The Effects of Simulated Catch-and-Release Angling on Adult Atlantic Salmon (*Salmo salar*) and their Offspring

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Abstract

Fish, including Atlantic salmon (*Salmo salar*), are released by anglers after capture as part of a fisheries management tool known as catch and release (C&R) angling. This has been introduced as a conservation measure to try and halt, or even reverse, the continuous decline in Atlantic salmon numbers. Consequently, pre-spawned salmon may now experience C&R during the freshwater migration to the spawning grounds. Yet, there is still limited information in this area, including how stress from capture prior to breeding can affect not only the fecundity of the parents, but also the phenotype of the offspring. This study explores how two of the main stressors associated with C&R angling, exercise and air exposure, experienced by the parents shortly (5 – 18 days) prior to spawning affect adult mortality, physiology and reproduction. It also investigates the effects of simulated C&R on the early developmental stages of the progeny, as well as examines its influences on key behavioural (risk-taking behaviour, activity, exploration, aggression, and dominance) and physiological (SMR, MMR, AS) traits in offspring.

An equal number of male and female wild adult Atlantic salmon were captured using a permanent fish trap, set up by the Cromarty Firth Fisheries, on the river Blackwater, N. Scotland, during their spawning migration. They then experienced one of three disturbance protocols that comprised of exercise (120 s) and air exposure (0, 60, or 120 s) of different duration, similarly to what they would encounter during a C&R angling event. There was also a fourth group present that did not experience any additional disturbance and was therefore used as baseline (control group). Each experimental fish (of either sex) was later mated (using IVF) with a non-experimental counterpart, and the offspring were reared under fixed conditions. Experimental parent mortality was unaffected by the simulated C&R, however the growth rate of the fungus *Saprolegnia* spp. on the body of the fish increased. Furthermore, males from the treatment group exercise + extended air produced sperm that survived for longer once activated (i.e. had an increased maximum duration of sperm motility). Females that experienced disturbance spawned at the usual time, but with smaller clutches.

An increase in egg and fry mortality was noted for the groups whose parents were exposed to air, mostly due to higher mortality during egg shocking (a normal

husbandry practice in hatcheries to separate non-viable eggs) and an increase in fry mortality during a 12-day fungal (*Saprolegnia* spp.) outbreak. Moreover, adults from the most extreme treatment group (exercise + extended air exposure) produced offspring that were smaller at first feeding. As for offspring behaviour, both the activity and exploration of a novel environment were lower in the treatments whose experimental parent was exercised and then air exposed for an extended period. Similar results in exploration were observed by the offspring in the exercise group. Yet, exploration in the most extreme disturbance group was improved as the fish became bigger. Progeny from the same treatments, 'exercise' and 'exercise + extended air' also displayed higher levels of aggression. Interestingly however, during the dominance trials, both these treatment groups were subordinate to offspring from the control treatment. Fish from the control treatment exhibited dominance over the fish from the disturbed parents during the trials on the first two days, but an absence of clear dominance was observed on the third day. There was no observable difference in dominance status between the treatment 'exercise' and 'exercise + extended air' treatments. The metabolism of the offspring was only affected in the exercise group, where both the MMR and AS were reduced.

These results suggest that stressing the parents shortly before spawning will not affect the timing of the spawning, but it could influence the reproductive success of the parents. Furthermore, it indicates that disturbing the parents, especially air exposing them for more than 60 s, could adversely affect the early developmental stages of the offspring, including those behavioural traits which could influence dispersal and competition from feeding territories, and thus reduce their chances of survival. The results therefore have implications for both the period over which C&R is allowed and the way in which it is implemented by anglers.

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Lastly, I would like to extend a big thank you to Dr. John Armstrong, my third supervisor, from Marine Scotland for his constructive feedback. Marine Scotland was also generous enough to fund this thesis, and I would therefore like to express my appreciation.

Candidate's Declaration

I declare that the work presented and described in this thesis is my own. The only exception is the recording of egg mortality at the SSE hatchery in Contin, Scotland, by the Cromarty Firth Fisheries Staff.

No part of thesis has been submitted for any other degree or qualification.

Signature of candidate:

Date: 27/10/21

Abbreviations

Aerobic Scope – AS

Adrenocorticotrophin Hormone – ACTH

Catch and Release Angling – C&R

Corticotrophin Hormone – CRH

Fishery Induced Evolution – FIE

General Linear Model – GLM

Glucocorticoids – GC

Hypothalamic-Pituitary-Interrenal Axis – HPI-Axis

Maximum Metabolic Rate – MMR

Passive Integrated Transponder – PIT

Prostaglandin E2 – PGE2

Scottish and Southern Energy - SSE

Specific Growth rate 1 – SGR1

Specific Growth rate 2 – SGR2

Standard Metabolic Rate – SMR

Transgenerational Phenotypic Plasticity – TPP

Yolk Sac Volume – YSV

Chapter 1 – General Introduction

1.1 Introduction

How can we be certain that species-specific conservation measures do not have unintended long-term consequences? Specifically, do we know whether wild animals released after capture suffer long-term adverse effects, and if these are transferred to their offspring? This is of particular relevance in catch-and-release (C&R) angling, during which fish captured by rod-and-line anglers are released on the assumption that they will then survive and reproduce normally. Yet almost nothing is known about whether this highly stressful event has an adverse effect on their ability to reproduce and generate viable offspring, or whether the phenotype, behaviour and physiology of these offspring will be altered. In this doctoral thesis I will explore these issues in wild Atlantic salmon.

1.2 What is Catch and Release?

1.2.1 Humans as Hunters and Fishery Induced Evolution (FIE)

Activities such as fishing can result in humans selectively removing from the wild individuals with particular traits (e.g. the size of mesh in nets may result in capture of only larger individuals), and as such pose a predatory type of pressure on natural populations (Killen et al., 2015). Commercial and recreational fishing actions do not only deplete fish stocks but can also trigger evolutionary shifts in fish populations and their key life-history traits; for example, changes in fecundity, metabolic rate, aggression, and size of maturity (Hard et al., 2008; Enberg et al., 2009; Killen et al., 2015; Hollins et al., 2018). This phenomenon is known as fisheries induced evolution (FIE; Hard et al., 2008; Enberg et al., 2009; Heino et al., 2015; Killen et al., 2015; Louison et al., 2017; Hollins et al., 2018; Koeck et al., 2018). In addition to their desirability to harvest, fish can also display discrete vulnerabilities to capture based on their physiological and behavioural traits, which can eventually contribute to FIE (Hard et al., 2008; Killen et al., 2015; Louison et al., 2017). For example, vulnerability of individuals to passive fishing gears, such as traps, is related to traits such as exploration, activity, and boldness (Klefoth et al., 2012; Killen et al., 2015; Pauli et al., 2015; Koeck et al., 2018).

1.2.2 Decline in Atlantic Salmon Population

Atlantic salmon populations have been suffering worldwide declines or extirpation since the mid-19 century (MacCrimmon and Got, 1979; Parrish et al., 1998; Gibson et al., 1993; Friedland e al., 2009; Chaput et al 2012; Mills et al., 2013; Lenders et al., 2016). The reduction in numbers cannot be tracked back to a single reason, but rather an assortment of causes, mainly anthropogenic (Parrish et al., 1998; Gibson, 1993; Chaput et al., 2012; Mills et al., 2013; Lenders et al., 2016; Nicola et al., 2018; Dadswell et al., 2021). The main examples of these include overfishing, dam construction, river pollution, ocean acidification, global warming, alterations of the marine food-web and intensive aquaculture (Parrish et al., 1998; Gibson, 1993; Friedland et al., 2009; Landers et al., 2016; Nicola et al., 2018 Dadswell et al., 2021). As a result, there have been attempts to restore wild Atlantic salmon stocks, dating back over many years (MacCrimmon and Got, 1979; Gibson, 1993).

1.2.3 Introduction to C&R Angling

A conservation initiative known as ‘catch and release’ (C&R) angling has been implemented by fisheries managers in several countries, to preserve stocks and maintain ecosystem balance (Cooke and Schramm, 2007; Wedemeyer and Wydoski, 2008; Smukall et al., 2019; Van Leeuwen et al., 2021). C&R requires fishers to release captured fish back into their natural environment while still alive (Cooke and Schramm, 2007; Wedemeyer and Wydoski, 2008; Smukall et al., 2019). This allows the socio-economic interests of recreational fisheries to be preserved, even at low stocks (Van Leeuwen et al. 2021). The success of this programme, however, relies on the ability of individual fish to survive an angling event, recover, and then breed successfully (Dempson et al., 2002; Richard et al., 2013; Richard et al., 2014; Lennox et al., 2016). It was calculated by Cooke and Cowx (2004) that about 60 % of the global recreational catches were released back into the wild, which corresponds to approximately 30 billion fish per year (Ferter et al., 2013). Yet, the implementation of C&R is both country and species dependent and may either be mandatory or voluntary (Ferter et al., 2013).

1.2.4 Catch-and-Release Regulations - Scotland

Atlantic salmon can be found within the rivers and coastal waters of England, Scotland, Wales, and Northern Ireland, and in 2012 the UK had a returning anadromous adult population ranging between 604,568 – 646,161 (JNCC, 2013a; DEFRA, 2018). Most UK Atlantic salmon (about 80 %) reside within Scotland, where populations can be found in most rivers and streams (JNCC, 2013b). As a conservation initiative for the declining population of salmon within Scotland, the Scottish Parliament endorsed 'The Conservation of Salmon (Scotland) Regulations 2016' as a first ever attempt to manage their killing in inland waters (Marine Scotland, 2018a; Scottish Government, 2018). This stated that it is legal to kill salmon only if the stock within the specified river is above the defined conservation limit; the point where the spawning fish stock falls below the desirable threshold, and future recruitment level starts declining (Marine Scotland, 2018a; 2018b; Scottish Government, 2018). If, however the population is below the set threshold, a mandatory C&R policy should be implemented. Additionally, all salmon caught in coastal waters should be released (Marine Scotland, 2018a; 2018b; Scottish Government, 2018). On February 8th, 2018, the legislation was revisited and revised into 'The Conservation of Salmon (Amendment) Scotland Regulations 2018'. This amendment prohibits the retention of salmon caught within inland waters, as well as states their immediate release back into their habitat with as minimal damage as possible (Marine Scotland, 2018c).

1.2.5 Physiological Impacts of Air Exposure

It has long been known that removing fish from the water can be detrimental or even lethal for most fishes, due to the fact that most fish species need to obtain oxygen from water (Olsen et al., 2010; Nguyen et al., 2013; Cook et al., 2015). The intensity of the effects of air exposure are species- and context-specific and based on several variables, such as water and air temperature, playing time (i.e. time taken to land), human handling and fish condition (Arlinghaus et al., 2007; Olsen et al., 2010). By removing the fish from the water, with the exception of air-breathing individuals, they are exposed to acute hypoxia (Cook et al, 2015). The gill filaments adhere together and collapse, making it difficult to impossible for gas exchange to take place across the gill lamellae, which consequently

inhibits aerobic respiration, and initiates metabolic deficit due to lack of oxygen availability (Ferguson and Tufts, 1992; Tufts et al., 1997; Cooke et al., 2002; Suski et al., 2004; Thompson et al., 2008; Cook et al., 2015). This results in an oxygen debt, with a carbon dioxide build-up and a drop in blood pH (Ferguson and Tufts, 1991; Cook et al., 2015). The fish also experience bradycardia while air exposed, followed by tachycardia when re-immersed in water (to deplete the oxygen dept; Cook et al., 2015). Lastly, air exposure activates the stress response (see chapter 1.5 Stress Response in Fish).

1.2.6 Catch-and-Release Practices

A great deal of research has been conducted on the best practices to minimize the effects of C&R on the adults. The optimal fishing practices need to be communicated to anglers to achieve maximum sustainability in C&R (Lennox, 2018). This can comprise fishing techniques and gear, as well as handling or releasing procedures (Lennox, 2018). Two ways to abate physiological disturbances associated with angling and allow fish to continue normal migratory behaviour are to reduce both the playing time and the post-angling air exposure (Olsen et al., 2010). The impacts of air exposure depend on the sensitivity of the species, the state of the individual fish (i.e. its level of exhaustion), environmental conditions (i.e. temperature) as well as situation; therefore there is no set limit on the duration of exposure that is valid for all capture events (Cook et al., 2015). However, based on the current knowledge of Atlantic salmon, the recommended duration of air exposure is 10 seconds or less (Olsen et al., 2010; Richard et al., 2013; Cook et al., 2015). With the correct technique and tools, this should be more than enough time, as little to no air exposure is necessary for unhooking (easiest done while fish is held in the water with a net), measuring and photographing (since it is possible to hold fish horizontally above the water, with a gentle grip around the base of the tail and under the front part of the belly) (Olsen et al., 2010; Cook et al., 2015). Moreover, landing fish is best done with a knotless nylon or rubber net with a small mesh size, and wet hands, so as to reduce any damage incurred to the skin, mucus, scales, or fins (Olsen et al., 2010). Also, in cases where the removal of the hook will cause more damage than the hook itself (i.e. if the hook is too deeply embedded), it is advised to leave the hook in place and cut the fishing line instead (Olsen et al., 2010). Even with all this knowledge gathered for the adults, we still know almost nothing about how C&R

of the parents affects the fitness of the offspring, and the best practices to minimize these effects.

1.3 Atlantic Salmon

1.3.1 Distribution and Importance

The natural geographic range of Atlantic salmon spreads across both sides of the North Atlantic Ocean, extending from the Northeast coast of North America and Canada to Greenland, Iceland and along to the whole Western European coast (MacCrimmon and Gots, 1979; Olsen et al., 2010; Tree for life, 2018). It can also be found in all countries bordering the Baltic (MacCrimmon and Gots, 1979). Over time it has been introduced to Australia, New Zealand, Chile and Argentina (Sutterby and Greenhalgh, 2005). Salmon play a critical socio-economic, cultural, and environmental role (Lennox et al., 2016). In former times this was in the form of bushmeat (wild species hunted for human consumption), whereas in the present it is primarily as game (fish caught by anglers for sport). Sport fishing not only offers a means of social bonding, but also provides commerce and income to local and national communities (Lenders et al., 2016; Lennox et al., 2016; Miramichi Salmon Association, 2018). Moreover, the death of 90 – 95 % of the adults (Fig. 1.1) after spawning is of ecological importance, since it transfers a considerable amount of energy and nourishment from the oceans to the rivers and streams, which provides sustenance to both freshwater and terrestrial ecosystems (Nislow et al., 2004; Lennox, 2018; Samways et al., 2018).

1.3.2 The Life cycle of Atlantic salmon

Most Atlantic salmon are iteroparous, anadromous fish that remain in freshwater streams until exceeding a size threshold that triggers the smolt transformation, at which point they migrate to sea. The life cycle of Atlantic salmon is relatively complex as it comprises eight distinct stages; egg, alevin, fry, parr, smolt, adult, spawning adult and kelt (Fig 1.1; Marine.ie, 2018; Miramichi Salmon Association, 2018; Scottish Natural Heritage, 2018). Adult salmon preferentially spawn during the fall and winter months (water temperature = 6 – 10 ^{o}C) in moderately to fast-flowing, well-oxygenated upstream rivers (Decola, 1970; Gibson, 1993; Webb and Mclay, 1996; Sauter et al., 2001; Jonsson and Jonsson, 2011). Smolt transformation is a process that covers several physiological (alterations to the

lipid storage, hormones, and ion-regulation), behavioural and morphological (more streamline) alterations (McCormick et al., 1998; Armstrong and Nislow, 2006; Jonsson and Jonsson 2011). The years spent in fresh water prior to seaward migration increase with latitude, since higher latitudes have shorter growing seasons (Metcalfe and Thorpe, 1990). Fish grow rapidly upon reaching the ocean (acquiring approximately 99 % of their final adult size, even though the time spent in fresh water and in sea water may be similar), and then return to their natal river to spawn (often to the same stretch of stream in which they were born) (Marine.ie, 2018; Miramichi Salmon Association, 2018). This type of migration is known as homing (Mobley et al., 2021). Consequently, this has led to genetically distinct populations within each river system (and even within parts of a catchment; Garcia de Leaniz et al., 2007). The majority of Atlantic salmon spend 1-2 (rarely 3 or 4) years at sea before returning to spawn, with females tending to stay at sea for longer than males, which permits them to store about 6x more energy reserves, that they can later use for gonad and egg development (Mobley et al., 2021). A minority of males may become sexually mature without ever going to sea and take part in spawning events as small ‘sneaker’ or ‘precocious’ males (Fleming 1996). While most individuals spawn in only one breeding season, they have the potential to spawn in up to five seasons (Olsen et al 2010; Lennox, 2018).

As a species they are also very phenotypically plastic, with a diverse life history (Olsen et al., 2010). Some examples of how they achieve these are through different reproductive strategies (i.e. sneaker males vs fighting), and durations of their freshwater (1-8 years) and marine phases (1-4 years), which can depend on both the individual and the environment (Olsen et al., 2010; Van Leeuwen et al., 2016). The likelihood of a male becoming precociously mature as a sneaker is influenced by two key factors, the size and fat reserves of the fish during spring (Rowe and Thorpe, 1990; Rowe et al., 1991; Simpson, 1992; Fleming, 1996). Anadromous males invest their resources into courtship and fighting, while precocious males invest more into sperm competition (quality and quantity; Fleming, 1996; Mobley et al., 2021). The different reproductive strategies displayed by the males, as well as the elaborate secondary sexual characteristics that they exhibit, is a consequence of the high male competition that has arisen due to the asynchronous female spawning (Fleming, 1996). The overall energetic investment in spawning is similar for males and females (Jonsson et al., 1991), but

the use of resources differs: males tend to arrive on the spawning grounds earlier, remain for longer, and are more active than females so as to maximize their reproductive success, and so tend to use up a greater amount of their reserves in spawning-related activity (Fleming, 1996). In contrast, females devote a greater proportion of their reserves to gametes (Fleming, 1996). They have an important trade-off to consider when producing eggs. They can either produce a smaller clutch but with larger and better-quality eggs, which generates offspring with a higher individual fitness, or produce a large clutch but with smaller eggs, which may provide a greater chance of some individuals surviving and reaching maturity (Armstrong and Nilsow, 2006). The first option benefits the individual offspring, while the second option benefits the mother.

