (2000). Some Suggested Guidelines for Geomorphic Aspects of Anadromous Salmonid Habitat Restoration Proposals. *Restoration Ecology* **8:** 48-56. doi: 10.1046/j.1526-100x.2000.80007.x

Korwin-Kossakowski, M. (2012). Fish hatching strategies: a review. *Rev. Fish Biol. Fisher.* **22**: 225-240. doi: 10.1007/s11160-011-9233-7

Kovats, R. S., Valentini, R., Bouwer, L.M., Georgopoulou, E., Jacob, D., Martin, E., Rounsevell, M. & Soussana, J.-F. (2014): Europe. In: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Barros, V.R. *et al*. (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 1267-1326.

Koyama, T., Takano, H., & Yokoyama, T. (2011). Micropores in the Vitteline Layer of the Eggs of the Dragonfly *Oligoaeshna pryeri*: A Preliminary Observation from the Viewpoint of Oxygen Uptake. In *Oxygen Transport to Tissue XXXII* (pp. 307-310). Springer US.

Krause, S., Heathwaite, L., Binley, A. & Keenan, P. (2009). Nitrate concentration changes at the groundwater-surface water interface of a small Cumbrian river. *Hydrol. Process.* 23: 2195–2211. doi: 10.1002/hyp.7213

Krause, S., Blume, T. & Cassidy, N. J. (2012). Investigating patterns and controls of groundwater up-welling in a lowland river by combining Fibre-optic Distributed Temperature Sensing with observations of vertical hydraulic gradients. *Hydrol. Earth Syst. Sci.* **16**: 1775-1792. doi: 10.5194/hess-16-1775-2012

Krause, S., Tecklenburg, C., Munz, M. & Naden, E. (2013). Streambed nitrogen cycling beyond the hyporheic zone: Flow controls on horizontal patterns and depth distribution of nitrate and dissolved oxygen in the upwelling groundwater of a lowland river. *J. Geophys. Res.: Biogeosci.* **118**: 54-67. doi: 10.1029/2012JG002122

Krogh, A. (1959). The comparative physiology of respiratory mech- anisms. Philadelphia, PA: University of Pennsylvania Press.

Kutchai, H., & Steen, J. B. (1971). Permeabilty of the shell and shell membraes of hens' eggs during development. *Resp. Physiol.* **11**: 265-278. doi: 10.1016/0034-5687(71)90001-6

Labat, D. (2008). Wavelet analysis of the annual discharge records of the world's largest rivers, *Adv. Water Resour.*, **31**: 109–117, doi:10.1016/j.advwatres.2007.67.004.

Lachniet, M. S., S. J. Burns, D. R. Piperno, Y. Asmerom, V. J. Polyak, C. M. Moy, and K. Christenson (2004), A 1500-year El Niño/Southern Oscillation and rainfall history for the Isthmus of Panama from speleothem calcite, J. Geophys. Res., 109, D20117, doi:10.1029/2004JD004694.

Lacroix, G. L. (1985). Survival of Eggs and Alevins of Atlantic Salmon (*Salmo salar*) in Relation to the Chemistry of Interstitial Water in Redds in some Acidic Streams of Atlantic Canada. *Can. J. Fish. Aquat. Sci.* **45**: 292-299. doi: 10.1139/f85-037.

Lapointe, M., Eaton, B., Driscoll, S. & Latulippe, C. (2000). Modelling the probability of salmonid egg pocket scour due to floods. *Can. J. Fish. Aquat. Sci.* **57**: 1120-1130. doi: 10.1139/f00-033.

Larsen, M. H., Johnsson, J. I., Winberg, S., Wilson, A. D. M., Hammenstig, D., Thörnqvist, P., Midwood, J. D., Aarestrup, K. & Höglund, E. (2015). Effects of Emergence Time and Early Social Rearing Environment on Behaviour of Atlantic Salmon: Consequences for Juvenile Fitness and Smolt Migration. *PLoS One* **10**: e0119127. doi:10.1371/journal.pone.0119127.

Le Cam, S., Perrier, C., Besnard, A.-L., Bernatchez, L., Evanno, G. (2015). Genetic and phenotypic changes in an Atlantic salmon population supplemented with non-local individuals: a longitudinal study over 21 years. *Proc .R. Soc. Lond. [Biol.]* **282**: 20142765. doi: 10.1098/rspb.2014.2765.

León-Velarde, F., Monge, C. & Carey, C. (1997). Physiological Strategies of Oxygen Transport in High Altitude Bird Embryos. *Comp. Biochem. Phys. A* **118**: 31-37. doi: http://dx.doi.org/10.1016/S0300-9629(96)00373-8.

Levasseur, M., Bergeron, N. E., Lapointe, M. F. & Bérubé, F. (2006). Effects of silt and very fine sand dynamics in Atlantic salmon (*Salmo salar*) redds on embryo hatching success. *Can. J. Fish. Aquat. Sci.* **63**: 1450-1459. doi: 10.1139/f06-050.

Lexer, C. & Fay, M. F. (2005). Adaptation to environmental stress: a rare or frequent driver of speciation? *J. Evol. Biol.* **18**: 893-900. doi: 10.1111/j.1420-9101.2005.00901.x

Lisle, T. E. (1989). Sediment transport and resulting deposition in spawning gravels, north coastal California. *Water Resour. Res.* **25**: 1303-1319. doi: 10.1029/WR025i006p01303.

Lisle, T. E. & Lewis, J. (1992). Effects of Sediment Transport on Survival of Salmonid Embryos in a Natural Stream: A Simulation Approach. *Can. J. Fish. Aquat. Sci.* **49**: 2337-2344. doi: 10.1139/f92-257

Louhi, P., Mäki-Petäys, A. & Erkinaro, J. (2008). Spawning habitat of Atlantic salmon and brown trout: general criteria and intragravel factors. *River Res. Appl.* **25**: 1304–1319. doi: 10.1002/rra.1072.

Louhi, P., Ovaska, M., Mäki-Petäys, A., Erkinaro, J. & Muotka, T. (2011). Does fine sediment constrain salmonid alevin development and survival? *Can. J. Fish. Aquat. Sci.* **68**: 1819-1826.

Luczynski, M. & Ostaszewska, T. (1991) Development of hatching gland cells in Coregoninae embryos. *Z. Angew. Zool*. **78**: 399–412.