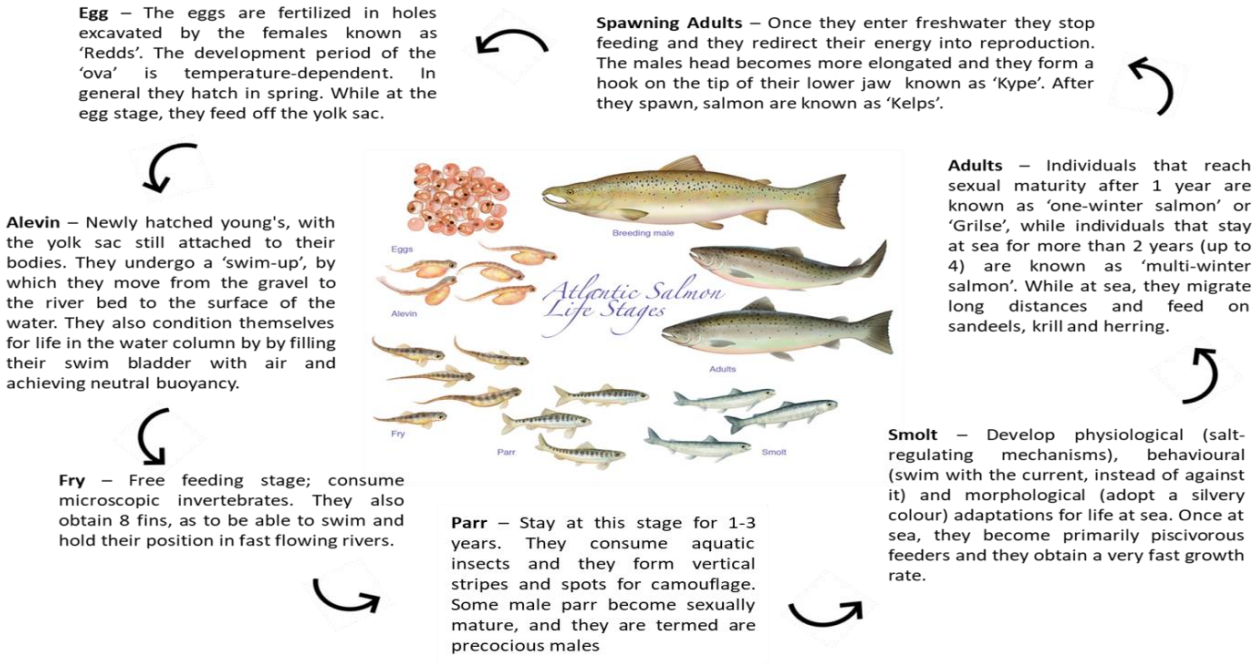

Figure 1.1 The life cycle of the Atlantic salmon, *Salmo salar* (Illustration taken from Miramichi Salmon Association, 2018, and the text is a configuration of the information taken from Rowe and Thorpe, 1990; Jonsson and Jonsson 2011; Marine.ie, 2018; Miramichi Salmon Association, 2018; Trees for Life, 2018; Scottish Natural Heritage, 2018)

1.3.3 The Ecology of Atlantic salmon

Juvenile salmonids living in streams form social hierarchical structures through competitive interactions, where individuals that out-compete others exhibit a

higher dominance status (Gilmour et al., 2005; Reid et al., 2011; Sanchez-Gonzales and Nicieza, 2021). Being dominant secures the most optimal feeding territories and all the benefits that accompany it, such as shelter from predation, greater access to food and preferential positions within the water current (Gilmour et al., 2005; Reid et al., 2011). There is also evidence of a link between the dominance status and metabolic rate of an individual (Hoogenboom et al., 2013; Metcalfe et al., 2016; Sanchez-Gonzales and Nicieza, 2021). Furthermore, distinct populations of salmonids may have metabolic rates suited to the environment they inhabit (Durtsche et al., 2021). For example, Sockeye salmon (*Oncorhynchus nerka*) populations within the Fraser River have dissimilar physiologies and aerobic scopes (AS = the maximum range of oxygen consumption that an individual can exhibit) based on the migration path that they follow, with the populations that follow the most challenging routes having the greatest AS and hence capacity for aerobic activity (Eliason et al., 2011).

After emerging from the nest (or "redd"), juvenile salmon feed predominantly on small invertebrates carried in the water current (Armstrong and Nislow, 2006; Einum et al., 2011; Andersson and Hoglund, 2012). They ontain feeding territories on the margins of shallow streams, where they are subjected to shadow competition (Armstrong and Nislow, 2006; Einum et al., 2011). Juvenile salmonids, like many other freshwater fish, have been forced to the margins by larger predatory fish (Armstrong and Nislow, 2006). The time of emergence from the gravel is related to several behavioural traits, where for instance early emerging offspring exhibit enhanced aggression and have a higher chance of becoming dominant (Andersson and Hoglund, 2012). A couple of weeks after first emergence offspring are subjected to density-dependent effects, where smaller individuals are either forced to migrate downstream or die off due to limited resources (Brudson et al., 2016). After the first year in fresh water and before the process of smolting, juvenile salmon move towards the centre of the stream and into deeper water (Heggenes, 1990; Verspoor et al, 2007).

1.4 Parental Effects and Epigenetic Inheritance

1.4.1 What are Parental Effects and Epigenetic Inheritance?

The term 'Parental effects' refers to the situation when the offspring's phenotype is adjusted by the parents through non-genetic means (McCormick et al., 1998; Eaton et al., 2015; Bautista and Burggren, 2019). The environmental conditions that the parents experience, either at birth or later in life, can be transferred to the next generation (intergenerational effects) or across several generations (transgenerational effects) either directly (i.e. nutrients and hormones) or indirectly (i.e. through parental care; Burton and Metcalfe, 2014; Eaton et al., 2015; Donelan and Trussell, 2018; Atherton and McCormick, 2020). These effects can either be adaptive or maladaptive for the offspring, based on the environment and conditions that they themselves experience (Burgess and Marshall, 2014; Burton and Metcalfe, 2014; Jonsson and Jonsson, 2014; Haussmann and Heidinger, 2015; Blount et al., 2016). In the case of advantageous changes, the phenotypic plasticity caused by this epigenetic inheritance is considered as an essential buffer to the harsh environmental conditions that offspring may encounter (Schreck et al., 2001; Jensen et al., 2014). If the parents have successfully predicted the potential conditions that the offspring will face, such that the phenotype of the offspring is suited to that environment, then the parental effects will be adaptive, as they will increase the offspring's viability (Eriksen et al., 2013; Burton and Metcalfe, 2014; Donelan and Trussell, 2018; Bautista and Burggren, 2019; Lehto and Tinghitella, 2019; Atherton and McCormick, 2020). If on the other hand there is a mismatch between the anticipated and actual environment experienced by the offspring then there may be a reduction in the offspring's chances of survival, and so the parental effects would be considered maladaptive (Eriksen et al., 2013; Burton and Metcalfe, 2014; Bautista and Burggren, 2019; Lehto and Tinghitella, 2019; Atherton and McCormick, 2020). The parents may also cause maladaptive nongenetic changes to their offspring as a result of constraints that they face (e.g. offspring may receive suboptimal resources as a result of the parents being poorly nourished).

All these phenomena can arise through either the father (when they are termed paternal effects), the mother (maternal effects), or a combination of both parents (parental effects; Burton and Metcalfe, 2014; Jonsson and Jonsson, 2014;

Haussmann and Heidinger, 2015). It is now accepted that such effects can be instigated both pre- and post-fertilization of the offspring, work across several physiological systems, and can be a reaction to various environmental stimuli (Donelan and Trussell, 2018; Mobley et al., 2021). The most vulnerable stages to stress are during early development. Most species of fish lay eggs and provide no parental care, and therefore potentially expose their offspring to harsh environmental conditions (Jensen et al., 2014). Epigenetic inheritance has the potential to assist offspring in the short-term, as it allows parents to adjust the phenotype of their offspring to suit anticipated conditions and so offers time and space for populations to adapt their phenotype to a changing environment (Immler et al., 2018; Bautista and Burggren, 2019). It is important to understand however, that adjustments to the phenotype of individuals at one life-stage will most probably have consequences for successive stages (Jensen et al., 2014).

1.4.2 Maternal and Paternal Effects

The circumstances that the parents experience throughout their life, or that the offspring encounter during early development, can potentially make the offspring more viable by enhancing their performance through adjustments to their physiology, morphology, and behaviour (Ghio et al., 2016; Donelan and Trussell, 2018; Atherton and McCormick, 2020). Parental and offspring adjustments however may have dissimilar results on the offspring's performance, due to differences in the timepoint at which those changes are triggered (Donelan and Trussell, 2018). Parental offspring phenotypic manipulation can start as early as through the gametes. An example of this is the adult marine tubeworm, *Hydroides diramphus*, which can regulate the properties of its gametes so as to maximize their performance in response to the osmotic concentration of the parents' environment (Jensen et al., 2014). However, offspring sired from those parents may pay a cost of an environmental mismatch, since they are more susceptible to salinities that do not match the concentrations of the parents' environment (Jensen et al., 2014). Offspring of mothers experiencing pre-natal stress can suffer from genetic abnormalities, immunosuppression, reduced weight at birth and lower survival rates (McCormick et al., 1998; Eriksen et al., 2007; Eriksen et al., 2013). McCormick et al. (1998), demonstrated this using the tropical damselfish, *Pomacentrus amboinensis*, where changes in the offspring's length and yolk sac size were associated with cortisol concentrations in the mother: higher maternal

cortisol concentrations produced shorter larvae with smaller yolk sacs. Paternal state can also influence offspring fitness, both during early and later life development (Immler, 2018). This is because sperm contains epigenetic markers, such as chromatin, proteins, and several families of RNAs, in addition to DNA, and these can influence gene expression in the developing offspring (Immler, 2018). Understanding how past experiences, of both parents and offspring, can shape future phenotypes will provide a better appreciation for the ecological significance of the dynamics and the interrelationships in nature (Donelan and Trussell, 2018).

1.4.3. Examples of Adaptive and Maladaptive Traits

Stressful environments have been assumed to be a negative influence for wild animals for many years, with numerous instances of parental stress negatively impacting offspring survival, performance (behavioural and physiological), morphology, and immunity (Eriksen et al., 2006; Schreck, 2010; McGhee et al., 2012; Madaro et al., 2015; Sopinka, 2015; Atherton and McCormick, 2020). This has been shown in adult female Atlantic salmon that were given cortisol implants, which later produced offspring with more morphological deformities, reduced yolk sac usage, and slower or stunted growth (Eriksen et al., 2006; Eriksen et al., 2007; Eriksen et al., 2013). This negative perspective however was recently re-evaluated, and it is now accepted that stressful events experienced by parents also have the potential to result in more adapted offspring when the environment they are established in is taken into consideration, or to expand the niches that they are capable of occupying (Madaro et al., 2015; Sopinka, 2015; Bautista and Burggren, 2019). Offspring of the carnivorous snail *Nucella lapillus* grew bigger and faster in size and spent less time hiding when faced with predation by the green crab *Carcinus maenas*, when the parents themselves also experienced the same threat (Donelan and Trussell, 2018). Prior parental exposure to a threat could enable a reliable non-genetic transmission of information to the offspring, or to future generations, regarding an active predator risk in the immediate area, allowing offspring to better appreciate present and future risk (Donelan and Trussell, 2018; Atherton and McCormick, 2020). There is also evidence that such influences do not only affect one distinct life stage, but rather can carry over across successive developmental stages (Atherton and McCormick, 2020).

1.5 Stress Responses in Fish

Stress is a universal feature of vertebrate life (McCormick et al., 1998), and is defined as the process where an organism's homeostasis is knocked out of balance by either external or internal stimuli (Iversen and Eliassen, 2014). Once the stress response is activated, the animal will experience three different phases: the primary, secondary, and tertiary response (Barton, 2002; Gilmour et al., 2005; Harper and Wolf, 2009; Sopinka et al., 2017). In fish, the primary neuroendocrine response involves a variety of physiological reactions including the release of catecholamines through the sympathetic-chromaffin pathway, and the activation of the Hypothalamic-Pituitary-Interrenal axis (HPI-axis) which triggers a hormonal cascade starting with the release of the neuropeptide corticotrophin hormone (CRH) from the hypothalamic cells, which activates the adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland, which finally synthesizes the glucocorticoids (GC) (e.g. cortisol) from the interrenal cells (Sloman et al., 2001; Overli et al., 2002; Gilmour et al., 2005; Thomas and Gilmour, 2006; Harper and Wolf, 2009; Schjolden et al., 2009; Sopinka et al., 2017). Both the chromaffin and interrenal cells are in the piscine anterior kidney (Harper and Wolf, 2009). Catecholamine concentration peaks within the first few minutes of activation, while the GC concentration increases at a slower rate (Schreck et al., 2001; Sloman et al., 2001; Thomas and Gilmour, 2006). The high circulating quantities of GCs and catecholamines initiate the secondary and tertiary responses (Schreck et al., 2001; Sloman et al., 2001; Thomas and Gilmour, 2006; Harper and Wolf, 2009). The secondary response acts on the metabolic and physiological pathways, and operates at the tissue level (Sloman et al., 2001; Thomas and Gilmour, 2006; Harper and Wolf, 2009). Some examples of secondary response include increased cardiac output, vasodilation of the arteries in the gill filament, ion balance, immunosuppression, and effects on metabolism (Sloman et al., 2001; Harper and Wolf, 2009; Sopinka et al., 2017). The primary and secondary responses take precedence and allow fish to adapt to acute stressors by increasing their vigilance, and upregulating the cardiorespiratory system and catabolic metabolism (Harper and Wolf, 2009). Finally comes the tertiary response, which is considered to have ecological significance as it acts on the whole organism or population level (Sloman et al., 2001; Thomas and Gilmour, 2006; Sopinka et al., 2017). The tertiary response is deemed maladaptive, and is initiated when the fish experience

a prolonged or re-current disturbance (Sloman et al., 2001; Thomas and Gilmour, 2006; Harper and Wolf, 2009). During this phase, fish may be unable to adjust to disturbances, which can result in reduced or repressed growth, reproductive output, swim performance, disease resistance, and overall survival (Sloman et al., 2001; Thomas and Gilmour, 2006; Harper and Wolf, 2009; Sopinka et al., 2017). If the original stress does not cause any permanent damage to the fish that would result in death, this can still occur through secondary effects (i.e. secondary opportunistic pathogens) (Schreck et al., 2001).

The role of GCs during a disturbance is to maintain or re-establish homeostatic equilibrium through behavioural, immunological, muscular, and cardio-physiological adjustments (Overli et al., 2002; Schjolden et al., 2009; Donaldson et al. 2014; Lennox, 2018). The plasma concentration of cortisol is a common marker of stress in teleost fish, since it can reliably channel information regarding the magnitude, duration and severity of the stressor affecting a specific organism (Schreck et al., 2001; Sloman et al., 2001; DiBattista et al., 2005; Gilmour et al., 2005; Eriksen et al., 2013). Plasma concentrations of hormones in the mother can be transmitted to her developing eggs, depending on the reproductive stage that the stressor was experienced as well as the duration and severity of the stressor (Schreck et al., 2001; Taylor et al., 2016). Here it is important to note that species differ in their tolerance to stress, which means that a specific stressor may affect species in a different manner, either in degree or direction (Schreck et al., 2001). Being able to quantify the level of stress an organism has experienced can assist in our understanding of carry-over and parental effects, personality traits and variations in life history (Sopinka et al., 2017).

1.6 Effects of Catch-and-Release Angling on Salmonids

The gear used in fisheries sits on a spectrum between passive (i.e. traps, such as creels) and active (i.e. trawling) fishing, where based on the equipment deployed fish with specific traits are selected (Hollins et al., 2018). In the case of rod-and-line angling, a salmon's vulnerability to capture is partly based on its phenotype, which is a combination of its physiological (i.e. swim performance and stress response) and behavioural traits (i.e. boldness, activity and dominance; Hollins et

al., 2018; Koeck et al., 2018; Lennox, 2018). Once hooked, most individuals will fight to exhaustion before being landed successfully (Olsen et al., 2010; Lennox, 2018). This will affect the fish's physiology as the attempt to escape will deplete the glycogen, phosphocreatine, and ATP stores from their muscles, produce a build-up of metabolic by-products, as well as result in a hormonal release of catecholamine and corticosteroids (see earlier section on stress responses; Donaldson et al., 2014; Raby et al., 2015; Lennox, 2018).

The stress response can result in adaptive short-term phenotypic alterations, such as 'flight or fight', but if experienced for prolonged time it can become maladaptive (Raby et al. 2015; Lennox, 2018). Chronic stress can cause reduced growth and reproduction, delayed maturity, reduced resistance to stressors and disease, modified behaviour and at times death (Donaldson et al., 2014; Lennox, 2018). The effects of stress are both individual- and species-dependent (Lennox, 2018). The duration of air exposure after capture can also significantly influence the behaviour and post-release survival of caught salmon; the longer the exposure the bigger the impact (Olsen et al., 2010; Cook et al., 2015). Fish removed from the water can experience desiccation, which can subsequently damage the gill lamellae and cause acute hypoxia (Cook et al., 2015). What is more, anadromous Atlantic salmon cease to feed once they return to fresh water, and so their spawning migration and reproductive investment is fuelled from a fixed amount of resources (Tufts et al., 2000; Olsen et al., 2010). During a C&R event, fish tend to use a quota of these non-renewable resources for recovery and survival (Tufts et al., 2000; Olsen et al., 2010; Lennox, 2018). Therefore, returning salmon are probably more vulnerable to the effects of C&R than nonmigratory fish, as they cannot restore the reserves expended in recovering from the stress of capture without depleting the amount available to fuel the remainder of the migration and the breeding attempt (Olsen et al., 2010; Lennox, 2018).

1.6.1 Effects on Mortality Risk

Most Atlantic and Sockeye (*Oncorhynchus nerka*) salmon that undergo C&R survive (~90-95 %) to reproduce, however the biotic and abiotic conditions under which they are caught are critical for their survival (Tufts et al., 2000; Dempson et al., 2002; Donaldson et al., 2010; Raby et al., 2015). About 90 % of post-release mortalities occur within the first 24 h (Tufts et al., 2000). The major contributors

to post-release mortality in descending order of magnitude are hooking location (higher if hooked in oesophagus or gill than in mouth) and gear type, water temperature and handling (Tufts et al., 2000; Lindsay et al., 2004; Olsen et al., 2010; Raby et al., 2015). The type of hook used can considerably influence the outcome, as it directly affects hooking location, bleeding, handling time and duration of air exposure (some hooks are harder to remove than others; Olsen et al., 2010). Several studies on Atlantic salmon also demonstrate that there is a higher probability of post-angling mortality at higher water temperatures (20 ± 2 ^{o}C) due to several physiological implications (Wilkie et al., 1996; Tufts et al., 2000; Dempson et al., 2002). These physiological disturbances can include elevated physiological disruption (e.g. loss of ions), and raised metabolic demand due to air exposure and exercise (Arlinghaus et al., 2013; Gale et al., 2013). Salmon released back into their natural habitat after capture can also die from increased vulnerability to predation or secondary opportunistic wound infection (Raby et at., 2015; Lennox, 2018). Increased stress as a result of C&R may cause immunosuppression, which can allow the proliferation of non-harmful or opportunistic pathogens, such as the fungi *Saprolegnia* spp. or *Aeromonas salmonicids*, to become infectious and cause death (Wedemeyer and Wydoski, 2008; Raby et al., 2015). The impact of C&R is also likely to depend on sex, maturity, prior experiences, fitness and pre-capture pathogen load (Raby et al., 2015).

1.6.2 Physiology

The fasting that adult salmon endure when returning to fresh water to spawn significantly diminishes their white muscle glycogen stores, which tend to determine the anaerobic capacity of individuals (Tufts et al., 2000). Hence, post-angling effects and rate of recovery will differ at various stages of migration, although the adverse effects of C&R do not necessarily increase with time since leaving the ocean (Tufts et al., 2000; Olsen et al., 2010). For instance, salmon that have only recently entered the river may fight capture for longer and so take longer to restore homeostasis after released than post-spawned fish that have very depleted energy reserves (Tufts et al., 2000). Salmon that have spent more than one year at sea have a lesser physiological disturbance to angling than salmon who have returned to spawn after just one winter at sea (Tufts et al., 2000). Water hardness, which is defined by the concentration of dissolved calcium and

magnesium, can also significantly influence the blood composition of caught salmon, with soft water having a bigger impact (Tufts et al., 2000). Likewise, salmon freshly returned to the river suffered a greater disturbance after capture than post-spawned fish in relation to plasma pH, osmotic and lactate concentrations, bicarbonate, sodium, potassium and chloride (Tufts et al., 2000; Donaldson et al., 2010; Gale et al., 2011), again likely because they fight for longer before exhaustion. Salmon angled at lower water temperatures (8 ^{o}C) had a heart rate 1.6 - 1.8 times lower than individuals caught at higher temperatures (16.5 and 20 ^{o}C), but the rate of recovery was similar (Anderson et al., 1998). However, high temperatures can be problematic: Sockeye salmon caught at about 20 ^{o}C had depressed ventilation rate, increased oxygen consumption, and were unable to sustain equilibrium once released back into their habitat (Gale et al., 2011). Fishing can also induce a selection pressure on the physiological phenotypes of fish, which could potentially lead to changes in fish distribution, population vulnerability to fishing and life history (Hollins et al., 2018). For example, Koeck et al. (2018) discovered that rainbow trout (*Oncorhynchus mykiss*) associated with a proactive phenotype (i.e. increased locomotor activity with reduced cortisol concentration, and decreased serotonergic and dopaminergic brain activity) were more susceptible to angling, thereby exerting a selection pressure in an angled wild population towards a less active, more stress sensitive individuals.

1.6.3 Behaviour

Behavioural modifications are an underlying mechanism with which individuals try to overcome a stressor (Raby et al., 2015; Lennox, 2018). These modifications can either be adaptive or maladaptive depending on the situation (Raby et al., 2015; Lennox, 2018). Post-release salmon can also behave aberrantly due to shock, injury, stress, physiological disturbance, or restoration of homeostasis, which can increase their vulnerability to predation once released (Olsen et al., 2010; Gale et al., 2011). Salmon can also experience increased predation if they are released in habitats in which they don't normally reside, such as calm shallow streams (Olsen et al., 2010). If they survive the initial post-release period, released Atlantic salmon tend to move and spawn in similar habitats as individuals that haven't experienced C&R, unless they are late season individuals which then tend to migrate shorter distances (Lennox et al. 2015). However, there is also evidence that salmonids that experience C&R may be less likely to pass barriers, such as

dams, and more likely to postpone their migration upstream or even withdraw downstream, and demonstrate irregular locomotor activity (Arlinghaus et al., 2013, Richard et al., 2014).

1.6.4 Reproduction

Environmental conditions experienced by the parents, both as juveniles and adults, can also be transmitted either directly or indirectly to future generations (Schreck, 2010; McGhee et al., 2012; Jensen et al., 2014; Burton and Metcalfe, 2014 Donelan and Trussell, 2018; Atherton and McCormick, 2020). For instance, rainbow trout (*Oncorhynchus mykiss*) treated with cortisol at the egg stage (simulating maternal stress exposure) displayed increased fearfulness to a sudden stimulus; however, the effects were dependent on the time of exposure (effects may be more pronounced if individuals are exposed to the stressor during critical windows; Colson et al., 2015). Coho salmon (*Oncorhynchus kisutch*) exposed to cortisol at the egg stage demonstrated increased boldness and dominance when face with conspecific intruders (Sopinka et al., 2015). Acute stress from C&R can also influence the reproduction of female Atlantic salmon through hormonal alterations, decreased gamete quality or quantity and suppressed ovulation (Olsen et al., 2010; Eriksen et al., 2013). This was also shown with other teleost females that had been exposed to a stressor prior to reproduction (Eriksen et al., 2007; Eriksen et al., 2013). Other reproductive traits in female teleost fish that have been shown to be affected by stress include reduced gonad and oocyte mass/ size, postponed ovulation and delayed gonadal maturation (Eriksen et al., 2017). To study the effects of stress, cortisol was artificially introduced in mature female Atlantic and Pacific salmon (Eriksen et al., 2015; Sopinka et al., 2015). The adults exhibited decreased fecundity (number of fertilized eggs), while the offspring had reduced survival rates and early growth, decreased swimming performance, as well as an increased probability of morphological deformities (Eriksen et al., 2015; Sopinka et al., 2015). Sopinka et al. (2015) also indicated that even though maternal stress had a significant impact on offspring performance, egg cortisol levels were unchanged. C&R has shown to have a bigger adverse influence on the reproductive success of male Atlantic salmon rather than that of females (Richard et al., 2013). Lastly, Atlantic salmon exposed to air for 10 seconds or more can produce two to three times fewer offspring compared to individuals that were not air exposed (Richard et al., 2013). However, the long-term intergenerational

effects of C&R have not yet been investigated in depth, and therefore there is a gap in knowledge on how this conservation initiative will affect the species in the long run. This is an issue not only for the Atlantic salmon, but fish in general experiencing C&R.

1.6.5 Effects of C&R Angling of Adult Salmonids on the Offspring

Various studies have illustrated how the parent's condition and environment can affect the development, behaviour, and physiology of the offspring (Schreck, 2010; McGhee et al., 2012; Jensen et al., 2014; Sopinka et al., 2014; Donelan and Trussell, 2018; Atherton and McCormick, 2020). Furthermore, many of these investigations highlight how maternal stress can affect the early developmental stages of the offspring (Eriksen et al., 2006; Allen et al., 2008; Eriksen et al., 2015; Thayer et al., 2018). There is, however, a gap in knowledge on how maternal stress affects the offspring phenotype, performance, and life history beyond this point (Eriksen et al., 2007; Andersson et al., 2011). Yet, what is even more interesting is the very scarce information on the effects of stress on fathers can have on the next generation. When it comes to C&R angling and our attempt to understand its ecological effects on wild populations, most of the effort to date has targeted the parents themselves, and not the offspring or the future survival of the population (Richard et al., 2013; Sopinka, 2015). So far, the effects of parental stress on offspring have been explored either through cortisol manipulation of the parents or direct manipulation of cortisol concentration in the eggs (Eriksen at al., 2006; Andersson et al., 2011; Burton et al., 2011; Sopinka et al., 2015; Sopinka et al., 2016).

1.7 Conclusion

The Atlantic salmon is an important species that plays a critical role for both the economy and the environment. It also has a very diverse life history and is phenotypically plastic, which allows it to adapt to challenging environments and changing conditions. However, many populations of this species, across wide geographical areas, are in serious decline as a result of over-exploitation, habitat loss, ecosystem disruption and climate change. As a result, there are now conservation measures in place to reduce the impact of fishing, including catch-and-release policies for angled fish in many river systems. A lot is known about

the effects of C&R on the physiology, behaviour, and reproduction of the adult population, as well as the post-release mortality rates. There is also a lot of information relating to the best fishing practices to minimize these effects. However, one component that has not been investigated yet is the influence and the impact that C&R of the parents has on the performance of future generations. My research therefore attempts to fill this gap, through a series of related studies on the effects of simulated C&R of adult wild Atlantic salmon on both the adults and their offspring.

1.8 Structure of Thesis

Following this introductory chapter, the thesis has the following structure:

Chapter 2 - Simulated Pre-Spawning Catch & Release of Wild Atlantic Salmon (*Salmo salar*) Results in Faster Fungal Spread and Opposing Effects on Female and Male Proxies of Fecundity

In this chapter I explore the impact that C&R angling can have on the survival, immunity, and reproduction of wild Atlantic salmon. Using a simulation that included different durations of exercise and air exposure, I mimicked two of the main stressors that can cause harm to fish during an angling event. Then, based on the cumulative disturbance that each of the treatments had suffered, I examined whether the mortality of experimentally affected males and females had increased, or whether the spread of the naturally occurring fungus *Saprolegnia* spp., that infected the fish at the time of capture, had a higher percentage body cover increase. Also, at the time of the external artificial fertilization, I took samples of male and female gametes as to investigate the effects parental treatment on gamete quantity of quality.

Chapter 3 – Effects of simulated catch-and-release angling of pre-spawning Atlantic salmon on the viability and development of their offspring

Chapter 3 investigates how parental stress immediately (5 – 18 days) prior to spawning can influence the early developmental stages of the next generation. To achieve this, I used the fertilized clutches from the experimentally affected parents in chapter 2, and subsequently examined the influence that the cumulative parental disturbance had on the survival of the offspring during

distinct developmental stages. Additionally, the experiment explored whether parental disturbance had any effect, or any disproportional impact (based on the level of exercise and air exposure the parents experienced) on the offspring yolk sac size, date and size of first feeding, and growth rate. Lastly, it examined the vulnerability of the offspring to the naturally occurring fungus *Saprolegnia* spp. during an unexpected 12-day fungal outbreak within the system.

Chapter 4 – Simulated catch-and-release angling of adult wild Atlantic salmon (*Salmo salar*), decreases the activity and exploration of a novel environment in offspring and increases aggression.

Here I investigate how simulated parental C&R angling of wild Atlantic salmon can affect key behaviour traits of the offspring during the early stages of their juvenile life. Offspring from the experimentally affected parents in Chapter 2 were transferred to the university of Glasgow, where under controlled conditions, a series of sequential tests examined the offspring's risk-taking behaviour, their locomotor activity and exploration of a novel environment, and aggression towards conspecifics. This was studied in relation to the cumulative disturbance experienced by the parents.

Chapter 5 - Simulated C&R Affects the Pairwise Dominance of Offspring

Chapter 5 explored how parental pre-spawning stress from a C&R simulation can affect the metabolic rate and dominance of the offspring. In doing so, I measured the standard metabolic rate (SMR), the maximum metabolic rate (MMR), and aerobic scope (AS) of the offspring from the experimentally treated parents. Moreover, the dominance (hierarchical structure) was determined through several sets of feeding trials across the offspring of dissimilar disturbance.

Chapter 6 – General Discussion

In this final chapter I synthesise the results from the entire project and discuss their implications for our understanding of parental effects and the impact of parental stressors on offspring, with a focus on the relevance for the management and conservation of wild salmon.

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Chapter 2 - Simulated Pre-Spawning Catch & Release of Wild Atlantic Salmon (*Salmo salar*) Results in Faster Fungal Spread and Opposing Effects on Female and Male Proxies of Fecundity

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2.1 Summary

Atlantic salmon are increasingly being released after capture by anglers, as a fisheries management tool. However, there is little information regarding the medium- to long-term impacts on the fish being subjected to the stress of capture. The greatest effects are likely to occur near the time of spawning and such data are important for setting appropriate closed seasons during which angling for salmon may be prohibited. This study examines how stressors related to catch and release angling experienced shortly before spawning affect adult salmon mortality, vulnerability to the fungus *Saprolegnia* spp, and reproductive traits. Adult salmon were caught using a permanent fish trap on the river Blackwater, N. Scotland, during their upstream spawning migration, and were then exposed to one of three stressor protocols comprising exercise and air exposure of different durations mimicking catch and release practices, with a fourth group used as a non-stressed control. There was no effect of the experimental stressors on the pre-spawning mortality rate of the fish, but they increased the rate of growth of the fungus *Saprolegnia* spp. over the body of the fish. Air exposure and exercise also influenced the reproductive traits of both male and female salmon. Unexpectedly, the sperm of the male salmon from the most intense disturbance protocol exhibited an increase in the maximum duration of sperm motility. Motility period also increased with time elapsed since the salmon were exposed to the stressor. Lastly, females that experienced exercise and/or air exposure of any duration spawned at the usual time but with fewer eggs (smaller clutch sizes). These results indicate that adult salmon would likely spawn within the right timeframe after having been exercised to exhaustion and exposed to air during

the late stages of upstream migration, but their reproductive success might be reduced as a result of smaller clutch sizes. This suggests that there may be benefits from preventing angling close to the time of spawning.

2.2 Introduction

Recreational fisheries have been implicated in the heavy exploitation of fish within marine and freshwater ecosystems (Cooke and Schramm, 2007; Arlinghaus et al., 2013). To preserve stocks, maintain ecosystem balance, and ensure long-term resilience of recreational fishing, fisheries managers in many areas have implemented the concept of 'catch-and-release' (C&R) angling, in which captured fish are returned to the water rather than killed (Cooke and Schramm, 2007; Wedemeyer and Wydoski, 2008; Smukall et al., 2019). With the global growth of both mandatory and voluntary C&R practices, it has been estimated that, depending on location and species, at least 60 % of all rod-caught fish are being returned after capture to the water (Cooke and Cowx, 2004; Ferter et al., 2013; Cowx et al., 2017; Simms et al., 2017), amounting to billions of fish being released on a yearly basis (Casselman, 2005; Arlinghaus et al., 2007; Arlinghaus et al., 2013). The success of this management practice is dependent on the capacity of the fish to recover from an angling, with minimal impact on their survival and reproductive success (Dempson et al., 2002; Richard et al., 2013; Richard et al., 2014; Lennox et al., 2016). In Atlantic salmon the exploitation rate is relatively low, typically figures in the order of 10 % (Cefas et al., 2018; ICES Advice, 2020). However, regardless of the fishing gear and technique, C&R may act as a significant acute stressor, with possible adverse effects over different timescales and biological levels (from cellular to ecosystem), and so its impact on conservation is unclear (Arlinghaus et al., 2013; Raby et al., 2015).

Research has indicated that mortality of fish immediately following release is usually low (Bartholomew and Bohnsack, 2005; Cook et al., 2015; Twardek et al., 2018, Smukall et al., 2019). However, there are several physiological and behavioural disturbances that a fish may experience during capture that may have sublethal effects (Donaldson et al., 2014; Cook et al., 2015; Raby et al., 2015; Lennox, 2018; Twardek et al., 2018; Smukall et al., 2019). Once hooked, many

fish will often fight to exhaustion as they are reeled in (Olsen et al., 2010; Lennox, 2018), causing depletion of energy reserves and production of metabolic by-products, which can result in a release of catecholamines and corticosteroids (Killen et al., 2003; Suski et al., 2003; Donaldson et al., 2014; Raby et al., 2015; Lennox, 2018). During recovery from angling, the ability to detect and escape predators, as well as capture prey may be compromised (Cooke and Schramm, 2007; Arlinghaus et al., 2013). This may be a result of physiological constraints caused by exhaustion, being released back into a novel environment or a consequence of acute stress (Arlinghaus et al., 2013). Air exposure following capture has been shown to be one of the most detrimental components of C&R in fish (Killen et al., 2006; Thorstad et al., 2007; Olsen et al., 2010; Richard et al., 2013; Cook et al., 2015). For example, for Shortnose Sturgeon (*Acipenser brevirostrum*) every minute of air exposure post-capture causes a 1.8-fold higher risk of reflex impairment (Struthers et al., 2018). Similar evidence of reflex impairment and compromised swimming capabilities were demonstrated in steelhead trout (*Oncorhynchus mykiss*) that were exposed to air after capture (Twardek et al., 2018). The same investigation also demonstrated a higher immediate post-release downstream movement during the spawning migration following release.

The effects of environmental conditions experienced by parents, whether around the time of breeding or earlier in life, can be transmitted directly or indirectly to future generations (Burton and Metcalfe, 2014). These are known as parental (or sometimes more narrowly as maternal) effects, and they can be either adaptive or maladaptive for offspring (Burgess and Marshall, 2014; Burton and Metcalfe, 2014; Jonsson and Jonsson, 2014; Haussmann and Heidinger, 2015; Blount et al., 2016). Parental effects have been observed in a wide range of organisms, both from the terrestrial and aquatic environment. For example, gravid common lizards (*Zootoca vivipara*) exposed to snake cues produced offspring with a phenotype, physiology, and behaviour better suited to a high-risk environment, since they grew longer tails, preferred colder temperatures, and increased their dispersal by 3-fold (Bestion et al., 2014; Benjamin and Mathieu, 2019). Parental influences have also been identified in highly competitive environments. One such example is the marine bryozoan, *Bugula neritina*, where in competitive environments it produces larvae that are bigger in size and which can disperse further (Allen et

al., 2008). Furthermore, the sex allocation of the offspring in Black howler monkeys (*Alouatta caraya*) can be influenced by the glucocorticoid (GC) levels of the mother at the time of conception, where low levels of GC (<200ng g^{-1}) result to female offspring (Rangel-Negrin et al., 2017). Since GC respond to maternal stress, more males are likely to be born under harsh environmental conditions, such as forest fragmentation. In Brown trout (*Salmo* trutta), exposure of the ova to cortisol pre-fertilization, can lead to increased oxygen consumption and aggression in the offspring (Sloman, 2010).