Lynch, M., & Walsh, B. (1998). *Genetics and analysis of quantitative traits*(Vol. 1). Sunderland, MA: Sinauer.

Mackenzie, C., & Moring. J. R. (1988). Estimating survival of Atlantic salmon during the intragravel period. *N. Am. J. Fish. Manage.* **8**:45–49. doi: 10.1577/1548-8675(1988)008<0045:ESOASD>2.3.CO;2.

Macklin, S., Haas, W. & Miller, W. (1995) Carbon dioxide and dissolved oxygen removal from makeup water by gas transfer membranes. *Proc. Int. Water Conf.* **56**: 401−407.

Malard, F. & Hervant, F. (1999). Oxygen supply and the adaptations of animals in groundwater. *Freshwater Biol.* **41**: 1-30. doi: 10.1046/j.1365-2427.1999.00379.x.

Malcolm, I. A., Youngson, A. & Soulsby, C. (2003). Survival of salmonid eggs in a degraded gravel-bed stream: Effects of groundwater – surface water interactions. *River Res. Appl.* **19**: 303-316. doi: 10.1002/rra.706

Malcolm, I. A., Soulsby, C., Youngson, A., Hannah, D. M., McLaren, I. S. & Thorne, A. (2004). Hydrological influences on hyporheic water quality: implications for salmon egg survival. *Hydrol. Process.* **18**: 1543-1560. doi: 10.1002/hyp.1405.

Malcolm, I. A., Soulsby, C., Youngson, A. & Hannah, D. M. (2005). Catchment-scale controls on groundwater–surface water interactions in the hyporheic zone: Implications for salmon embryo survival. *River Res. Appl.* **21**: 977-989. doi: 10.1002/rra.861

Malcolm, I. A., Soulsby, C. & Youngson, A. F. (2006) High-frequency logging technologies reveal state-dependent hyporheic process dynamics: implications for hydroecological studies. *Hydrol. Process.* **20**: 615-622. doi: 10.1002/hyp.6107.

Malcolm, I. A., Middlemas, C. A., Soulsby, C., S. J. Middlemas, S. J. & Youngson, A. F. (2010). Hyporheic zone processes in a canalised agricultural stream: implications for salmonid embryo survival. *Fundam. Appl. Limnol. Arch. Hydrobiol.* **176**: 319-336. doi: 10.1127/1863-9135/2010/0176-0319.

Malcolm, I. A., Youngson, A. F., Soulsby, C., Imholt, C., & Fryer, R. J. (2011). Is interstitial velocity a good predictor of salmonid embryo survival? *T. Am. Fish. Soc.* **140**: 898-904. doi: 10.1080/00028487.2011.601216.

Malcolm, I. A., Gibbins, C. N., Soulsby, C., Tetzlaff, D., & Moir, H. J. (2012). The influence of hydrology and hydraulics on salmonids between spawning and emergence: implications for the management of flows in regulated rivers. *Fisheries Management and Ecology* **19**: 464–474. doi:10.1111/j.1365-2400.2011.00836.x

Marchal, P., Andersen, J. L., Aranda, M., Fitzpatrick, M., Goti, L., Guyader, O., Haraldsson, G., Hatcher, A., Hegland, T. J., Le Floc'h, P. *et al*. (2016). A comparative review of fisheries management experiences in the European Union and in other countries worldwide: Iceland, Australia, and New Zealand. *Fish and Fisheries* **17**: 803-824. doi: 10.1111/faf.12147.

Martínez, M. L., Chapman, L. J., & Rees, B. B. (2009). Population variation in hypoxic responses of the cichlid *Pseudocrenilabrus multicolor victoriae*. *Can. J. Zool.* **87**: 188-194. doi: 10.1139/Z09-002

Matschak, T. W., Stickland, N. C., Mason, P. S. & Crook, A. R. (1997). Oxygen availability and temperature affect embryonic muscle development in Atlantic salmon (*Salmo salar*). *Differentiation* **61**: 229-235. doi:10.1046/j.1432-0436.1997.6140229.x

Matschak, T. W., Hhopcroft, T., Smason, P., Crook, A. R., & Stickland, N. C. (1998). Temperature and oxygen tension influence the development of muscle cellularity in embryonic rainbow trout. *J. Fish Biol.* **53**: 581-590. doi: 10.1111/j.1095-8649.1998.tb01003.x

May, C. L., Pryor, B., Lisle, T. E. & Lang, M. (2009). Coupling hydrodynamic modeling and empirical measures of bed mobility to predict the risk of scour and fill of salmon redds in a large regulated river. *Water Resour. Res.* **45**. doi: 10.1029/2007WR006498

McClure, M. M., Utter, F. M., Baldwin, C., Carmichael, R. W., Hassemer, P. F., Howell, P. J., Spruell, P., Cooney, T. D., Schaller, H. A. & Petrosky, C. E. (2008). Evolutionary effects of alternative artificial propagation programs: implications for viability of endangered anadromous salmonids. *Evol. Appl.* **1**: 356-375. doi: 10.1111/j.1752-4571.2008.00034.x

McGinnity, P., Prodöhl, P., Ferguson, A., Hynes, R., ó Maoiléidigh, N., Baker, N., ... & Taggart, J. (2003). Fitness reduction and potential extinction of wild populations of Atlantic salmon, Salmo salar, as a result of interactions with escaped farm salmon. *Proc R Soc Lond [Biol]* **270**: 2443-2450. doi: 10.1098/rspb.2003.2520

McMillan, H., Krueger, T. and Freer, J. (2012), Benchmarking observational uncertainties for hydrology: rainfall, river discharge and water quality. *Hydrol. Process.* **26**: 4078–4111. doi:10.1002/hyp.9384

Meehan, W. R. & Swanston, D. N. (1977). Effects of gravel morphology on fine sediment accumulation and survival of incubating salmon eggs. USDA For. Serv. Res. Pap. PNW-220, 16pp. Pacific Northwest Forest and Range Experiment Station, Portland, Oregon.

Mengistu, S. G., Creed, I. F., Kulperger, R. J. & Quick, C. G. (2013), Russian nesting dolls effect—Using wavelet analysis to reveal non-stationary and nested stationary signals in water yield from catchments on a northern forested landscape, *Hydrol. Processes*, **27**: 669–686, doi:10.1002/hyp9552.