In fish, adults that experience stress prior to reproduction have been shown to exhibit changes in hormonal profiles, delayed spawning, decreased gamete quality and quantity, and suppressed ovulation (Sopinka et al., 2016; Smukall et al., 2019). Evidence of similar effects are found in Atlantic salmon exposed to acute stress from C&R (Olsen et al., 2010; Richard et al., 2013). Moreover, egg viability of caught and released fish seems to depend on both the species of fish and the timing of the fishing event. For example, there were no effects of C&R on the egg viability of Atlantic and sockeye (*Oncorhynchus nerka*) salmon caught by angling during the final stages of migration to their spawning grounds (Booth et al., 1995; Smukall et al., 2019), but the viability of the eggs decreased in rainbow trout (*Oncorhynchus mykiss*) after a brief period of emersion during gamete development (Campbell et al., 1992). In salmonids there is also evidence that C&R can affect the migration patterns and total distance travelled by the fish, and cause fish to drop back down stream, as well as impair their ability to leap over barriers (Arlinghaus et al., 2013; Richard et al., 2014). This could indicate that fish can have the capacity to survive an angling event, but with repercussions for their reproduction (i.e. not being able to reach ideal spawning grounds). There remains uncertainty regarding the effects of C&R angling on the reproductive fitness of fish, particularly when capture occurs during the gamete developmental stage (Richard et al., 2013; Arlinghaus et al., 2013; Smukall et al., 2019).

In species with external fertilization and polyandrous mating systems, such as salmonids, male mating success is governed by the balance of three key sperm factors; quantity, longevity, and velocity (Gage et al., 2004; Crean et al. 2012; Beirao et al., 2019). In sockeye salmon (*Oncorhynchus nerka*) for example, 80 % of eggs are fertilized in the first 5 s of sperm-egg mixing (Hoysak and Liley, 2001). Therefore, if stressors experienced by the male prior to spawning affect the

activity levels of his sperm this could have a major impact upon his fertilization success. However, the attributes of the sperm do not only influence the reproductive success of the parents, but the fitness of the offspring as well. In Atlantic salmon, offspring fertilized by sperm of intermediate longevity developed at an accelerated rate, and therefore emerge from the gravel earlier (Immler et al., 2014). This in turn allows them to establish a territory sooner and enhance their competitiveness against other conspecifics (Immler et al., 2014). Comparably, zebrafish (*Danio rerio*) sired by long-lived sperm acquired an increased lifespan and obtained a reduced deterioration of both their fecundity and their offspring fitness in late life (Alavioon et al., 2019).

Salmon returning to fresh water have to cope with several natural (i.e. osmoregulatory and temperature) and anthropogenic (i.e. pollution and artificial barrier) stressors in addition to angling, which could result in an additive or synergistic impact on the ability of salmon to recover during C&R (Lennox, 2018). Moreover, adults cease feeding upon entering fresh water during their spawning migration, so that the body reserves that they carry at that point must be allocated among migration, maintenance, and reproduction (Tufts et al., 2000; Olsen et al., 2010). When experiencing C&R however, fish will use a quota of these non-reusable resources for recovery and survival (Tufts et al., 2000; Olsen et al., 2010; Lennox, 2018). In addition, C&R can lead to immunosuppression and leave fish more vulnerable to pathogens like the ubiquitous, opportunistic fungi *Saprolegnia* spp. (Casselman, 2005; Wedemeyer and Wydoski, 2008; Olsen et al., 2010; Arlinghaus et al., 2013; Havn et al., 2015; Smukall et al., 2019). Fish can become immunocompromised either directly through physical damage of the skin through scale loss, abrasions, or hook injury during handling, or indirectly through metabolic, osmoregulatory and hormonal disturbances due to stress (Olsen et al., 2010; Wedemeyer and Wydoski, 2008; Smukall et al., 2019). Therefore, it is important to not only understand the specific effects that C&R has on salmon, but also the confounding effect of angling on the other stressors experienced *en route* to their spawning grounds.

Catch and release schemes clearly have less of an impact on the population than the alternative of captured fish being killed. However, given our limited knowledge of the effects of C&R on gamete development and reproduction of Atlantic salmon, further studies are necessary to understand the immediate

effects that this fisheries management policy will have on spawning (Olsen et al., 2010). The greatest effects of C&R are likely to occur if the capture occurs near the time of spawning, therefore data on pre-spawned salmon are important in considerations of appropriate closed seasons during which angling may be prohibited. I hypothesized that adult salmon experiencing the most cumulative disturbance from C&R angling prior to spawning, would be most susceptible to fungal infection and would experience the greatest impacts on reproduction. I therefore examined whether simulated C&R (adult pre-spawning stress) influences their mortality rate and vulnerability to pathogens (*Saprolegnia* spp.), as well as quantified its effects on the reproductive traits of both sexes (time of spawning, clutch and egg size, and sperm quality and quantity).

2.3 Methods

2.3.1 Salmon collection

Mature anadromous Atlantic salmon (fork length = 667.5 ± 217.5mm; weight = 3.061 ± 2.266kg) were collected from November to December 2018 during their upstream spawning migration, using the permanent fish trap set up by the Cromarty Firth Fishery Board on the river Blackwater, Scotland. They were transferred using large individual water-filled bags from the fish trap to large circular holding tanks (diameter 4 m; depth 1.5 m; water flow 60 litres/min; maximum stocking density = 60 fish per tank, but exact number varied based on the timings of fish capture and the onset of treatments), where they were held in single-sex groups and given 24-48 h to recover. There was a total of four holding tanks (two per sex; one for experimental and one for non-experimental fish – see below for definitions of fish categories). The water in the holding tank was supplied directly from the river Blackwater, and the temperature (6 ± 1.5 ^{o}C) was recorded on an hourly basis using a temperature data logger (HOBO Pendant Temperature/Light 64K Data Logger, Onset Computer Co., USA). All procedures carried out in this study were approved under UK Home Office Project License PB948DAAO.

2.3.2 Catch and Release Simulations

Fish were subjected to treatments that simulated the experiences potentially encountered by fish during C&R angling, following the approaches used by previous studies (Struthers et al., 2018; Smukall et al., 2019). Male fish were selected haphazardly from the pool of captured anadromous migrants, as were females with the proviso that they had not yet released their eggs into the body cavity (i.e. were 'hard') and so were not immediately ready to spawn. Selected fish were anaesthetised on the day of capture using clove oil and measured for fork length (to the nearest 0.5 cm), weight (to the nearest 0.001 kg; using a DEFENDER 5000 XTREMEW electronic balance) and photographed on both sides (using a Sony Cyber-shot DSC-WX100 camera) for later calculation of the percentage of the body covered by the fungus *Saprolegnia* spp. (see below; Table S2.1). Individuals were then randomly (Number generator random; version 2.0) allocated to one of four pre-determined treatment groups based on stressor protocols, before being tagged with an individually colour-coded Floy tag (indicating treatment) and passive integrated transponder (PIT) tag (individual ID) and allowed to recover for 24-48h. Any fish that died after the C&R simulations were applied to them, but still within the overall trapping period (n = 5 males and 1 female) were replaced, so maintaining the sample size of 15 fish per treatment per sex (n = 120 in total). A flow chart summary for the number of experimental fish used in each procedure can be seen in Figure S2.1.

An equal number of males and females were assigned to each of four treatments (Table 2.1): (1) a control treatment in which fish were not exercised or air-exposed; (2) a treatment in which fish were exercised for 210 s (see supplementary information for the reasoning behind the timeframes of exercise and air exposure) without rest by manually chasing them in an arena (diameter 4 m; depth 1.5 m; depth 0.18 m), and lightly tapping the fish on its sides or tail using a hand and glove, intended to simulate the exercise that occurs during angling; (3) a treatment which consisted of this exercise, plus air-exposure for 60 s (by being held in a knotless net), simulating the experience of being caught and then held up for hook removal or to be measured and photographed; or (4) a treatment which consisted of exercise plus air-exposure for 120 s. Following treatment, fish were placed in one of two new holding tanks (one per sex) containing fish that been through the protocols (Struthers et al., 2018; Smukall et

al., 2019). Control fish went through the process of being transferred to the post-treatment holding tanks but using water-filled plastic bags to minimise the disturbance that they experienced. The water in both the arena and holding tanks was supplied directly from the river blackwater (temperature = 6 ± 1.5 ^{o}C).

Table 2.1 Summary of the four treatment groups used in the experiment and the cumulative levels of acute disturbance they represent, indicated by the number of asterisks.

Treatment	Exercise	Air exposure	Cumulative disturbance
Control	No	No	
Exercise	210 sec	No	*
Exercise + Air Exposure	210 sec	60 sec	**
Exercise + Extended Air Exposure	210 sec	120 sec	***

Fish were left undisturbed in the post-protocol holding tanks for a minimum of five days (Table 2.2). They were then stripped of gametes once the females became ripe, i.e. had released their eggs into the abdominal body cavity such that their belly was soft, as determined by staff from the hatchery who were blind to experimental treatment groups.

Table 2.2 Description of the mean time delay (days) between the date that the fish were exposed to the treatments up to the day they were artificially stripped of their gametes. Presented are also the number of fish per treatment group (N), and the maximum minimum range of this delay for each of the treatment groups.

Treatment	Mean time delay until mating (days)	
	Males	Females
Control	12.5 (N = 17), range = 7 - 18	10.3 (N = 16), range = 6 - 13
Exercise	11.5 (N = 18), range = 7 - 18	9.1 (N = 15), range = 6 - 12
Exercise + Air Exposure	13.6 (N = 14), range = 10 - 18	9.5 (N = 15), range = 5 - 12
Exercise + Extended Air Exposure	12.9 (N = 15), range = 7 - 17	10.2 (N = 15), range = 6 - 15

2.3.3 Artificial Fertilization and Gamete Collection

Each experimental fish was mated with a single non-experimental fish taken from the holding tanks; the time at which male experimental fish were mated was determined by when female non-experimental fish were ripe. A total of 112 crossings were conducted (Experimental females = 60, and experimental males = 52). Fish were initially anesthetised in clove oil. Their identity was noted from the PIT tag and they were then photographed on both sides as before for later calculation of the increase in percentage body cover of the *Saprolegnia* spp. fungus. To prevent contamination and activation of the gametes, the ventral surface of the salmon was dried of any excess water. The body mass of all experimental fish was then measured pre-stripping to the nearest 0.001 kg.

Egg collection was achieved by gently massaging the abdomen in a unidirectional manner, from below the pectoral fins to just above the urogenital opening, until all eggs had been released into a bowl. About 1 ml of milt (semen) was then collected from a single male, using a similar approach, and placed directly onto ice for assessment of sperm quality and quantity. All remaining milt from the male was stripped and released on top of the eggs, and both the eggs and milt were

gently mixed together. Water was then added to activate the sperm, and the eggs left for c.60 min before being rinsed. This allowed the eggs to swell up and the shell to harden. Once adult fish had been stripped of their gametes they were re-weighed (somatic mass) and released back into the lake close to the site of capture.

The eggs were then transferred to the SSE (Scottish and Southern Energy) hatchery in Contin, Scotland, where they were drained of water and ovarian fluid. Subsequently, the weight of a counted subsample of eggs (to the nearest 0.01 g; Scout Pro electronic balance, OHAUS) and of the total clutch (to the nearest 1 g) were measured to estimate the total number of eggs produced by each crossing (see below). The eggs were then allowed to incubate at the hatchery, with each family being reared in a separate tray.

The person responsible for stripping adults of eggs and sperm was blind to the treatment group of the experimental fish. Each of the fish were used only once during the crossings, with two exceptions: on one occasion two non-experimental males were used to fertilise an experimental female since the first male proved to have insufficient milt to be guaranteed to fertilise all the eggs. Secondly, a shortage of ripe non-experimental females led to the clutch of one non-experimental female being split in half and fertilized by two experimental males.

2.3.4 Mortality and Vulnerability to Disease

Adult pre-spawn mortality (between capture and spawning) was recorded throughout the experiment. The fungus *Saprolegnia* spp. was quantified by calculating the percentage body cover with fungus (including fins) from the photographs taken at capture and at mating, using the software ImageJ (version 1.51r). The analysis was blind to treatment and conducted in a random sequence. The fungal spread was then determined by calculating the increase in the percentage body cover between these two time points.

2.3.5 Effects on Male Reproduction

The volume of milt produced by experimental males was determined using the change in the body mass of the fish pre- and post- stripping. This was then converted to a volume by assuming that 1 g of milt was equal to 1 mL. Sperm

concentration in diluted milt was quantified using a Neubauer haemocytometer (milt solution dilution factor 1:75), counting the number of spermatozoa per grid square in the field of view using ImageJ (version 1.51r). Then, the approximate concentration was calculated using the following formula:

$$\text{Sperm concentration } \left(\frac{\text{cells}}{\text{L}}\right) = \text{Avg. number of sperm cells per square } \times \text{Dilution factor } \times 10^4$$

The quantity of sperm produced by a male could then be estimated using the formula below:

$$\text{Sperm Quantity (cells)} = \text{Sperm Concentration } (\frac{\text{cells}}{\text{L}}) \times \text{Milt Volume (L)}$$

Sperm quality was assessed in terms of its period of activity. Within 60 min of being collected, the chilled milt samples were warmed to ambient air temperature and the sperm were activated by adding 20 µl of milt to 1.5 ml (20:1500) of fresh water. The movements of the activated sperm were then filmed under a light microscope (40x, HM-Lux Pol. Monocular microscope, Leitz) using a digital camera (14MP HDMI HD 1080P Digital Microscope Magnifier Industrial Camera). Sperm quality was quantified in terms of maximum duration of sperm motility, which was defined as the total time (s) taken from the initial activation until all sperm in the microscope's field of view had stopped moving (Alavi and Cosson, 2005; Fauvel et al., 2010). Maximum duration of sperm motility for each experimental male was determined by measuring the maximum duration of motility in two separately activated samples, and then calculating their mean value. A few males (n = 17) only had one recorded measurement of sperm activity. A table (Table S2.2) is provided in the supplementary information indicating the average time (s) per male at which most sperm cells within the microscope's field of view stopped swimming, and the average time (s) per male when all cells within the microscopes field of view had stopped.

2.3.6 Effects on Female Reproduction and Spawning

One measure taken of a female's reproductive investment was the mean volume of her fertilized eggs. The clutch of each female was photographed (Sony Cyber-shot DSC-WX100 camera) within their individual family trays alongside a size

reference; the photograph was taken on the day of fertilization but after the eggs had finished swelling after exposure to water. The diameter of 20 randomly selected eggs was subsequently measured blind to experimental treatment from the photographs in ImageJ (version 1.51r); this was used to calculate the average volume (mm^3) of an individual egg from each family using the formula of a sphere:

$$\text{Volume of egg } (mm^3) = \frac{4}{3}\,\pi\, r^3$$

Where r = egg radius. Clutch size was calculated based on the measurements of total clutch weight and weight of the counted subsample of eggs using the formula below:

$$\text{Total clutch size} = \frac{\text{Weight of total clutch (kg) x Number of eggs in subsample}}{\text{Weight of subsample of eggs (kg)}}$$

2.4 Statistical Analyses

Adult mortality (from the time the treatments were applied up until the time of spawning) was examined in R (Version Ri386 3.4.4) using a Cox Proportional Hazards model. The analysis included all fish (n=125) that had gone through the stress protocols, including the 110 fish that survived to mating. Along with the treatment, percentage fungal infection of the fish at the time of trapping was also added to the model as an explanatory variable. General linear models (GLM) were used to examine whether the experimental treatments had a significant effect on the fish vulnerability to disease (defined as the increase in the percentage of body of salmon covered in fungus *Saprolegnia* spp.). The model also included days elapsed between the day the stressor was applied and stripping, the fork length of the fish at the time of trapping and the percentage fungal infection of the fish at the time of trapping as explanatory variable. GLMs were also used to investigate whether the stressor protocols influenced reproductive traits of males (sperm quantity and maximum duration of sperm motility) and females (days elapsed until females were ready to spawn, egg volume and clutch size). The model for sperm quantity included spread of fungus as an explanatory variable, while the model for maximum duration of sperm motility used days elapsed and date of trapping. Moreover, the model for days elapsed until females were ready to spawn include

percentage fungal infection of the fish at the time of trapping as an explanatory variable. Finally, the model for egg volume used clutch size and fork length of the female at the time of trapping as explanatory variables, while female clutch size used fork length and egg volume. All GLMs used treatment as the main explanatory variable. To normalise the residuals of the data, some of the dependent variables underwent either logarithmic (spread of fungus and maximum duration of sperm motility) or squared transformation (clutch size). The variables sex, date of trapping (Julian Date), fish fork length and somatic mass, initial % body cover with fungus, spread of fungus, order at which fish underwent experimental stressor, date fish experienced the stressor, clutch size, and days elapsed from the time of the stressor to spawning (Days elapsed), were tested within the models as explanatory variables, and used as indicated in the GLMs. The interactions between treatment and each of the variables sex, days elapsed from the stressor protocol to spawning, and spread of fungus were also initially included. The models with the lowest AIC scores were selected, and assumptions of linearity, normality of residuals, and homogeneity of variance were verified by inspection of model residual-fits plots. Significance was then determined using p-values ($\alpha = 0.05$), and if a treatment effect was found, differences among treatment groups were explored using a Tukey multiple comparison of means. A Levene's test for homogeneity of variance was run for each model. Lastly, the correspondence between the two recorded measurements for the maximum duration of sperm motility was investigated using a Pearson's correlation test. The strong positive correlation between these two measurements (see below), allowed for the single recorded measurements of sperm motility to be included in the rest of the statistical analysis.

2.5 Results

2.5.1 Mortality and Vulnerability to Disease

While mortality was not affected by C&R simulations, individuals that had a higher percentage of the body covered by the fungus *Saprolegnia* spp. on the date of trapping had a higher probability of mortality (Table 2.3).

Table 2.3 Summary of the Cox Proportional Hazards model for the effects of the stressor protocols on salmon mortality from the time the treatment was applied up until the time of spawning; the Control treatment group was the reference category. M = Males, F = Females.