Merz, J. E., Setka, J. D., Pasternack, G. B., & Wheaton, J. M. (2004). Predicting benefits of spawning-habitat rehabilitation to salmonid (Oncorhynchus spp.) fry production in a regulated California river. *Can. J. Fish. Aquat. Sci.* **61**: 1433-1446. doi: 10.1139/f04-077

Metcalfe, N. B., Huntingford, F. A. & Thorpe, J. E. (1987). The influence of predation risk on the feeding motivation and foraging strategy of juvenile Atlantic salmon. *Anim. Behav.* **35**: 901-911. doi: 10.1016/S0003-3472(87)80125-2

Miller, S. C., Reeb, S. E., Wright, P. A. & Gillis, T. E. (2008). Oxygen concentration in the water boundary layer next to rainbow trout (Oncorhynchus mykiss) embryos is influenced by hypoxia exposure time, metabolic rate, and water flow. *Can. J. Fish. Aquat. Sci.* **65**: 2170-2177. doi: 10.1139/F08-123

Miller, B., & Kendall, A. W. (2009). *Early life history of marine fishes*. University of California Press.

Mills, N. E., Barnhart, M. C., & Semlitsch, R. D. (2001). Effects of hypoxia on egg capsule conductance in Ambystoma (Class Amphibia, Order Caudata). *J. Exp. Biol*. **204**: 3747-3753. doi:

Milne, I., Seager, J., Mallett, M., & Sims, I. (2000). Effects of short-term pulsed ammonia exposure on fish. *Environ. Toxicol. Chem.* **19**: 2929-2936. doi: 10.1002/etc.5620191213

Milner, N. J., Elliott, J. M., Armstrong, J. D., Gardiner, R., Welton, J. S. & Ladle, M. (2003). The natural control of salmon and trout populations in streams. *Fish. Res.* **62**: 111-125. doi:10.1016/S0165-7836(02)00157-1.

Milner, N. J., Russell, I. C., Aprahamian, M., Inverarity, R., Shelley, J. & Rippon, P. (2004). *The role of stocking in the recovery of the River Tyne salmon fisheries.* Environment Agency, fisheries Technical Report No. 2004/1.

Moffett, I. J. J., Allen, M., Flanagan, C., Crozier, W. W., & Kennedy, G. J. A. (2006). Fecundity, egg size and early hatchery survival for wild Atlantic salmon, from the River Bush. *Fish. Manage. Ecol.* **13**: 73-79. doi: 10.1111/j.1365-2400.2006.00478.x

Moir, H. J., Soulsby, C., & Youngson, A. F. (2002). Hydraulic and sedimentary controls on the availability and use of Atlantic salmon (*Salmo salar*) spawning habitat in the River Dee system, north-east Scotland.*Geomorphology* **45**: 291-308. doi: 10.1016/S0169-555X(01)00160-X.

Moir, H. J., Gibbins, C. N., Soulsby, C., & Youngson, A. F. (2005). PHABSIM modelling of Atlantic salmon spawning habitat in an upland stream: testing the influence of habitat suitability indices on model output. *River Res. Applic.* **21**: 1021-1034. doi: 10.1002/rra.869

Montgomery, D. R., Buffington, J. M., Peterson, N. P., Scheutt-Hames, D. & Quinn, T. P. (1996). Stream-bed scour, egg burial depths, and the influence of salmonid spawning on bed surface mobility and embryo survival. *Can. J. Fish. Aquat. Sci.* **53**: 1061-1070. doi: 10.1139/f96-028.

Montgomery, D. R., Beamer, E. M., Pess, G. R. & Quinn, T. P. (1999). Channel type and salmonid spawning distribution and abundance. doi: 10.1139/f98-181.

Morlet, J. (1983). Sampling theory and wave propagation. In *Issues in Acoustic Signal—Image Processing and Recognition* (pp. 233-261). Springer Berlin Heidelberg.

Motha, J. A., Wallbrink, P. J., Hairsine, P. B. & Grayson, R. B. (2003). Determining the sources of suspended sediment in a forested catchment in southeastern Australia**.** *Water Resour. Res*. **39**, 1056. doi: 10.1029/2001WR000794.

Mueller, C. A., Joss, J. M., & Seymour, R. S. (2011). The energy cost of embryonic development in fishes and amphibians, with emphasis on new data from the Australian lungfish, *Neoceratodus forsteri. J. Comp. Physiol. B* **181** 43-52. doi: 10.1007/s00360-010-0501-y

Mulholland, P. J. & Webster, J. R. (2010). Nutrient dynamics in streams and the role of *J-NABS*. *J. North Am. Benthological Soc.* **29**: 100-117. doi: 10.1899/08-035.1

Naden, P., Bell, V., Carnell, E., Tomlinson, S., Dragosits, U., Chaplow, J., May, L. & Tipping, E. (2016). Nutrient fluxes from domestic wastewater: A national-scale historical perspective for the UK 1800–2010. *Sci. Total. Environ.* doi: 10.1016/j.scitotenv.2016.02.037

Nakamura, Y. (2003). Sediment oxygen consumption and vertical flux of organic matter in Seto Inland Sea, Japan. *Estuar. Coast. Shelf Sci.* **56**: 213-220. doi: 10.1016/S0272-7714(02)00158-0

National River Flow Archive. (2016). *National River Flow Archive.*Available: http://nrfa.ceh.ac.uk/. Last accessed 31st October 2016

Naura, M., Hornby, D. D., Collins, A. L., Sear, D. A., Hill, C., Jones, J. I. & Naden, P. S. (2016). Mapping the combined risk of agricultural fine sediment input and accumulation for riverine ecosystems across England and Wales. *Ecol. Indic.* **70**: 209-221. doi: 10.1016/j.ecolind.2016.03.055

Nikinmaa, M., & Rees, B. B. (2005). Oxygen-dependent gene expression in fishes. *Am. J. Physiol – Reg., Integ. Comp. Physiol.* **288**: R1079-R1090. doi: 10.1152/ajpregu.00626.2004

Ninness, M. M., Stevens, E. D., & Wright, P. A. (2006). Removal of the chorion before hatching results in increased movement and accelerated growth in rainbow trout (*Oncorhynchus mykiss*) embryos. *J. Exp. Biol.* **209**: 1874-1882. doi: 10.1242/jeb.02200

Olsson, T. I., & Persson, B. G. (1986). Effects of gravel size and peat material concentrations on embryo survival and alevin emergence of brown trout, *Salmo trutta* L. *Hydrobiologia* **135**: 9-14. doi: 10.1007/BF00006453

Oppen-Berntsen, D. O., Bogsnes, A. & Walther, B. T. (1990). The effects of hypoxia, alkalinity and neurochemicals on hatching of Atlantic salmon (*Salmo salar*) eggs. *Aquaculture* **86**: 417-430. doi: 10.1016/0044-8486(90)90330-P.

Otterson, G., Planque, B., Belgrano, A., Post, E., Reid, C. & Stenseth, N. C. (2001). Ecological effects of the North Atlantic Oscillation. *Oecologia* **128**: 1-14. doi:10.1007/s004420100655.

Owens, P. N., Walling, D. E. & Leeks, G. J. L. (1999). Use of floodplain sediment cores to investigate recent historical changes in overbank sedimentation rates and sediment sources in the catchment of the River Ouse, Yorkshire, UK. *CATENA* **36**: 21-47. doi: 10.1016/S0341-8162(99)00010-7

Owens, P. N., Batalla, R. J., Collins, A. J., Gomez, B., Hicks, D. M., Horowitz, A. J., Kondolf, G. M., Marden, M., Page, M. J., Peacock, D. H., Petticrew, E. L., Salomons, W. & Trustrum, N. A. (2005). Fine-grained sediment in river systems: environmental significance and management issues. *River Res. Applic.,* **21**: 693–717. doi:10.1002/rra.878.

Packman, A. I., & Salehin, M. (2003). Relative roles of stream flow and sedimentary conditions in controlling hyporheic exchange. In *The Interactions between Sediments and Water* pp. 291-297. Springer Netherlands.

Packman, A., Salehin, M., & Zaramella, M. (2004). Hyporheic Exchange with Gravel Beds: Basic Hydrodynamic Interactions and Bedform-Induced Advective Flows. *J. Hydraul. Eng.* **130**: 647-656 doi: 10.1061/(ASCE)0733-9429(2004)130:7(647).

Padilla, P. A. & Roth, M. B. (2001). Oxygen deprivation causes suspended animation in the zebrafish embryo. *Proc. Natl. Acad. Sci. USA* **98**, 7331- 7335. doi: 10.1073/pnas.131213198

Paganelli, C. V., Olszowka, A., & Ar, A. (1974). The avian egg: surface area, volume, and density. *The Condor* **76**: 319-325. doi: 10.2307/1366345

Pahlow, M., Riebesell, U. & Wolf-Gladrow, D. A. (1997). Impact of cell shape and chain formation on nutrient acquisition by marine diatoms. *Limnol. Oceanogr.* **42**: 1660-1672. doi: 10.4319/lo.1997.42.8.1660.

Palm, D., Lindberg, M., Brännäs, E., Lundqvist, H., Östergren, J., & Carlsson, U. (2009). Influence of European sculpin, *Cottus gobio*, on Atlantic salmon *Salmo salar*, recruitment and the effect of gravel size on egg predation–implications for spawning habitat restoration. *Fish. Manage. Ecol.* **16**: 501-507. doi: 10.1111/j.1365-2400.2009.00705.x

Parker, R.R. (1971). Size selective predation among juvenile salmonid fishes in a British Columbia inlet. *J. Fish. Res. Board Can.* **28**: 1503-1510.

Parrish, D. L., Behnke, R. J., Gephard, S. R., McCormick, S. D., Reeves, G. H. (1998). Why aren't there more Atlantic salmon (*Salmo salar*)? *Can. J. Fish. Aquat. Sci.* **55:** 281–287. doi: 10.1139/cjfas-55-S1-281.

Peck, L. S. & Maddrell, S. H.P. (2005), Limitation of size by hypoxia in the fruit fly *Drosophila melanogaster*. *J. Exp. Zool.,* **303A**: 968–975. doi:10.1002/jez.a.211.

Pilgaard, L., Malte, H., & Jensen, F. B. (1994). Physiological effects and tissue accumulation of copper in freshwater rainbow trout (*Oncorhynchus mykiss*) under normoxic and hypoxic conditions. *Aquat. Toxicol.* **29**: 197-212. doi: 10.1016/0166-445X(94)90068-X

Polymeropoulos, E. T., Elliott, N. G. & Frappell, P. B. (2016). The maternal effect of differences in egg size influence metabolic rate and hypoxia induced hatching in Atlantic salmon eggs: implications for respiratory gas exchange across the egg capsule. *Can. J. Fish. Aq. Sci*. 0, 0. doi: 10.1139/cjfas-2015-0358.

Pörtner, H. O. & Knust, R. (2007). Climate Change Affects Marine Fishes Through the Oxygen Limitation of Thermal Tolerance. *Science* **315**: 95-97. doi: 10.1126/science.1135471.

Pulliam, H.R. and Caraco, T. (1984) Living in groups: is there an optimal group size? In *Behavioral ecology; an evolutionary approach,* 2nd edn (J.R. Krebs and N.B. Davies, eds), pp. 122-48. Blackwell Scientific Publications, Oxford.

Rahn, H., Carey, C., Balmas, K., Bhatia, B., & Paganelli, C. (1977). Reduction of pore area of the avian eggshell as an adaptation to altitude. *Proc. Nat. Acad. Sci.* **74**: 3095-3098. doi:

Reid, J. E. & Chaput, G. (2012). Spawning history influence on fecundity, egg size, and egg survival of Atlantic salmon (*Salmo salar*) from the Miramichi River, New Brunswick, Canada. *ICES J. Mar. Sci.* **56**: 1678–1685. doi: 10.1093/icesjms/fss091

Reisenbichler, R. R. & Rubin, S. P. (1999). Genetic changes from artificial propagation of Pacific salmon affect the productivity and viability of supplemented populations. *ICES J. Mar. Sci.* **56**: 459-466. doi: 10.1006/jmsc.1999.0455.