	Number of mortalities		Concordance	exp(coef)	lower. 95	upper. 95	z	p
	M	F						
Control	6	0						
Exercise	4	0		1.297	0.348	4.483	0.39	0.70
Exercise + Air	2	2		1.447	0.366	5.731	0.53	0.60
Exercise + Extended Air	1	0		0.312	0.035	2.746	-1.05	0.29
Fungus Pre Treatment				5.814	2.291	14.753	3.71	<0.001
	15		0.87					

The increase in coverage of *Saprolegnia* spp. fungus on the body of the fish after the experimental treatments was greater in fish that had a higher percentage body cover of fungus at the time of trapping, and those that were caught in the trap early in the experiment. However, fungal spread was greater in individuals from the 'Exercise' and 'Exercise + Extended Air' groups, as indicated by a significant interaction between the treatment and days elapsed between the date the stressor was applied and spawning (Table 2.4, Figure 2.1).

Table 2.4 Summary of General Linear Models (GLM) investigating the factors influencing the increase in the percentage of the body covered by the fungus *Saprolegnia* spp. from the day of capture until the day of spawning. Shown are the comparisons of each of the three stressor protocols to the control, together with the effect of fish fork length, date of trapping, days elapsed from capture until spawning and percentage body cover of the fungus *Saprolegnia* spp. on the date of trapping.

	d.f.	Estimate	Std. Error	t	p
Intercept		6927	757.6	9.143	<0.001
Exercise	1	-2.034	0.754	-2.696	0.01
Exercise + Air	1	-0.789	0.796	-0.991	0.32
Exercise + Extended Air	1	-1.505	0.752	-2.001	0.047
Days Elapsed	1	-0.019	0.051	-0.375	0.71
Fork Length	1	6.478×10^{-04}	9.611×10^{-04}	-0.674	0.50
Date of Trapping	1	-0.160	0.017	-9.141	<0.001
Fungus Pre Treatment	1	0.610	0.132	4.635	<0.001
Exercise: Days Elapsed	1	0.183	0.067	2.736	0.01
Exercise + Air: Days Elapsed	1	0.082	0.069	1.200	0.23
Exercise + Extended Air: Days Elapsed	1	0.132	0.064	2.064	0.04
Residuals	106				

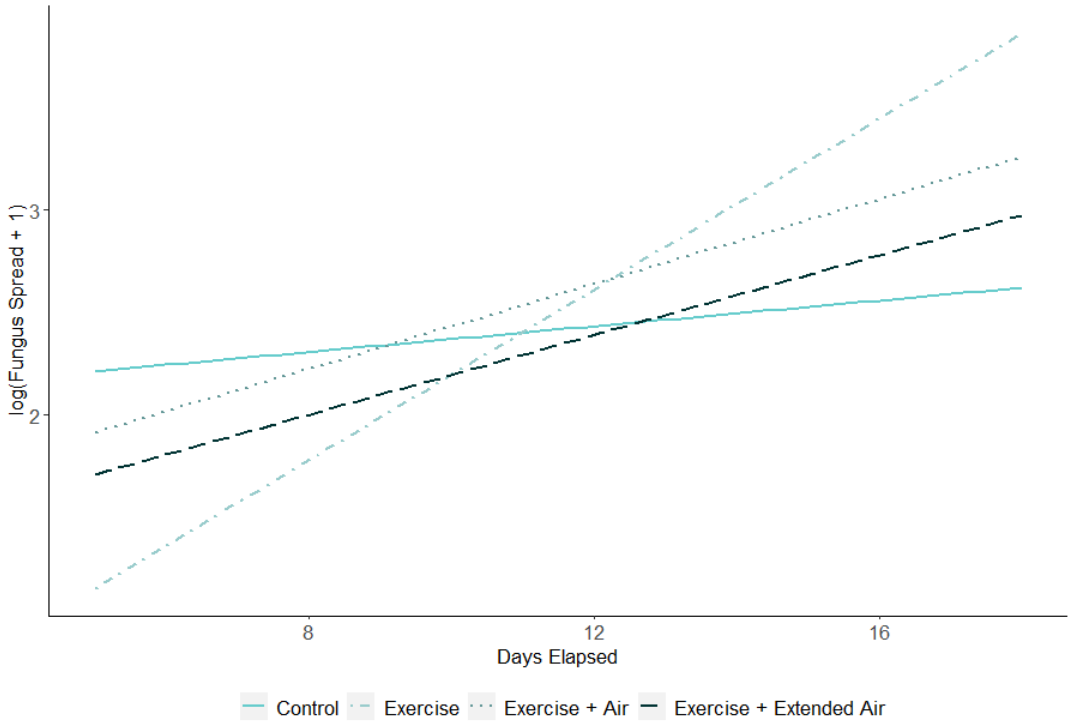

Figure 2.1 Effects of the interaction between the stressor protocols and days elapsed, on the increase in the percentage (%) of the body covered by the fungus *Saprolegnia* spp. from the date of capture until the date of spawning. The coloured lines indicate treatment. The coloured lines indicate the four treatment groups; note the faster increase in fungal spread in stressor treatments compared to the control group (see Table 2.4 for statistical analysis)

2.5.2 Effects on Male Reproduction

There was a strong positive correlation between the two independent measurements of the maximum sperm survivability (Figure 2.3), indicating that the assay was robust. Males from the Exercise + Extended Air group produced sperm that survived for a longer period once activated than did sperm from control males (Tables 2.6, Figure 2.4a). Furthermore, males in the Exercise + Extended Air group showed a positive relationship between the days elapsed from experiencing the stressor protocol until spawning and maximum duration of sperm motility, but the pattern was the opposite direction in the other three treatment groups (Tables 2.5 and 2.6, Figure 2.4b). Thus, the viability of sperm from the males exposed to the highest levels of disturbance increased with the duration of the male's recovery period, whereas males exposed to lesser disturbance displayed a decline in sperm viability over time after the catch and release

simulation. Additionally, later-trapped males had a lower duration of sperm motility compared to those caught early on. Sperm quantity was unaffected by either exercise or air exposure (Table 2.5, Figure 2.2).

Table 2.5 Summary of General Linear Models (GLM) for the effects of the stressor protocols on the quantity of sperm (cells) produced by male salmon. Included in the model was the variable percentage increase in the spread of fungus on the body of the fish from date of trapping to stripping.

	d.f.	Estimate	Std. Error	t	p
Intercept		177277	68587	2.585	0.01
Exercise	1	37758	79016	0.478	0.64
Exercise + Air	1	160601	86600	1.855	0.07
Exercise + Extended Air	1	79537	75100	1.059	0.30
Fungal Spread	1	3023	2617	1.155	0.25
Residuals	42				

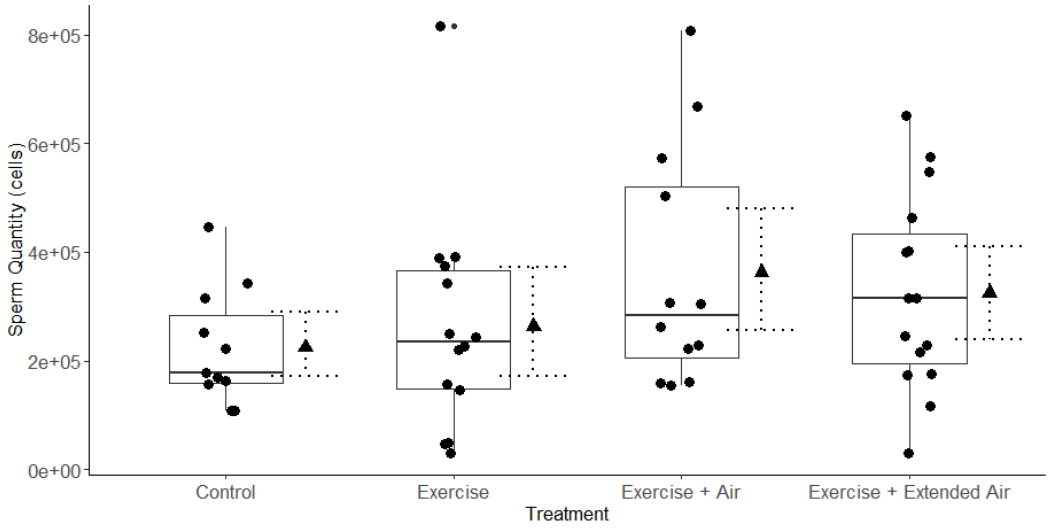

Figure 2.2 Effects of the Stressor Protocol on Sperm Quantity (cells). Each circular data point represents the sperm from an experimental male fish. The boxplot indicates the mean, the interquartile range, and maximum and minimum values for each of the treatments. The dotted line represents the 95 % Confidence Interval and the triangle represents the mean (see Table 2.5 for statistical analysis).

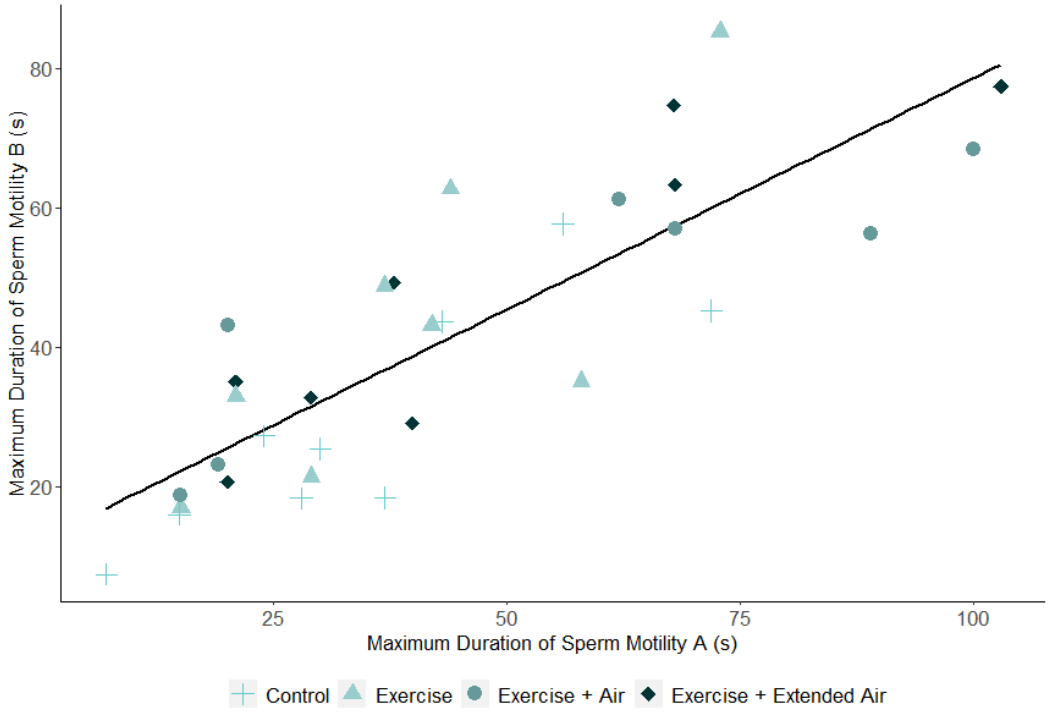

Figure 2.3 Pearson's product-moment correlation for survivability of the two spe rm samples (A and B) taken from the same male; r = 0.832, 30 d.f., p <0.001 at 9 5 % significance level. The signs and the colours indicate the treatment.

Table 2.6 Summary of General Linear Models (GLM) for the factors influencing the maximum duration (s) of sperm motility. The model also accounts for the date the fish were collected from the trap and days elapsed from capture until spawning.

	d.f.	Estimates	Std. Error	t	p
Intercept		2684.686	971.747	2.763	0.01
Exercise	1	-0.121	0.603	-0.200	0.84
Exercise + Air	1	-0.352	0.773	-0.456	0.65
Exercise + Extended Air	1	-1.240	0.589	-2.107	0.04
Days Elapsed	1	-0.102	0.035	-2.876	0.01
Date of Trapping	1	-0.062	0.022	-2.758	0.01
Exercise: Days Elapsed	1	0.024	0.045	0.541	0.59
Exercise + Air: Days Elapsed	1	0.037	0.054	0.688	0.50
Exercise + Extended Air: Days Elapsed	1	0.121	0.044	2.730	0.01
Residuals	39				

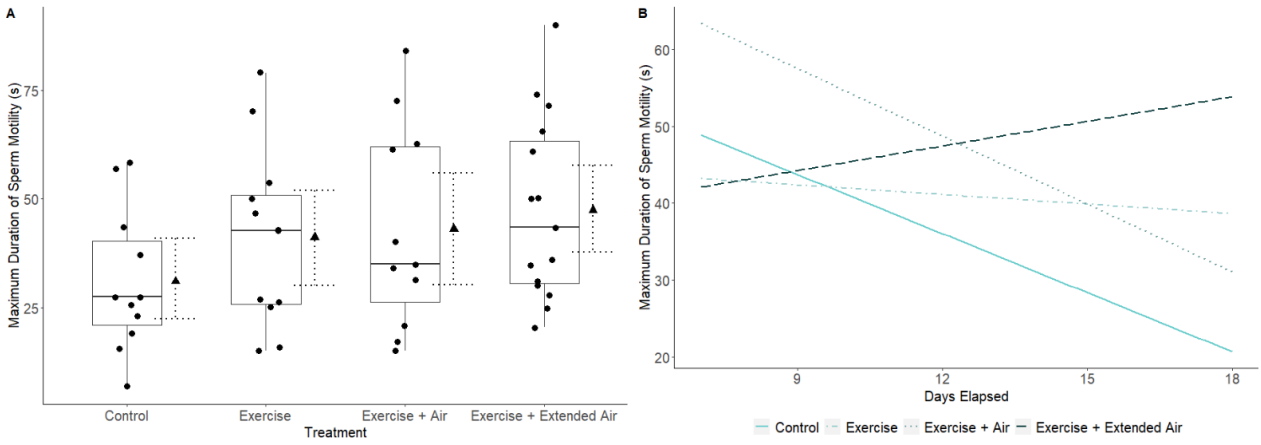

Figure 2.4 A. Effects of the stressor protocols on the Maximum Duration of Sperm Motility (s). Each circular data point represents an experimental fish. The boxplot indicates the mean, the interquartile range, and maximum and minimum values for each of the treatments. The dotted line represents the 95 % Confidence Interval and the triangle represents the mean. **B.** Effects of the stressor protocols on the relationship between Maximum Duration of Sperm Motility (s) and Days Elapsed since the protocols took place. The coloured lines indicate treatment; see Table 2.6 for statistical analysis.

2.5.3 Effects on Female Reproduction and Spawning

For females, there was no difference across the treatments in the time elapsed (number of days) between the date of the C&R simulation and the date they were considered ripe for mating. The date of trapping had a positive effect on the time elapsed, with later-trapped fish being ripe later, while the level of fungus on the fish at the time of trapping had a negative effect, such that fish with more fungus became ripe sooner (Table 2.7).

Table 2.7 Summary of General Linear Model (GLM) for the effects of the stressor protocols on the time elaspsed between capture and spawning (ripe). The date of capture, clutch size and the level of fungal cover at that point on the number of elapsed days between the date the stressor was applied and the date when female salmon were ripe for stripping of eggs, were used as explanatory variables.

	d.f.	Estimate	Std. Error	t	p
Intercept		-13890	4170	-3.331	0.002
Exercise	1	-1.445	0.747	-1.935	0.06
Exercise + Air	1	-0.835	0.730	-1.145	0.26
Exercise + Extended Air	1	-0.460	0.745	-0.617	0.54
Date of Trapping	1	0.320	0.096	3.334	0.002
Fungus Pre treatment	1	-0.920	0.405	-2.270	0.03
Clutch Size	1	$-0.001e^{-1}$	$0.002e^{-1}$	-0.781	0.44
Residuals	51				

Larger females produced larger eggs, but there was no effect of treatment on egg size (Table 2.8a; Figure 2.5). After controlling for the effect of female size, females with relatively larger clutches for their size produced smaller eggs. Females that experienced C&R simulations produced smaller clutch sizes relative to their body size (Tables 2.8b and 2.9, Figure 2.5). The Levene's test also indicated a decreased variability in the number of eggs produced by stressed females ($p = 0.04$).

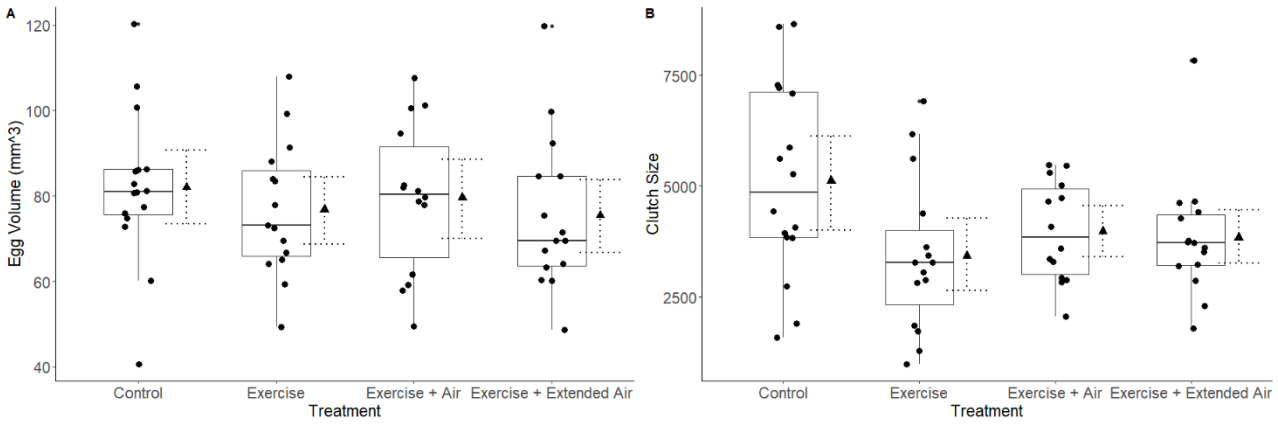

Figure 2.5 Effects of the stressor protocols on female reproduction. **A.** Average Egg Volume (mm^3), and **B.** Clutch Size. Each circular data point represents an experimental fish. The boxplot indicates the mean, the interquartile range, and maximum and minimum values for each of the treatments. The dotted line represents the 95 % Confidence Interval and the triangle represents the mean (see Table 2.6 and 2.8 for statistical analysis).