Reiser, D. W., Olson, A. & Binkler, K. (1998). Sediment Deposition within Fry Emergence Traps: A Confounding Factor in Estimating Survival to Emergence. *N. Am. J. Fish. Manage.* **18**: 713-719. doi: 10.1577/1548-8675(1998)018<0713:SDWFET>2.0.CO;2

Riehl, R., & Kock, K. H. (1989). The surface structure of Antarctic fish eggs and its use in identifying fish eggs from the Southern Ocean. *Polar Biol.* **9**: 197-203. doi: 10.1007/BF00297176

Riley, W. D. & Moore, A. (2000). Emergence of Atlantic salmon, *Salmo salar* L., fry in a chalk stream. *Fisheries Manag. Ecol.* **7**: 465-468. doi: 10.1046/j.1365-2400.2000.00228.x

Roberts, J. R. (2004). Factors affecting egg internal quality and egg shell quality in laying hens. *The Journal of Poultry Science* **41**: 161-177. doi: 10.2141/jpsa.41.161

Roff, D. A. (1992). *The evolution of life histories; theory and analysis*. New York: Chapman & Hall

Rollinson, N. & Hutchings, J. A. (2010). Why does egg size increase with maternal size? Effects of egg size and egg density on offspring phenotypes in Atlantic salmon (*Salmo salar*). *Evol. Ecol. Res.* **12**: 949-960.

Roni, P., Johnson, C., DeBoer, T., Pess, G. R., Dittman, A. H. & Sear, D.A. (2015). Interannual variability in the effects of physical habitat and parentage on Chinook salmon egg-to-fry survival. *Can. J. Fish. Aquat. Sci.* doi: 10.1139/cjfas-2015-0372.

Rombough, P. J. (1988). Growth, aerobic metabolism and dissolved oxygen requirements of embryos and alevins of steelhead, Salmo giardinieri. *Can. J. Zool.* **66**: 651-660. doi: 10.1139/z88-097.

Rombough, P. J. (1994). Energy Partitioning During Fish Development: Additive or Compensatory Allocation of Energy to Support Growth? *Funct. Ecol.* **8**: 178-186. doi: 10.2307/2389901.

Romero-Lankao, P., Smith, J. B., Davidson, D. J., Diffenbaugh, N. S., Kinney, P. L., Kirshen, P. Kovacs, P., and Villers Ruiz, L. (2014): North America. In: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Barros, V.R., C.B. Field, D.J. Dokken, M.D. Mastrandrea, K.J. Mach, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea, and L.L. White (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 1439-1498.

Rosenberg, A., Bigford, T. E., Leathery, S., Hill, R. L. & Bickers, K. (2000). Ecosystem approaches to fishery management through essential fish habitat. *Bull. Mar. Sci.* **66**: 535-547.

Roussel, J.-M. (2007). Carry-over effects in brown trout (*Salmo trutta*): hypoxia on embryos impairs predator avoidance in experimental channels. *Can. J. Fish. Aquat. Sci.* **64**: 786-792. doi: 10.1139/F07-055.

Russell, M. A., Walling, D. E. & Hodgkinson, R. A. (2001). Suspended sediment sources in two small lowland agricultural catchments in the UK. *J. Hydrol*. **252**: 1-24. doi: 10.1016/S0022-1694(01)00388-2.

Rutherford, J. C., Marsh, N. A., Davies, P. M., & Bunn, S. E. (2004). Effects of patchy shade on stream water temperature: how quickly do small streams heat and cool? *Mar. Freshwater Res.* ***55***: 737-748. doi: 10.1071/MF04120

Ryan, R. J., & Packman, A. I. (2006). Changes in streambed sediment characteristics and solute transport in the headwaters of Valley Creek, an urbanizing watershed. *J. Hydrol.* ***323***: 74-91. doi: 10.1016/j.jhydrol.2005.06.042

Salehin, M., Packman, A. I. & Paradis, M. (2004), Hyporheic exchange with heterogeneous streambeds: Laboratory experiments and modeling, *Water Resour. Res.* **40**: W11504, doi:10.1029/2003WR002567.

Saltveit, S. J., & Brabrand, Å. (2013). Incubation, hatching and survival of eggs of Atlantic salmon (Salmo salar) in spawning redds influenced by groundwater. *Limnologica-Ecology and Management of Inland Waters* **43**: 325-331. doi: 10.1016/j.limno.2013.05.009

Schindler, D. E., Leavitt, P. R., Brock, C. S., Johnson, S. P. & Quay, P. D. (2005). Marine-derived nutrients, commercial fisheries, and production of salmon and lake algae in Alaska. *Ecology* **86**: 3225-3231. doi: 10.1890/04-1730.

Schindler Wildhaber, Y., Michel, C., Burkhardt-Holm, P., Bänninger, D. & Alewell, C. (2012). Measurement of spatial and temporal fine sediment dynamics in a small river. *Hydrol. Earth Syst. Sci.* **16**: 1501–1515. doi: 10.5194/hess-16-1501-2012

Schindler Wildhaber, Y., Michel, C., Epting, J., Wildhaber, R. A., Huber, E., Huggenberger, P., Burkhardt-Holm, P. & Alewell, C. (2014). Effects of river morphology, hydraulic gradients, and sediment deposition on water exchange and oxygen dynamics in salmonid redds. *Sci. Total Envrion.* **470-471**: 488-500. doi: 10.1016/j.scitotenv.2013.09.100.

Schmehl, M. K., & Graham, E. F. (1987). Comparative ultrastructure of the zona radiata from eggs of six species of salmonids. *Cell Tiss. Res.* **250**: 513-519. doi: 10.1007/BF00218942

Schreck, C. B., Olla, B. L. & Davis, M. W. (1997). Behavioural responses to stress. In: Iwama, G. K., Pickering, A. D., Sumpter, J. P. & Schreck, C. B. *Fish stress and health in aquaculture*. UK: University Press, Cambridge. 145-170.

Scibek, J., Allen, D. M., Cannon, A. J. & Whitfield, P. H. (2007). Groundwater–surface water interaction under scenarios of climate change using a high-resolution transient groundwater model. *J. Hydrol.* **333**: 165-181. doi: 10.1016/j.jhydrol.2006.08.005.

Sear, D. A. (1996) Sediment transport processes in pool-riffle sequences. *Earth Surf. Process. Landf.* **21**: 241-262. doi: 10.1002/(SICI)1096-9837(199603)21:3<241::AID-ESP623>3.0.CO;2-1.

Sear, D. S., Frostick, L. B., Rollinson, G. & Lisle, T. E. (2008). The Significance and Mechanics of Fine-Sediment Infiltration and Accumulation in Gravel Spawning Beds. In, Sear, David A. and DeVries, Paul (eds.) *Salmonid Spawning Habitat in Rivers: Physical Controls, Biological Responses, and Approaches to Remediation.* Bethesda, USA, American Fisheries Society, 149-174.

Sear, D. A., Pattison, I., Collins, A. L., Newson, M. D., Jones, J. I., Naden, P. S. & Carling, P. A. (2014). Factors controlling the temporal variability in dissolved oxygen regime of salmon spawning gravels. *Hydrol. Process*. **28**: 86-103. doi: 10.1002/hyp.9565.

Sear, D. A., Jones, J. I., Collins, A. L., Hulin, A., Burke, N., Bateman, S., Pattison, I. & Naden, P. S. (2016). Does fine sediment source as well as quantity affect salmonid embryo mortality and development? *Sci. Total Environ.* **541**: 957-968. doi: 10.1016/j.scitotenv.2015.09.155

Secor, D. H. & Gunderson, T. E. (1998). Effects of hypoxia and temperature on survival, growth, and respiration of juvenile Atlantic sturgeon *Acipenser oxyrinchus*. *Fish. Bull.* **96**: 603-613.

Segura, C. J., Chaparro, O. R., Paschke, K. A., & Pechenik, J. A. (2010). Capsule walls as barriers to oxygen availability: implications for the development of brooded embryos by the estuarine gastropod *Crepipatella dilatata* (Calyptraeidae). *J. Exp. Mar. Ecol.* **390**: 49-57. doi: 10.1016/j.jembe.2010.04.007

Seymour, R. S. (1994). Oxygen diffusion through the jelly capsules of amphibian eggs. *Israel J. Zool.* **40**: 493-506.

Shumway, D. L., Warren, C. E., & Doudoroff, P. (1964). Influence of oxygen concentration and water movement on the growth of steelhead trout and coho salmon embryos. *T. Am. Fish. Soc.* **93**: 342-356. doi: 10.1577/1548-8659(1964)93[342:IOOCAW]2.0.CO;2

Sierra, M. V. & Gomez, N. (2007). Structural Characteristics and Oxygen Consumption of the Epipelic Biofilm in Three Lowland Streams Exposed to Different Land Uses. *Water Air Soil Pollut..* **186**: 115. doi: 10.1007/s11270-007-9469-y

Silver, S. J., Warren, C. E., & Doudoroff, P. (1963). Dissolved oxygen requirements of developing steelhead trout and chinook salmon embryos at different water velocities. *T. Am. Fish. Soc.* **92**: 327-343. 10.1577/1548-8659(1963)92[327:DORODS]2.0.CO;2

Simpson, S. C. & Meixner, T. (2012). Modeling effects of floods on streambed hydraulic conductivity and groundwater-surface water interactions. *Water Resour. Res.* **48**: W02515. doi: 10.1029/2011WR011022.

Sinha, V., & Li, K. (2000). Alternative methods for dissolved oxygen removal from water: a comparative study. *Desalination* **127**: 155-164. doi: 10.1016/S0011-9164(99)00200-3

Soderberg, R.W., Flynn, J.B., Schmittou, H.R. (1983). Effects of ammonia on the growth and survival of rainbow trout in static-water culture. *Trans. Am. Fish. Soc.* **112**: 448–451. doi: 10.1577/1548-8659(1983)112<448:EOAOGA>2.0.CO;2

Solbé, J. F., & Shurben, D. G. (1989). Toxicity of ammonia to early life stages of rainbow trout (*Salmo gairdneri*). *Water Res.* **23**: 127-129. doi: 10.1016/0043-1354(89)90071-7

Solomon, A. K. (1968). Characterization of biological membranes by equivalent pores. *The Journal of general physiology* **51**: 335. doi:

Songe, M. M., Willems, A., Sarowar, M. N., Rajan, K., Evensen, Ø., Drynan, K., Skaar, I. & van West, P. (2016). A thicker chorion gives ova of Atlantic salmon (*Salmo salar* L.) the upper hand against *Saprolegnia* infections. *J. Fish. Dis.* **39**: 879-888. doi: 10.1111/jfd.12421.

Sogard, S. M. (1997). Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bull. Mar. Sci.* **60**: 1129-1157.

Soulsby, C., Malcolm, I. A. & Youngson, A. F. (2001). Hydrochemistry of the hyporheic zone in salmon spawning gravels: a preliminary assessment in a degraded agricultural stream. *Regul. River.* **17**: 651-665. doi: 10.1002/rrr.625.

Soulsby C., Malcolm I. A., Tetzlaff D. & Youngson A. F. (2009) Seasonal and inter-annual variability in hyporheic water quality revealed by four years continuous monitoring in a salmon spawning stream. *River Res. Appl.* **25**: 1304–1319. doi: 10.1002/rra.1241.

Soulsby, C., Grant, J., Gibbins, C., & Malcolm, I. A. (2012). Spatial and temporal variability of Atlantic salmon (Salmo salar L.) spawning activity in braided river channels: a preliminary assessment. *Aquat. Sci.* **74**: 571-586. doi: 10.1007/s00027-012-0249-4

Stahlschmidt, Z. R., Heulin, B., & DeNardo, D. F. (2010). The Role of Python Eggshell Permeability Dynamics in a Respiration-Hydration Trade-Off. *Physiol. Biochem. Zool.* **83**: 576-586. doi: 10.1086/652425

Stanford, J. A. & Ward, J. V. (1993). An ecosystem perspective of alluvial rivers: connectivity and the hyporheic corridor. *J. North Am. Benthological Soc* **12**: 48-62. doi: 10.2307/1467685

Stanley, J. G., & Trial, J. G. (1995). *Habitat Suitability Index Models: Nonmigratory Freshwater Life Stages of Atlantic Salmon*. National biological service great lakes science center.