Table 2.8 Summary of General Linear Models (GLM) for the effects of the stressors on the females' reproductive traits; **a.** Egg volume (mm^3); the model also corrects for the effects of the females' size (length) and the total clutch size produced by the female. **b.** Clutch size (total number of eggs in the clutch); the model also corrects for the effects of the females' size (length), and egg volume.

	d.f.	Estimate	Std. Error	t	p
a. Egg Volume					
Intercept		4.644	0.099	46.773	<0.001
Exercise	1	-0.004	0.030	-0.125	0.90
Exercise + Air	1	-0.012	0.029	-0.404	0.69
Exercise + Extended Air	1	-0.017	0.029	-0.600	0.55
Length	1	0.001	$0.002e^{-1}$	4.822	<0.001
Clutch Size	1	$-0.002e^{-2}$	$0.001e^{-2}$	-1.829	0.07
Residuals	54				
b. Clutch Size					
Intercept		-70750171	9784808	-7.231	<0.001
Exercise	1	-7061270	3412198	-2.069	0.04
Exercise + Air	1	-10106921	3383560	-2.987	0.004
Exercise + Extended Air	1	-7691627	3369692	-2.283	0.03
Length	1	185098	18573	9.966	<0.001
Egg Volume	1	-219639	89191	-2.463	0.02
Residuals	55				

Table 2.9 Summary of Tukey Multiple Comparisons of Means output (95 % family-wise confidence level) for the effects of the stress protocols on female clutch size.

	Lwr.	Upr.	p
Exercise – Control	-25087804	-7561030	0.000
Exercise + Air – Control	-22762449	-4915525	0.001
Exercise + Extended Air – Control	-23131890	-5605117	0.000
Exercise + Air – Exercise	-6575778	11546638	0.89
Exercise + Extended Air – Exercise	-6947697	10859523	0.94
Exercise + Air – Exercise + Extended Air	-9590725	853691	1.00

2.6 Discussion

The results demonstrate that the set of stressors used as a representative disturbance of a typical catch and release practice, for salmon angling, do not necessarily lead to immediate lethal effects, at least under the conditions examined. Notably, however, there were a range of important sublethal effects that could potentially impact the fecundity of salmon that experience capture and release near to spawning time. There was evidence that the stressors influenced the reproductive capacity of female and male salmon, so leading to the potential for intergenerational effects of catch and release angling. Furthermore, fish that experienced the greatest degree of disturbance during simulated catch and release subsequently experienced the fastest growth of the fungus *Saprolegnia* spp..

The pre-spawning mortality of the Atlantic salmon in the benign holding tank environment was low regardless of treatment group. A relatively high survival rate (> 90 %) of adult Atlantic salmon exposed to C&R angling or an equivalent stressor has been exhibited in several other studies (e.g. Thorstad et al., 2007, Havn et al., 2015, and Lennox et al., 2016; Van Leeuwen et al., 2021). This has been shown in other salmonids, including pacific salmon (pink and chum) and steelhead trout (Booth et al., 1995; Raby et al., 2013; Donaldson et al., 2014; Whitney et al., 2019). One possible explanation for salmonids being especially able to endure and tolerate angling might be that they are physiologically equipped to deal with

shifting environmental conditions (e.g. moving from saltwater to freshwater) and undergo extreme exertion during upstream migration. This includes biochemical adaptation, such as increased anaerobic metabolism and protein catabolism, during their progressively harsher journey to the spawning grounds, which might equip them with the necessary adaptations to handle acute stressors such as C&R angling (Raby et al., 2013; Elmer, 2020; Whitney et al., 2019). The salmon in our treatment groups were able to recover under relatively stable conditions, as they could recuperate without needing to continue their upstream migration. Additionally, the water temperature (6 ± 1.5 ^{o}C) that the fish experienced during the C&R simulations and in the holding tanks was within the thermal optimum for Atlantic salmon spawning (Pankhurst and King, 2010). In contrast, there is some evidence of a higher post-release mortality in salmonids (including Atlantic salmon) that experience the same stressors during the summer, or at higher temperatures (> 16 ^{o}C) (Dempson et al., 2002; Thorstad et al., 2007; Olsen et al., 2010; Arlinghaus et al., 2013; Gale et al., 2013; Twardek et al., 2018; Van Leeuwen et al., 2021). Temperature plays a key regulatory role in all physiological processes within ectotherms, including fish, therefore the physiological stress caused by C&R may be intensified by water temperatures beyond the thermal optimum for a species (Olsen et al., 2010; Havn et al., 2015). Mortality at higher water temperature may be triggered by increased metabolic demand, increased physiological disturbance (e.g. fuel depletion, ion loss) due to exercise and air-exposure, and a reduced aerobic scope for recovery (Arlinghaus et al., 2013; Gale et al., 2013).

The catch and release stressor protocols affected the rate of increase of the fungus *Saprolegnia* spp. on the body of the fish. Fish from the control group suffered virtually no increase in fungus, whereas fish exposed to angling simulations showed significant increases in fungal infection over time. Similarly, Pacific salmon caught in gillnets show an increased infection of secondary pathogens, such as *Saprolegnia*, which exploit the damage to epithelial tissue caused by the combined effects of handling stress and physical damage caused by the fishing gear (Teffer, 2018). Moreover, even though fish in the current study experienced no direct effects on mortality due to simulated C&R, mortality may have been higher if the fish were living in the wild rather than in the benign holding tanks. Elmer (2020) suggested that the burden of infection (presence and

load) may influence the ability of pacific salmon to survive other stressors encountered during the spawning migrations. In fact, previous research on salmonids (including Atlantic salmon) infected with *Saprolegnia* spp. has revealed higher secretion levels of Prostaglandin E2 (PGE2), a hormone that has been linked to the deactivation of several immune-related genes (Bordeleau et al., 2018). Furthermore, previous research has shown that *Saprolegnia* spp. affects different body regions on male and female salmonids (Fleming, 1996; Cieplinski et al., 2018). Females show signs of infection mostly in the dorsal half of the peduncle and tail, due to epithelial damage caused from the effort of building redds, while males are affected predominantly on the flanks, because of spawning behaviour, territoriality, and competition for females (Fleming, 1996; Hardie et al., 2007; Cieplinski et al., 2018). Moreover, infection rate tends to be higher during breeding due to the reduced body condition and immunocompromised nature of salmonids during the spawning migration (Hardie et al., 2007; Baker et al., 2013; Matthews, 2019). *Saprolegnia* can also contribute to fish mortality in several ways, including via haemodilution, respiratory and osmoregulatory distress, and organ failure (West, 2006; Lone and Manohar, 2018). *Saprolegnia*-infected individuals also exhibit lethargic behaviour, which increases their risk of predation (Lone and Manohar, 2018). As indicated above, higher temperature can negatively influence physiological processes of fish, and as such can leave an individual immunocompromised (Havn et al., 2015; Elmer, 2020). In conjunction with this, warmer temperature can enhance the virulence of several infectious agents against their host, one of which is *Saprolegnia* spp. (Elmer, 2020; Bateman, 2018). Thus, the migration success of fish such as salmonids could be governed by multiple interrelated variables, such as water temperature, fisheries interaction, infectious agents, and the overall fitness of an individual (Elmer, 2020).

The quantity of sperm produced by ripe males was unaffected by the treatments involving exercise and air exposure of any duration. However, salmon exposed to extended air exposure produced sperm with a longer period of motility once activated. This motility period tended to increase with number of elapsed days after the salmon were exposed to the stressor. This could be an outcome of severely stressed males redirecting more of their limited resources into reproduction, to provide their gametes with the ability to overcome the perceived environmental stressor they are facing and so have a higher chance of fertilizing

the ova (Elgee et al., 2010; Duffield et al., 2017). Stressed and unstressed males should both maximise their reproductive success following migration, but since their experiences have been different, it is plausible that their resource allocation decisions might differ as well. Salmon that have experienced physiological stress can experience reduced immune function (Wedemeyer and Wydoski, 2008; Olsen et al., 2010; Ardia et al., 2011; Arlinghaus et al., 2013; Havn et al., 2015), and altered movement patterns (Arlinghaus et al., 2013; Richard et al., 2014; Twardek et al., 2018). For example, atlantic salmon that have gone through C&R exhibit difficulty crossing barriers, show erratic activity, postponed upstream migration, increased immediate post-release downstream movement, and shorter overall travelled distances (Arlinghaus et al., 2013, Richard et al., 2014). Since these effects will tend to reduce access to mates and optimal spawning grounds, it is possible that they respond by enhancing the activity of their sperm to enhance the likelihood of fertilization. The longevity of the sperm can be related to the physiological and developmental attributes of the offspring (Immler et al., 2014; Alavioon et al., 2019). For example, atlantic salmon offspring produced from sperm with an intermediate duration of motility were found to have an accelerated early developmental phase, which in turn would allow for an earlier establishment of a territory, which would be advantageous in a harsh environment (Immler et al., 2014).

In contrast, unstressed males may be able to spend more energy and resources combating the fungus and maintaining upstream migration as to reach the natal spawning grounds within the right timeframe to spawn and compete for mates. The reproductive success of these individuals will be more influenced by the conspecific competition for mating opportunities rather than the ability to reach a spawning ground. Since there seems to be a trade-off between sperm velocity and longevity (Levitan, 2000; Lehnert et al., 2018; Taborsky et al., 2018), it would be more advantageous for these males to produce fast-swimming rather than long-lived sperm. In this case the catch-and-release simulations may have acted as a eustressor, where a short-term stressor could have positively affected the reproductive function of the salmon under the environment in which it lives (e.g. sperm survivability; Schreck, 2010). The eggs of external fertilizing fish, like atlantic salmon, tend to be fertilised extremely quickly (Hoysak and Liley, 2001; Islam and Akhter, 2011; Beirao et al., 2019). Sockeye salmon for instance, have

exhibited a high fertilization success within the first 5-10 seconds after the eggs are released (Hoysak and Liley, 2001). Allowing the gametes to be exposed for a longer period of contact prior to burial in the substrate may leave the eggs vulnerable to predation or to being washed away by the currents (Hoysak and Liley, 2001).

Clutch sizes were reduced by both exercise and air exposure. Other studies have also found that female salmon produce smaller clutch sizes after exposure to the air or to higher levels of cortisol (McConnachie et al., 2012; Richard et al., 2013; Cook et al., 2015). For example, Richard et al. (2013) demonstrated that air exposure of just 10 s during the summer, when water temperatures were relatively high, could reduce clutch size of female atlantic salmon by half, or more if the 10 s threshold was surpassed. In our study, however, the stressor was applied a short period prior to spawning, where the eggs had already developed, rather than earlier in the migration journey (e.g. during the preceding summer). This suggests that the effect was mediated not through changes to egg production (or resorption), but through females losing eggs prior to spawning. This could not have happened while experiencing the stressors, since none of the selected females had released eggs into the body cavity at the time of the stressor protocols. It is most likely to have happened some days later, while the fish were in the holding tanks, but this could not be corroborated since any free eggs located at the bottom of the tanks would have been flushed out due to the constant water turnover. Investigations of rainbow trout have illustrated that repeated acute stress during the early stages of vitellogenesis or during the nine months prior to spawning can influence oocyte development and result in smaller eggs (Campbell et al., 1992; Contreras-Sanchez et al., 1998). Parental treatment had no effect on egg volume in the present experiment, but that was probably due to the eggs already having developed by the time of the stressor treatments.

Our study indicates that even though C&R angling of atlantic salmon during the spawning season may not affect the mortality of fish, angling related stressors have sublethal effects on fungal infection and proxies of reproduction, with possible indirect effects on fecundity or reproductive success. Previous work has shown that C&R angling of atlantic salmon during the late stages of migration can affect the exploratory behaviour of angled fish, alter their migration patterns (reduced total distance travelled and stress-induced fallback), and impact their

ability to cross barriers and obstacles (Tufts et al., 1997; Richard et al., 2014; Havn et al., 2015; Lennox et al., 2015; Lennox et al., 2016). Changes in behaviour combined with influences in the reproductive traits could result in reduced reproductive fitness of the adults (Thorstad et al., 2003; Lennox et al., 2016). C&R angling might be an improvement over the alternative policy of killing the salmon caught, however the conditions under which the adult salmon are angled should be taken into consideration for regulations and management of C&R, as these can influence the intensity of impacts C&R will have on both the parents and offspring.

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2.7 Supplementary Information

Timeframes for Exercise and Air Exposure

Preliminary time frames for exercise (t = 300 s) and air exposure (t = 60 and 120 s) were decided prior to any fieldwork, with the assistance of Prof. Shaun Killen and Prof. Neil Metcalfe, and by reading previous investigations on C&R angling of Atlantic salmon. This was further discussed with Simon McKelvey (senior marine and freshwater biologist) and Edward Rush (bailiff) at Cromarty Firth Fisheries Trust, who suggested an exercise timeframe between 2 – 4 mins (120 – 240 s) as per their experience on the matter with fishermen. After running 2 fish though the protocol, it was observed that the fish were exhausted by 3 mins (180 s) into the exercise timeframe, therefore it was decided that the final time for the exercise would be 3.5 mins (210 s), so as to ensure that all the fish would be exhausted by the end of the simulation. Both Simon McKelvey and Edward Rush agreed on the time for the air exposure.

Finally, the timeframes were later collaborated by another experiment that used the assistance of experienced fishermen in the Galloway catchment that undertook actual C&R angling of Atlantic salmon. The angling times that the fishermen reported ranged between 60 – 300 s, with most of the times falling around 200 s. Similarly, the air exposure that they reported was between 60 – 120 s.

Table S2.1 Summary of the baseline data for of the adult salmon according to treatment and sex. Presented are the mean and standard deviations of the mass (kg), length (mm) and percentage body covered by the fungus *Saprolegnia* spp. on the date of trapping (pre-treatment).

	Mass Pre-treatment (kg)		Length Pre-treatment (mm)		Fungus Pre-treatment (%)	
	Male	Female	Male	Female	Male	Female
Control	2.13 ± 0.97	2.68 ± 1.15	613 ± 87	646 ± 86	23.62 ± 14.54	0.84 ± 0.65
Exercise	1.73 ± 0.58	1.98 ± 0.98	588 ± 62	590 ± 87	25.82 ± 15.33	0.73 ± 0.55
Exercise + Air	1.92 ± 0.63	2.31 ± 0.91	595 ± 25	617 ± 81	29.01 ± 9.29	0.78 ± 0.43
Exercise + Extended Air	2.27 ± 0.91	2.06 ± 0.84	639 ± 78	602 ± 67	22.70 ± 9.94	0.62 ± 0.88

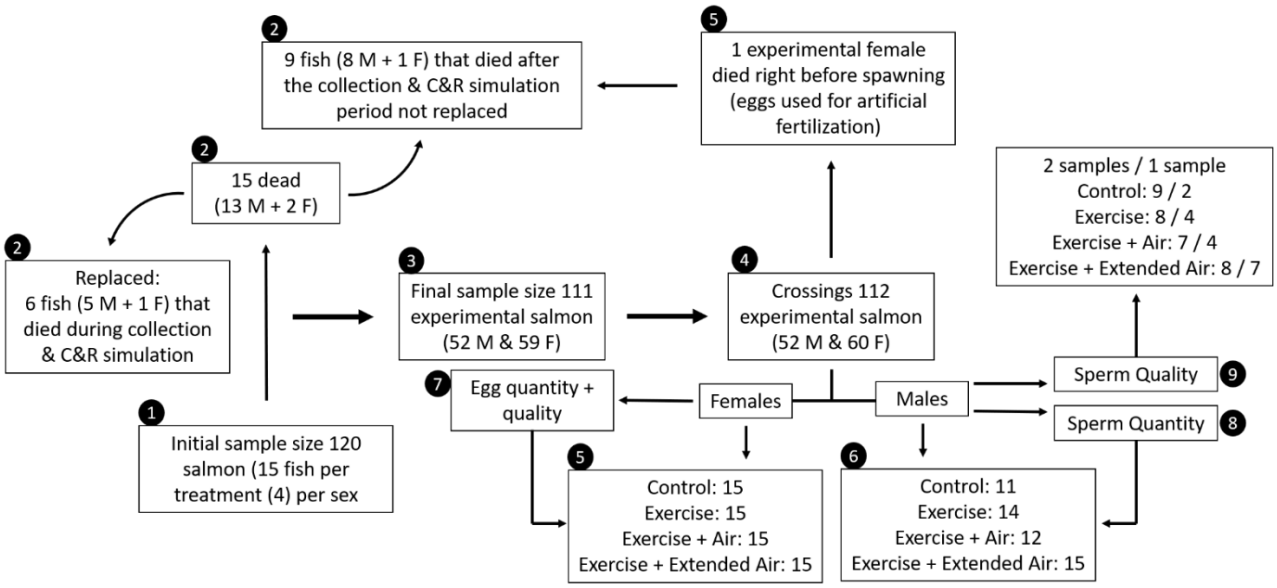

Figure S2.1 Flow chart summary for the number of experimental fish used in each procedure. (1) Initial sample size n = 120, 15 experimental salmon per 4 treatments (Control, Exercise, Exercise + Air, Exercise + Extended Air) per sex (Male, Female), (2) A total of 15 fish died during the investigation (13 males + 2 females). Six of those fish (5 males + 1 female) died during the collection period/ C&R simulation, and were therefore replaced (these fish had undergone the C&R simulation and were used during the adult mortality analysis). Nine of the fish (8 males + 1 female) died after the collection period/ C&R simulation and were therefore not replaced (these fish had undergone the C&R simulation and were used during the adult mortality analysis). (3) The final number of live experimental salmon (sample size) was n = 111 (52 males + 59 females). (4) The total number of crossing during the artificial fertilization was n = 112 (52 males + 60 females – see number 5). (5) A total of 60 experimental females (15 per treatment) were used in the crossings (artificial fertilization), and for date of spawning (number of days between the C&R simulation and the date the fish were considered to be ripe for mating). The female that died and was not replaced, had died on the day of the artificial fertilization, therefore her eggs were used for both the gamete quantity/ quality analysis, as well as for the crossings (n = 60 experimental females). (6) A total of 52 experimental males (Control: 11, Exercise: 14, Exercise + Air: 12, Exercise + Extended Air: 15) were used in the crossings. (7) A total of 60 experimental females (15 per treatment) were used in the gamete quantity (clutch size) and quality (egg volume). (8) A total of 52 experimental males (Control: 11, Exercise: 14, Exercise + Air: 12, Exercise +

Extended Air: 15) were used in sperm quantity. (9) A total of 49 experimental males (Control: 11, Exercise: 12, Exercise + Air: 11, Exercise + Extended Air: 15) were used in gamete quality (maximum duration of sperm motility). A total of 32 experimental males (Control: 9, Exercise: 8, Exercise + Air: 7, Exercise + Extended Air: 8) had 2 recordings for maximum duration of sperm motility and were used for Pearson's product-moment correlation for survivability of the two sperm samples (A and B). A total of 17 experimental males (Control: 2, Exercise: 4, Exercise + Air: 4, Exercise + Extended Air: 7) had only 1 recording for maximum duration of sperm motility. Three experimental males (Exercise: 2, Exercise + Air: 1) had no recordings of sperm quality and were not used in any of the analysis.