Stehr, C. M., & Hawkes, J. W. (1979). The comparative ultrastructure of the egg membrane and associated pore structures in the starry flounder, *Platichthys stellatus* (Pallas), and pink salmon, Oncorhynchus gorbuscha (Walbaum). *Cell Tissue Res.* **202**: 347-356. doi: 10.1007/BF00220430

Storey, R. G., Howard, K. W. F. & Williams, D. D. (2003), Factors controlling riffle-scale hyporheic exchange flows and their seasonal changes in a gaining stream: A three-dimensional groundwater flow model. *Water Resour. Res.* **39**, 1034, doi:10.1029/2002WR001367, 2

Stuart, M., Lapworth, D., Crane, E., & Hart, A. (2012). Review of risk from potential emerging contaminants in UK groundwater. *Sci. Total Environ.* **416** 1-21. doi: 10.1016/j.scitotenv.2011.11.072.

Tessier, N., Bernatchez, L. & Wright, J. M. (1997). Population structure and impact of supportive breeding inferred from mitochondrial and microsatellite DNA analyses in land-locked Atlantic salmon *Salmo salar* L. *Mol. Ecol.* **6**: 735-750. doi: 10.1046/j.1365-294X.1997.00244.x

Thorstad, E. B., Heggberget, T. G., Økland, F. (1998). Migratory behaviour of adult wild and escaped farmed Atlantic salmon, Salmo salar L., before, during and after spawning in a Norwegian river. *Aquacult*. *Res*. **29***:*419–428. doi: 10.1046/j.1365-2109.1998.00218.x.

Thorstad, E. B., Økland, F., Aarestrup, K. & Heggberget, T. G. (2008). Factors affecting the within-river spawning migration of Atlantic salmon, with emphasis on human impacts. *Rev. Fish Biol. Fish.* **18**: 345-371. doi: 10.1007/s11160-007-9076-4.

Timmons, M. B., Ebeling, J. M., Wheaton, F. W., Summerfelt, S. T. & Vinci, B. J. (2002). Recirculating Aquaculture Systems. 2nd ed. Cayuga Aqua Ventures, Ithaca, NY 14850, USA (800 pp. NRAC Publication No. 01–002).

Tonina, D. (2005). *Interaction between river morphology and intra-gravel flow paths within the hyporheic zone*. Ph.D thesis, University of Idaho.

Tonina, D., & Buffington, J. M. (2007). Hyporheic exchange in gravel bed rivers with pool-riffle morphology: Laboratory experiments and three-dimensional modeling. *Water Resour. Res.* 43, W01421, doi:10.1029/2005WR004328.

Tonina, D. & Buffington, J. M. (2009a). Hyporheic Exchange in Mountain Rivers I: Mechanics and Environmental Effects. *Geography compass* **3**: 1069-1086. doi: 10.1111/j.1749-8198.2009.00226.x.

Tonina, D. & Buffington, J. M. (2009b). A three-dimensional model for analyzing the effects of salmon redds on hyporheic exchange and egg pocket habitat. *Can. J. Fish. Aquat. Sci.* **66**: 2157-2173. doi: 10.1139/F09-146.

Trudell, M. R., Gillham, R. W. & Cherry, J. A. (1986). An in-situ study of the occurrence and rate of denitrification in a shallow unconfined sand aquifer. *J. Hydrol.* **83**: 251-268. doi: 0.1016/0022-1694(86)90155-1

Valente, L. M. P., Rocha, E., Gomes, E. F. S., Silva, M. W., Oliveira, M. H., Monteiro, R. A. F. & Fauconneau, B. (1999). Growth dynamics of white and red muscle fibres in fast- and slow-growing strains of rainbow trout. *J. Fish Biol.* **55**: 675-691. doi : 10.1111/j.1095-8649.1999.tb00710.x

Valiente, A. G., Beall, E., & GARCIA-VAZQUEZ, E. V. A. (2010). Population genetics of south European Atlantic salmon under global change. *Global Change Biol.* **16**: 36-47. doi: 10.1111/j.1365-2486.2009.01922.x

van Vliet, M. T., Franssen, W. H., Yearsley, J. R., Ludwig, F., Haddeland, I., Lettenmaier, D. P., & Kabat, P. (2013). Global river discharge and water temperature under climate change. *Glob. Environ. Chang.* **23**: 450-464. doi: 10.1016/j.gloenvcha.2012.11.002

Vaquer-Sunyer, R., Conley, D. J., Muthusamy, S., Lindh, M. V., Pinhassi, J. & Kritzberg, E. S. (2015). Dissolved Organic Nitrogen Inputs from Wastewater Treatment Plant Effluents Increase Responses of Planktonic Metabolic Rates to Warming. *Environ. Sci. Technol.* **49**: 11411-11420. doi: 10.1021/acs.est.5b00674

Vaux, W. G. (1968). Intragravel flow and interchange of water in a streambed. *United States Fish And Wildlife Service Fishery Bulletin **66*** pp. 479.

Verberk, W. C., Bilton, D. T., Calosi, P., & Spicer, J. I. (2011). Oxygen supply in aquatic ectotherms: partial pressure and solubility together explain biodiversity and size patterns. *Ecology* **92**: 1565-1572. doi: 10.1890/10-2369.1

Viant, M. R., Pincetich, C. A., & Tjeerdema, R. S. (2006). Metabolic effects of dinoseb, diazinon and esfenvalerate in eyed eggs and alevins of Chinook salmon (*Oncorhynchus tshawytscha*) determined by 1 H NMR metabolomics. *Aquat. Toxicol.* **77**: 359-371. doi: 0.1016/j.aquatox.2006.01.009

Visschedijk, A. H., Girard, H., & Ar, A. (1988). Gas diffusion in the shell membranes of the hen's egg: lateral diffusion in situ. *J. Comp. Physiol. B* **158**: 567-574. doi: 10.1007/BF00692565

Wangensteen, O. D. (1972). Gas exchange by a bird's embryo. *Resp. Physiol.* **14**: 64-74. doi: 10.1016/0034-5687(72)90017-5.

Warburton, S. J., Hastings, D., & Wang, T. (1995). Responses to chronic hypoxia in embryonic alligators. *J. Exp. Zool.* **273**: 44-50. doi: 10.1002/jez.1402730106

Ward, J. V., Malard, F., Tockner, K. & Uehlinger, U. (1999). Infuence of ground water on surface water conditions in a glacial flood plain of the Swiss Alps. Hydrol. Process. **13**: 277-293.

Washington Department of Fish and Wildlife (2013). *Habitat Restoration & Protection.* Available: http://wdfw.wa.gov/conservation/habitat/spawningbed_protection/redd.html. Last accessed August 2nd 2015.

Webb, J. H. & McLay, H. A. (1996). Variation in the time of spawning of Atlantic salmon (*Salmo salar*) and its relationship to temperature in the Aberdeenshire Dee, Scotland. *Can. J. Fish. Aquat. Sci.* **53**: 2739-2744. doi: 10.1139/f96-240.

Webb, B. W., Hannah, D. M., Moore, R. D., Brown, L. E., & Nobilis, F. (2008). Recent advances in stream and river temperature research. *Hydrol. Process*, **22**: 902-918. doi: 10.1002/hyp.6994.

Weider, L. J. & Lampert, W. (1985). Differential response of *Daphnia* genotypes to oxygen stress: respiration rates, hemoglobin content and low-oxygen stress. *Oecologia* **65**: 487-491.

Welton, J. S., Beaumont, W. R. C. & Ladle, M. (1999). Timing of migration and changes in age structure of Atlantic salmon, Salmo salar L., in the River Frome, a Dorset chalk stream, over a 24-year period. *Fisheries Manag. Ecol.* **6**: 437-458. doi: 10.1046/j.1365-2400.1999.00166.x

Westin, D.T. (1974). Nitrate and nitrite toxicity to salmonid fishes. *Prog. Fish Cult.* **36**: 86–89. doi: 10.1577/1548-8659(1974)36[86:NANTTS]2.0.CO;2

Whitney, C. K., Hinch, S. G. & Patterson, D. A. (2013). Provenance matters: thermal reaction norms for embryo survival among sockeye salmon *Oncorhynchus nerka* populations. *J. Fish. Biol.* **82**: 1159-1176. doi: 10.1111/jfb.12055.

Wickett, W.P. (1975). Mass transfer theory and the culture of fish eggs. *Chemistry and Physics of aqueous solutions*, pp.419-434.

Widmer, S., Moore, F. G., & Bagatto, B. (2006). The effects of chronic developmental hypoxia on swimming performance in zebrafish. *J. Fish Biol.* **69**: 1885-1891. doi: 10.1111/j.1095-8649.2006.01242.x

Winter, T. C. (2007). The Role of Ground Water in Generating Streamflow in Headwater Areas and in Maintaining Base Flow. *J. Am. Water Resour. Assoc.* **43**: 15-25. doi: 10.1111/j.1752-1688.2007.00003.x

Wolpert, L. (1966). The mechanical properties of the membrane of the sea urchin egg during cleavage. *Exp. Cell Res.* **41**: 385-396. doi: 10.1016/S0014-4827(66)80146-5

Wondzell, S. M. (2006), Effect of morphology and discharge on hyporheic exchange flows in two small streams in the Cascade Mountains of Oregon, USA. *Hydrol. Process*. **20**: 267–287. doi:10.1002/hyp.5902

Wondzell, S. M., J. LaNier, R. Haggerty, R. D. Woodsmith, & R. T. Edwards (2009), Changes in hyporheic exchange flow following experimental wood removal in a small, low-gradient stream *Water Resour. Res.* **45**: W05406, doi:10.1029/2008WR007214.

Wood, P. J. & Armitage, P. D. (1997). Biological Effects of Fine Sediment in the Lotic Environment. *Environ. Manage*. **21**: 203-217. doi: 10.1007/s002679900019.

Wootton, R. J. (1990) Ecology of teleost fishes. Chapman & Hall, London. pp. 1–404.

Wörman, A., Packman, A. I., Marklund, L., Harvey, J. W., & Stone, S. H. (2006). Exact three-dimensional spectral solution to surface-groundwater interactions with arbitrary surface topography. *Geophys. Res. Lett.,* **33**, L07402, doi:10.1029/2006GL025747.

Wroblicky, G. J., Campana, M. E., Valett, H. M. & Dahm, C. N. (1998). Seasonal variation in surface-subsurface water exchange and lateral hyporheic area of two stream-aquifer systems. **34**: 317-328. doi: 10.1029/97WR03285.

Wu, R. S. S. (2002). Hypoxia: from molecular responses to ecosystem responses. *Marine Poll. Bull.* **45**: 35-45. doi: 10.1016/S0025-326X(02)00061-9

WWF (2001). The Status of Wild Atlantic Salmon: A River by River Assessment. 184 pp. Available online at: http://wwf.panda.org/wwf_news/?3729/TheStatus-of-Wild-Atlantic-Salmon-A-River-by-River-Assessment.

Xu, J., Chen, Y., Li, W., Nie, Q., Song, C., & Wei, C. (2014). Integrating wavelet analysis and BPANN to simulate the annual runoff with regional climate change: a case study of Yarkand River, Northwest China. *Water Resour. Manage.* **28**: 2523-2537. doi: 10.1007/s11269-014-0625-z

Yi, X., Liang, Y., Huerta-Sanchez, E., Jin, X., Cuo, Z. X. P., Pool, J. E., Xu, X., Jiang, H., Vinckenbosch, N., Korneliusen, T. S. *et al*. (2010). Sequencing of 50 human exomes reveals adaptation to high altitude. *Science* **329**: 75-78. doi: 10.1126/science.1190371

Youngson, A. F., Malcolm, I. A., Thorley, J. L., Bacon, P. J. & Soulsby, C. (2004). Long-residence groundwater effects on incubating salmonid eggs: low hyporheic oxygen impairs embryo development. *Can. J. Fish. Aquat. Sci.* **61**: 2278-2287. doi: 10.1139/f04-217.

Zarnetske, J. P., Haggerty, R., Wondzell, S. M. & Baker, M. A. (2011a), Dynamics of nitrate production and removal as a function of residence time in the hyporheic zone, *J. Geophys. Res.*, **116**: G01025, doi:10.1029/2010JG001356.

Zarnetske, J. P., Haggerty, R., Wondzell, S. M. & Baker, M. A. (2011), Labile dissolved organic carbon supply limits hyporheic denitrification. *J. Geophys. Res.*: **116**: G04036, doi:10.1029/2011JG001730.

Zia, H., Harris, N. R., Merrett, G. V., Rivers, M. & Coles, N. (2013). The impact of agricultural activities on water quality: A case for collaborative catchment-scale management using integrated wireless sensor networks. *Comput. Electron. Agric.* **96**: 126-138. doi: 10.1016/j.compag.2013.05