Table S2.2 Summary of the average sperm survivability per male. Presented are the individual identifier for each male (PIT Tag number), their treatment (A – Control; B – Exercise, C – Exercise + Air Exposure; D – Exercise + Extended Air Exposure), the time point at which most sperm in the microscope's field of view stopped swimming – this measurement was the author's subjective assessment (Average survivability of most sperm cells - s), the time point where all the sperm in the microscope's field of view had stopped swimming (Average survivability for all sperm cells - s), and the difference between the two measures of sperm survivability (s).

PIT Tag	Treatment	Average survivability of most sperm cells	Average survivability for all sperm cells	Survivability difference
486	A	35	37	2
488	B	16	16	0
489	B	21	26	5
490	B	20.5	25	4.5
2278	D	20.5	28	7.5
520982	B	58	79	21
522333	A	7	7	0
523664	A	22	25.5	3.5
528561	B	39	53.5	14.5
528814	D	71.5	90	18.5
529087	A	20.5	23	2.5
529960	C	19.5	21	1.5
532917	B	15	15	0
02D7	C	33	34	1
040A	D	17.5	31	13.5

055A	D	20.5	20.5	0
0B9B	D	47	50	3
0CF6	D	54	74	20
0DB6	D	29	36	7
0FE5	A	17	19	2
1EF2	D	21	30	9
233B	B	34.5	70	35.5
2A65	D	37.5	43.5	6
2A7F	C	32	40	8
2BC0	D	50	50	0
37A1	D	57	71.5	14.5
52001E	C	35	35	0
5200C2	A	26.5	27.5	1
520A35	C	59.5	62.5	3
520C4A	B	34	50	16
520DAC	A	25.5	27.5	2
520DB8	B	26	27	1
520FC2	C	60	72.5	12.5
520FE3	B	43	43	0
5214F5	C	23	31.5	8.5
52172B	D	49	61	12
521D96	D	34.5	34.5	0
521E3D	A	15.5	15.5	0
5221CC	C	13	17	4
52278D	A	33	43.5	10.5
5228FD	B	21.5	42.5	21
5229B5	B	39.5	46.5	7
525F2C	C	78	84	6
5296D6	C	15	15	0
52987E	D	25	25	0
530B35	D	43.5	65.5	22
53290E	C	44	61.5	17.5
5337C5	A	47	58.5	11.5
9CDE	A	33	57	24

Chapter 3 – Effects of Simulated Catch-and-Release Angling of Pre-Spawning Atlantic Salmon on the Viability and Development of their Offspring

3.1 Summary

A common measure introduced to help conserve salmon populations is the requirement to release fish captured by recreational angling. As a result, many adult salmon may experience catch and release angling during their journey to the spawning grounds. If this occurs during key developmental stages of gamete development, it could result in unforeseen effects on the offspring. This investigation explores how parental stress immediately (5 – 18 days) prior to spawning could influence the early developmental stages of the next generation. Wild Atlantic salmon were captured using a permanent fish trap on the river Blackwater, N. Scotland, during their spawning migration. They were then exposed to one of four disturbance protocols intended to simulate elements of catch and release (C&R) angling. These comprised exercise (0 or 210 s) and air exposure (0, 60, or 120 s) of different durations. In each case an experimental fish (of either sex) was mated using IVF with a non-experimental fish and the fate of the resulting offspring tracked. The results indicate that both egg and fry mortality were higher in the families where one of the parents was air exposed, however this was primarily driven by the mortality caused at distinct stages of their development. For egg mortality this was mainly caused by the shocking of the eggs during the eyed stage, while the increase in fry mortality occurred mostly during a 12-day fungal outbreak within the system. Moreover, embryos arising from parents exposed to the stressors had smaller yolk sacs compared to offspring of control parents. Finally, offspring whose parents were air exposed for 120 s were shorter in length at the time of first feeding, but these differences had disappeared by 5 months of age. These results indicate that C&R of either parent Atlantic salmon close to the time of spawning, especially if it involves air exposure, could have an adverse influence on the early developmental stages of their offspring.

3.2 Introduction

Pre-spawning Atlantic salmon migrate over long distances and overcome several obstacles, both natural and man-made, during the journey to their natal spawning grounds (Olsen et al., 2010; Lennox, 2018). These salmon enter the freshwater environment several months before breeding, where they encounter an evolutionarily unforeseen complication known as catch and release (C&R) angling (Olsen et al., 2010). The practice of C&R is intended as a conservation measure and assumes individual fish will recover from an angling event and reach their natal grounds within the right period to spawn (Jensen et al., 2010; Lennox et al., 2015; Twardek et al., 2018). Once they are hooked on a line, most individual fish will fight to exhaustion before being landed (Olsen et al., 2010; Lennox, 2018). While this process can adversely affect fish physiology, there is abundant evidence that adult salmon can recover from C&R within a short period of time and restore homeostasis, partially through the activation of the HPI axis (Olsen et al., 2010; Raby et al., 2013; Donaldson et al., 2014; Whitney et al., 2019) and the resultant hormonal cascade which includes the release of catecholamines and glucocorticoids (GC) (Donaldson et al., 2014; Raby et al., 2015; Lennox, 2018). The high survival rates, however, do not necessarily mean that the salmon have not been adversely affected by C&R, since the combined effects of increased hormone release, usage of their limited resources and absence of replenishment of energy reserves could result in poorer condition of fish (Olsen et al., 2010; Twardek et al., 2018).

Any sublethal effects of C&R on migrating salmon could have a range of direct or indirect effects on reproduction once fish reach their spawning grounds. Non-reusable resources that would typically be used for migration (from feeding to breeding grounds), maintenance (i.e. fighting off disease) and reproduction (i.e. defending breeding positions, courtship, gamete production and release) are potentially redirected into recovery from the angling event (Jonsson and Jonsson, 1991; Tufts et al., 2000; Olsen et al., 2010; Lennox, 2018). Additionally, female salmon injected with cortisol or exposed to air for different durations of time prior to spawning, as can occur during C&R, produce smaller clutches (McConnachie et al., 2012; Richard et al., 2013; Cook et al, 2015, Chapter 2). Reproduction is energetically costly, with Atlantic salmon of both sexes utilising on average 50 % of their stored energy reserves during spawning (Jonsson and Jonsson, 1991). Birds

subjected to stress before reproduction may face a trade-off between reproductive success (producing the most viable offspring possible) and self-maintenance/ survival (Ricklefs and Wikelski, 2002). Since salmon may face a similar pre-spawned stress through C&R, they might also have to face a similar trade-off. Depending on the stage of gamete production at which a salmon experiences C&R, this trade-off may have different implications for the resulting offspring (Lennox et al. 2015). For example, if a female is subjected to C&R when her eggs are in the early stages of development, then it could reduce the rate at which she provisions them; studies in other contexts have shown that the performance and probability of survival of young fish is dependent on the nutritional state of the mother during the period of yolking (McDermott et al., 2011; Burton et al., 2013; Sopinka, 2015; Auer et al., 2018). To date, however, most research on the effects of C&R has examined impacts on the physiology and behaviour of the captured adults, and there remains substantial uncertainty on the effects on future generations (Richard et al., 2013; Sopinka, 2015; Sopinka et al., 2016b).

Parental experiences can adjust offspring phenotype without altering their genetic makeup (Burton, 2012; Lennox et al. 2015; Burton and Metcalfe, 2014; Stringwell, 2015). This can either prepare the offspring for the environment they will encounter (through adaptive traits) or decrease their overall chances of survival (through maladaptive trait alterations; Burton and Metcalfe, 2014; Haussmann and Heidinger, 2015; Atherton and McCormick, 2020; Bautista and Burggren, 2019; Lehto and Tinghitella, 2019). The phenomenon is known as parental effects and includes influences from both the mother (maternal) and father (paternal; Burton and Metcalfe, 2014; Jonsson and Jonsson, 2014; Haussmann and Heidinger, 2015). In oviparous taxa, such as many teleost fish, maternally synthesised sex steroids, thyroid hormones, GC, and many nutrients necessary for a developing embryo are absorbed into the yolk sac during the process of vitellogenesis (Sopinka, 2015; Sopinka et al. 2016a; Taylor et al. 2016). Moreover, there is evidence that the pre-spawning female's environment and experiences can influence offspring physiology, morphology, behaviour, and social status (Burton, 2012; Sopinka, 2015; Ghio et al. 2016; Taylor et al. 2016). If a pre-spawning female experiences a stressful event, such as C&R, at this time, the activation of the HPI axis results in an increase in synthesis of these adrenal and

non-adrenal hormones such that higher amounts are transmitted to the developing embryo, ultimately influencing its phenotype (Stringwell, 2015; Ghio et al., 2016; Sopinka et al. 2016a; Taylor et al. 2016). The quantity of hormones transmitted to the offspring can depend on the severity and duration of the stressor, as well as the reproductive stage that it was introduced (Taylor et al., 2016). Adult salmon can potentially influence their offspring's phenotype by altering the provisions they input into the gametes during the spawning migration. Since this happens to coincide with the timing that the pre-spawned salmon experience C&R, it is vital for us to know if this stressful event has any effects on the early developmental stages of the offspring. Since the date at which all angling of spawners is prohibited varies between fisheries management organisations, we also need to know whether it is harmful to allow C&R angling very close to the time of spawning.

There are several examples of parental pre-spawning stress or associated responses in salmonids affecting offspring. For instance, rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon that experience exposure to air (i.e. by being lifted out of the water) during gamete development, or that are fitted with intraperitoneal cortisol implants during this stage, show reduced egg survival between fertilization and hatching (Campbell et al., 1992; Eriksen et al., 2006; Eriksen et al., 2013). In contrast, Atlantic and sockeye (*Oncorhynchus nerka*) salmon offspring whose parents experienced C&R at the final stages of migration showed no change in egg mortality during the same period (Booth et al., 1995; Smukall et al., 2019). However, female salmonids with elevated plasma cortisol prior to spawning produced smaller offspring with smaller yolk sacs (McCormick, 1998; Eriksen et al., 2006; Eriksen et al., 2007; Andersson et al., 2011; Sopinka et al., 2014). The offspring of cortisol-implanted female Atlantic salmon also displayed evidence of increased morphological malformations, reduced utilization of yolk sac nutrition, and diminished growth (Eriksen et al., 2006; Eriksen et al., 2013). Nonetheless, it should not be assumed that all such changes are maladaptive. Although stressful events are usually considered to be negative for wild animals, emerging evidence suggests that when the environment that offspring will inhabit is taken into consideration, such events might act as eustressors and actually increase the fitness of the offspring (Schreck, 2010; Madaro et al., 2015; Sopinka, 2015; Sopinka et al., 2016a). Moreover, most

investigations are inclined to focus on maternal effects, and they tend to forget about paternal influences on the offspring (Olsen et al., 2010).

The aim of this study is therefore, to bridge the gap in knowledge that the effects of being subjected to stressors close to the time of spawning can have on the early life stages (fertilization to five months post feeding) of the offspring. We hypothesized that the higher disturbance that the adult salmon experience shortly before spawning, the bigger of an effect this will have on the viability and development of the offspring. This was examined by having wild adult Atlantic salmon (male and female) experience different cumulative levels of disturbance from a C&R simulation just prior to spawning, and subsequently investigate its influences on the survival of the offspring over specific developmental stages. Moreover, the experiment investigated whether parental treatment affected the time taken to complete the yolk sac stage, the yolk sac volume of the offspring, and their size at first feeding. Lastly it explored the growth rates of the offspring within the first 5 months post feeding, as well as their vulnerability to the fungus *Saprolegnia* spp.

3.3 Methods

3.3.1 Mature Salmon Collection and Exposure to Angling Simulation (Stressor Protocol)

Wild Atlantic salmon were collected from the river Blackwater, Scotland, during their upstream migration to their spawning grounds, between November and December 2018. The fish were collected using the permanent fish trap set-up by the Cromarty Firth fishery board (see Chapter 2). Shortly before being able to spawn an equal number (15 fish per treatment per sex, n = 120 in total) of both male and female salmon underwent through the stressor protocols, which were meant to simulate the stressors that fish experience during C&R angling and were comprised of exercise and air exposure of different durations (Table 3.1). The timing of this simulation was shortly after the end of the authorized angling season in this catchment, but angling at this time of year is allowed elsewhere. Following previous authors (Struthers et al., 2018; Smukall et al., 2019), I refer to this protocol as a C&R simulation, although it should be acknowledged that in a real

catch and release scenario the fish would also experience being hooked and so potentially incur some physical damage to the mouth. The protocol may therefore be a conservative assessment of the impact of C&R angling.

Table 3.1 Summary of the four treatment groups used in the experiment and the cumulative levels of acute disturbance they represent, indicated by the number of asterisks; see Chapter 2 for full description of treatments.

Treatment	Exercise	Air exposure	Cumulative disturbance
Control	No	No	
Exercise	210 sec	No	*
Exercise + Air Exposure	210 sec	60 sec	**
Exercise + Extended Air Exposure	210 sec	120 sec	***

3.3.2 Artificial Fertilization and Gamete Collection

A total of 112 fertilization crossings were carried out by mating each experimental fish (60 females, 52 males) to a single non-experimental fish taken from the stock population; the timing of mating of male experimental salmon was based on the ripeness of non-experimental females. During the crossings, each salmon was used only once. The fish were anesthetised in clove oil, and their ventral surface was patted down to remove surplus water, to avoid activation and contamination of gametes. The gametes, of both sexes, were then collected by softly massaging the abdomen, from just below the pectoral fins all the way down to the urogenital opening. This was repeated until all eggs/semen had been released. The gametes of each pair were then gently mixed before being soaked in water for approximately 60 min. This guaranteed that the fertilized eggs swelled up and the shells hardened. Once the adults were stripped of their gametes, they were weighed for somatic mass to the nearest 0.001 kg (DEFENDER 5000 XTREMEW electronic balance) and released back into the loch that was immediately adjacent to the trapping site. Each family of fertilized eggs was then incubated and reared at the Scottish and Southern Energy (SSE) hatchery in Contin, in separate trays.

3.3.3 Fungal Infection of the Parents

Experimental adult salmon were photographed (Sony Cyber-shot DSC-WX100 camera) on both sides, on the date of trapping and at mating. A size reference was also included in the photos for calculation of the percentage body area covered by the ubiquitous fungus *Saprolegnia* spp., which was calculated using the software ImageJ (version 1.51r). The sequence in which the photos were analysed was random, and the analysis was done blind to treatment. The spread of the fungus between capture and mating was later calculated by determining the percentage increase in the body area covered by the *Saprolegnia* spp.

3.3.4 Egg Mortality

Egg mortality at various developmental stages was calculated as a percentage of the total initial clutch size. The size of the clutch was established at the SSE Hatchery, after the eggs were immersed in water for c.60 min to allow them to swell up and for the shell to harden. Each clutch was initially drained of any excess water and ovarian fluid. To estimate the total number of eggs produced by each crossing, the weight of both a known subsample of eggs (to the nearest 0.01 g; Scout Pro electronic balance, OHAUS), and of the total clutch (to the nearest 0.001 kg) were measured. Clutch size was then determined using the formula below:

$$\text{Total clutch size} = \frac{\text{Weight of total clutch (kg) x Number of eggs in subsample}}{\text{Weight of subsample of eggs (kg)}}$$

Egg mortality was calculated over four distinct intervals: 1) Immediate mortality (egg mortality within the first 72 hours after fertilization); 2) Prior to eyed stage (mortality from the day of fertilization to the day before shock treatment); 3) Egg viability (mortality within 48 h after shock treatment); and 4) Total egg mortality (mortality incurred from the day of fertilization all the way to hatching). Shocking of the eggs (a standard hatchery procedure to remove unfertilised or unviable eggs) was conducted once they reached the eyed stage and involved placing the eggs in a bucket with water and gently stirring by hand. This procedure ruptured the yolk sac of any infertile or dying eggs, turning them white. The shocking was repeated three consecutive times for each family.

Dead eggs were removed from the trays daily, to prevent any fungal growth (i.e., *Saprolegnia* spp.) from spreading to healthy neighbouring eggs.

3.3.5 Offspring Transport and Maintenance

The monitoring of family-specific survival and developmental rates beyond the egg stage was conducted in the aquarium rooms of the University of Glasgow. It was not possible for logistical reasons to house all the experimental families, and so 7 families per treatment per sex of parental experimental fish were selected at random. The alevins (hatched embryos that are still dependent on their yolk sac for nutrition) of these 56 families (25 alevins per family) were transported to the University of Glasgow on April 9th, 2019. They were then housed in a flow-through stream system (water flow = 0.988 L/s), where each family was randomly allocated (Number generator random; version 2.0) to its own compartment (dimensions: 19 x 13 cm; water depth: 15.5 cm). The alevins were kept at a water temperature of 7 ^{o}C and in darkness until their yolk sacs had been used up and they were ready to commence feeding. The temperature was then gradually increased to 12 ^{o}C over the next two months (May – June) to simulate natural conditions, under a 12 h photoperiod (8:00 – 20:00).

3.3.6 Yolk Sac Volume

An additional 336 alevins (n = 6 per family per sex of parental experimental fish) were euthanized (same date as alevins were moved to Glasgow) using an overdose of clove oil and preserved in absolute Ethanol. Their yolk sac volume (YSV) was estimated using the prolate spheroid formula as indicated below, where 'l' and 'h' are the maximum length and height of the yolk sac respectively (Ching et al., 2012; Baron-Aguilar et al., 2013; Sulaeman, 2017; Thomas et al., 2020):

$$YSV = \left(\frac{\pi}{6}\right) \times l \times (h)^2$$

The length and height were measured (to the nearest 0.001 mm) by taking a photo (Sony Cyber-shot DSC-WX100) of the fish from a fixed distance, with a known size reference, and analysing the photos using the software ImageJ.

3.3.7 Date of First Feeding and Size at First Feeding

Fish in the stream system were monitored daily for behaviour and stage of development. Once members of a family had mostly used up their yolk sac a small test amount of pelleted food [EWOS, West Lothian, UK] was presented each day assess their responsiveness to food. The average date of first feeding for each family was based on the following three criteria: 1) none of the fish in that family still had a yolk sac; 2) at least some of the fish were swimming in the water column, and 3) at least 5 fry were actively searching and consuming the food that was provided. Date of first feeding was recorded on a family (compartment) basis. Moreover, the fork and head length of all surviving fry within each family was also recorded on the same day that first feeding was recorded. This was achieved by placing the fish in a water filled container (water depth = 1 cm) with a known size reference submerged inside. Then, a photo (Sony Cyber-shot DSC-WX100) of the fish was taken from a set distance, which was later analysed in the software ImageJ. The head length of the fish was defined as the distance between the tip of the snout and the posterior edge of the operculum. The proportional head length of the offspring was calculated by dividing the head length of the fish by its fork length.

3.3.8 Growth Rate

The salmon were fed pellets to excess in their original family compartment in the flow-through steam system from first feeding up to July 2nd, 2019, and then were fed on small bloodworms from that point on (again to excess). From first feeding (28/4/19 – 16/5/19, depending on family) up to June 9th, 2019, the fish were fed 3x a day (at 9:00, 13:00 and 17:00), from June 10th to July 1st they were fed 2x day (at 9:00 and 17:00), and then once a day (at 12:00). The fork length of all surviving offspring was also measured at months 3 (early July 2019) and 5 (end of August - early September 2019) after first feeding, while the head and proportional head length was only measured in July. The fork length measurements were then used to calculate the mean growth rates for each family. The first interval over which growth rate was measured (SGR1) covered the growth of the fish over the first three months post-feeding (end of April to beginning of July), while the second growth rate interval (SGR2) covered the growth of the fish between the 3rd and 5th month post feeding (beginning of July to mid-September).

The measurement in July was achieved in a similar manner to that at first feeding to minimize air exposure of the fish. The last measurement was achieved using water-resistant electronic callipers (Electronic Calliper Waterproof IP67, 0-150 mm) to nearest 0.01 mm. To minimize air exposure, fish were placed in individual water-filled zip bags for the duration of the measurement. Specific growth rates over each interval were calculated using the following formula, where 'ln1' is the initial length, 'ln2' is the final length, and 't' is the period in days between the two measurements:

$$\mathrm{SGR} = \left(\frac{\ln 2 - \ln 1}{\mathrm{t}}\right) x\ 100$$

3.3.9 Fry Mortality

Fry mortality was categorized in 3 ways: 1) Total Mortality, which was the percentage mortality of the offspring within the first three months post feeding; 2) Vulnerability to fungus, which was the percentage mortality caused by the fungus *Saprolegnia* spp. during a 12-day outbreak in the system during that period (from 06/05/19 to18/05/19) and 3) Residual Mortality, which was the 'Overall Mortality' but without the mortality caused by the fungus. The data were expressed as deaths as a percentage of the total number of offspring present in each family at the start of the interval.

3.4 Statistics

General Linear Models (GLMs) were used in R (Version Ri386 3.4.4) to investigate whether the experimental protocols applied to the parents had a significant effect on egg mortality (total, immediate, prior to the eyed stage, and after shocking) and offspring yolk sac volume (YSV). GLMs were also used to examine whether the C&R simulations on the parents affected the date of first feeding of the offspring (expressed as Julian date) and their size (fork and proportional head length) at first feeding, as well as their size at three (fork and proportional head length) and five months (fork length) post first feeding. Furthermore, GLMs were used to analyse the specific growth rates of the fish within the first three months after first feeding (SGR 1 fork length and SGR 1 absolute head length), and between the 3rd and 5th month post feeding (SGR 2 fork length). Finally, models were used to

investigate the fry mortality post feeding (total mortality, vulnerability to fungus, and residual mortality). The main explanatory variable in all models was the treatment group of the experimental parent (C&R simulation) – note that each offspring was the result of a mating between an experimental and a control fish. In addition all initial models had as explanatory variables date of trapping (Julian Date) of the experimental parent, its sex and initial % body area covered with fungus, % spread of fungus on the experimental parent, days elapsed from the time of the C&R simulation to spawning (days elapsed), date of fertilization (Julian Date), period between fertilization and preservation of the alevins in absolute ethanol (hatchery duration), yolk sac volume (YSV), and size (fork length) at first feeding and date (Julian Date) on which first feeding occurred (taken as the average for the family). The model for egg mortality caused by the shock treatment also included the percentage egg mortality prior to the eyed stage, while that for YSV also included the interval (in days) between fertilization and preservation. The size of the offspring at three and five months post first feeding were incorporated into a single GLM, with number of offspring in each compartment at the time of measurement used as an additional explanatory variable to control for density effects. This model also included an interaction between month of measurement (3^{rd} or 5^{th}) and parental treatment. The model for SGR 1 absolute head length also used head size at first feeding as an explanatory variable, whereas the model for SGR 2 included size at three months.

In all cases, the models that obtained the lowest AIC value were selected and assessed for normality of residuals, linearity, and homogeneity of variance through the residual fit plots. Values for egg mortality (total, fecundity, prior to the eyed stage, and after shocking) and fry residual mortality underwent logarithmic transformation to normalise the residuals of the data. The interaction between treatment and experimental parent sex was also considered. The significance of the variables in the final models was established using p-values (with $p = 0.05$ taken as the threshold for significance). If a categorical variable was found to be significant, the categories were investigated further using a Tukey multiple comparison of means. Homogeneity of variance across treatments was determined by running a Levene's tests for all final models. A summary of all the final models can be found in the supplementary information (Table S3.1).

3.5 Results

3.5.1 Egg Mortality at Distinct Developmental Stages

There was a higher percentage egg mortality, from the date of fertilization up to hatching, if the parents (of either sex) were exposed to the air for an extended period (120 s) prior to stripping (Table 3.2, Figure 3.1). Egg mortality over this period was lower if the parent subjected to the experimental treatment had been trapped towards the end of the collection period, and/or had been left undisturbed in the holding tanks for longer after the C&R simulation protocols were applied before being stripped (Table 3.2). Additionally, there was greater variability among families in the percentage egg mortality if the parents had experienced the C&R stressors compared to if they had not (Levene's test: $p = 0.02$).

Table 3.2 Final General Linear Model (GLM) investigating the effects of pre-spawning stressors associated with C&R on total egg mortality from fertilization to hatching (as a % of the initial clutch size; see Table S3.1 for model structure). Shown are the comparisons of each of the three C&R treatments to the control, together with the effect of days elapsed from the stressor protocol until spawning, date of trapping and sex of the experimental parent, and percentage increase in fungal spread on the experimental parents' body.

	d.f.	Estimate	Std. Error	t	p
Intercept		3598.097	1500.068	2.399	0.02
Exercise	1	0.302	0.267	1.130	0.26
Exercise + Air Exposure	1	0.463	0.278	1.674	0.10
Exercise + Extended Air Exposure	1	0.754	0.344	2.189	0.03
Days Elapsed	1	-0.105	0.038	-2.770	0.007
Date of Trapping	1	-0.083	0.035	-2.397	0.02
Fungal Spread - Parent	1	0.015	0.010	1.452	0.15
Parental Sex - Male	1	-0.433	0.310	-1.398	0.17
Residuals	82				

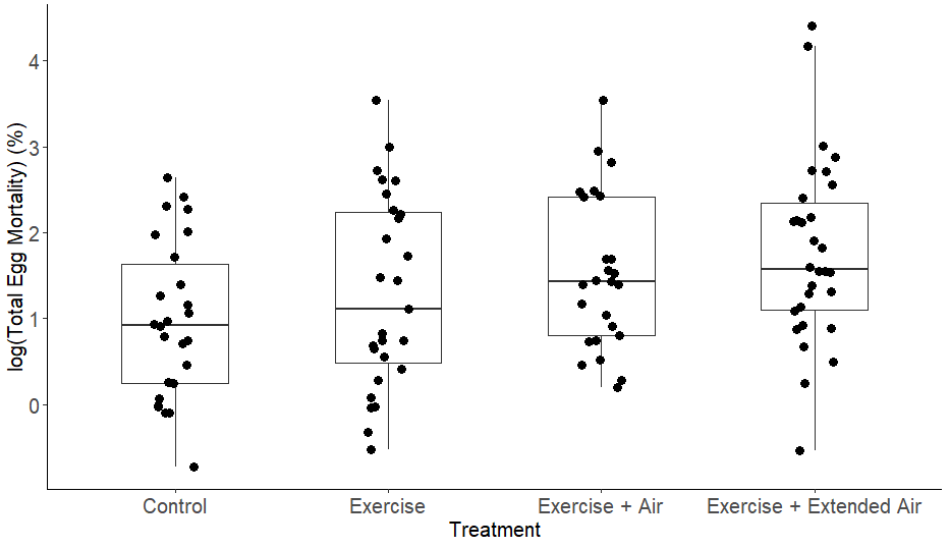

Figure 3.1 Effects of the C&R simulation on total egg mortality (% clutch) from fertilization to hatching. Each circular data point represents an experimental family. The boxplot indicates the median, the interquartile range, and maximum and minimum values for each of the treatments (see Table 3.2 for statistical analysis).

Egg mortality prior to the eyed stage, including immediate mortality, was unaffected by parental treatment (Table 3.3b). As with total egg mortality, however, both date of trapping of experimental parent and days lapsed between the angling simulation and stripping had a negative effect on immediate mortality (egg mortality within the first 72 h after fertilization, as a percentage of the total clutch). The Levene's test also illustrated higher variability in egg mortality prior to the eye stage among families derived from a parent that experienced exercise or air exposure of any duration, in comparison to control families ($p = 0.03$), however this effect was not found for immediate mortality.

Egg mortality after shocking was higher in families derived from a parent that had been exposed to air for 120 s, compared to families whose parents experienced no stressor (Table 3.3c, Figure 3.2). The sex of the experimental parent and percentage body cover of the fungus *Saprolegnia* spp. on the date of trapping also affected the percentage egg mortality after shocking. Egg mortality at this stage was lower in families where the experimental parent had a lower *Saprolegnia* spp. infection and when the parent experiencing the C&R simulation was the father. Additionally, families that had a high egg mortality prior to the eye stage also had a greater egg mortality after shocking.

Table 3.3 Final General Linear Models (GLM) investigating the effects of simulated C&R of salmon parents on the mortality rates in their eggs at different developmental stages: (a) Immediate egg mortality after fertilization, and (b) Egg mortality prior to the eyed stage. In both cases each of the three C&R simulations are compared to the control, together with the effect of date of trapping, days elapsed from the simulated C&R event until spawning, and percentage increase in fungal spread on the experimental parents' body. (c) Egg viability (mortality due to shocking), with the same treatment comparisons together with the effect of the extent of experimental parent fungal infection on the date of trapping, the sex of the experimental parent, the days elapsed from the simulated C&R event until spawning and egg mortality prior to the eyed stage.

	d.f.	Estimate	Std. Error	t	p
a. Immediate Mortality					
Intercept		2751	1191	2.309	0.02
Exercise	1	0.316	0.244	1.296	0.19
Exercise + Air Exposure	1	0.179	0.252	0.709	0.48
Exercise + Extended Air Exposure	1	0.441	0.301	1.467	0.14
Days Lapsed	1	-0.082	0.034	-2.441	0.02
Date of Trapping	1	-0.063	0.027	-2.308	0.02
Fungal Spread - Parents	1	0.003	0.010	0.209	0.77
Residuals	83				
b. Prior to Eyed Stage					
Intercept		1148	1354	0.848	0.40
Exercise	1	-0.148	0.277	-0.533	0.60
Exercise + Air Exposure	1	0.437	0.287	1.522	0.13
Exercise + Air Exposure	1	-0.122	0.342	-0.357	0.72

Days Lapsed	1	-0.025	0.038	-0.643	0.52
Date of Trapping	1	-0.026	0.031	-0.849	0.40
Fungal Spread - Parent	1	$-0.004e^{-1}$	0.011	-0.040	0.97
Residuals	83				

c. Egg viability due to shocking

Intercept		-1.285	0.542	-2.369	0.02
Exercise	1	-0.086	0.275	-0.312	0.76
Exercise + Air Exposure	1	0.418	0.285	1.469	0.15
Exercise + Extended Air Exposure	1	0.758	0.350	2.167	0.03
Days Elapsed	1	0.044	0.041	1.069	0.29
Parental Fungus Pre – Treatment	1	0.483	0.210	2.304	0.02
Parental Sex – Male	1	-0.548	0.257	-2.132	0.04
Egg Mortality Prior to Eyed Stage	1	0.358	0.125	2.871	0.005
Residuals	83				

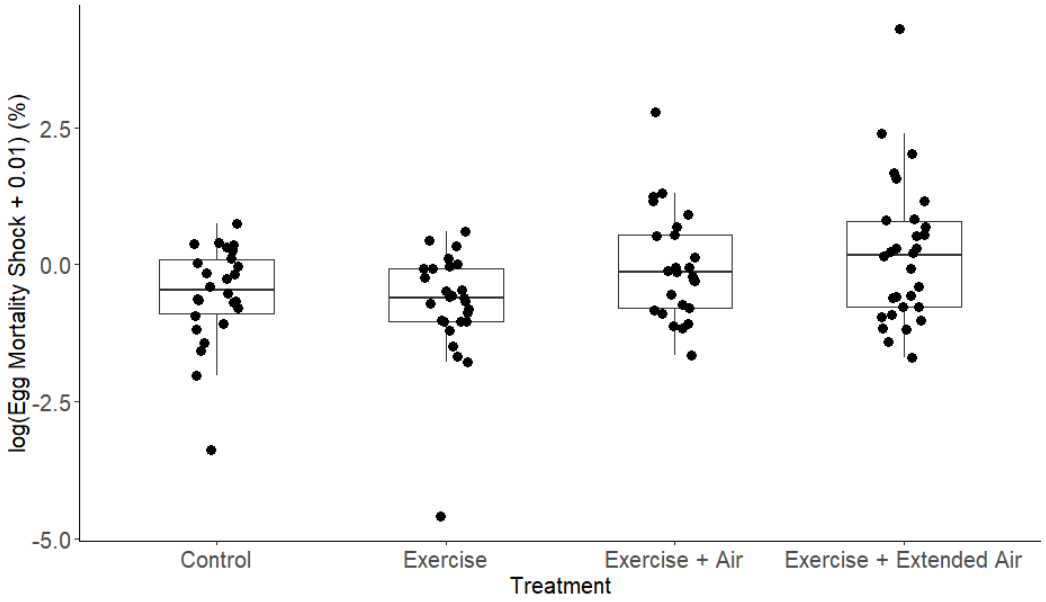

Figure 3.2 Effects of simulated C&R of parent salmon on egg mortality (% clutch) in the first 48 h following shocking. Each data point represents an experimental family. The boxplot indicates the median, the interquartile range, and maximum and minimum values for each of the treatments (see Tables 3.3 & 3.4 for statistical analysis).

3.5.2 Yolk Sac Volume

After controlling for the effect of developmental time (i.e. days from fertilization until preservation), offspring that had a parent exposed to the C&R simulations had a yolk sac that was smaller in volume compared to alevins from control parents (Table 3.4, Figure 3.3). There was also less within-family variability in yolk sac volume for alevins whose parents were exposed to the air (Figure 3.3).

Table 3.4 Summary of General Linear Models (GLM) investigating the effects of adult C&R simulations on the yolk sac volume of their alevins. Shown are the comparisons of each of the three C&R treatments to the control, together with the effect of time elapsed between the date the eggs were fertilized and the date the offspring were euthanized and preserved.

	d.f.	Estimate	Std. Error	t	p
Intercept		0.241	0.048	5.012	<0.001
Exercise	1	-0.011	0.005	-2.049	0.046
Exercise + Air	1	-0.011	0.005	-2.066	0.04
Exercise + Extended Air	1	-0.019	0.005	-3.532	0.001
Period Between Fertilization and Preservation	1	-0.001	0.000	-3.839	<0.001
Residuals	49				

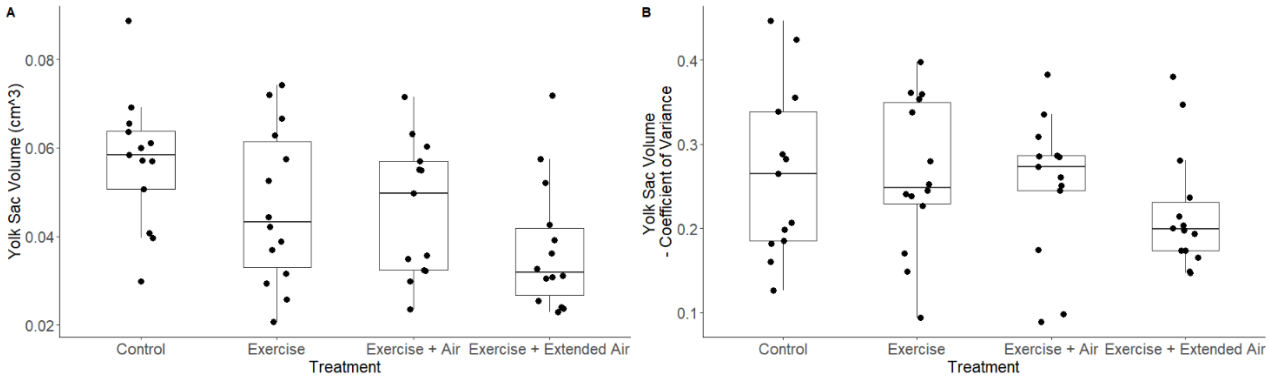

Figure 3.3 Effects of the adult C&R simulations on (a) the yolk sac volume (cm^3) of their alevins and (b) the coefficient of variance for the yolk sac volume within each family. Each data point represents an experimental family. The boxplot indicates the median, the interquartile range, and maximum and minimum values for each of the treatments (see Table 3.5 for statistical analysis).

3.5.3 Date of First Feeding and Size at First Feeding

Date of first feeding was unaffected by whether the experimental parent was exposed to exercise or air exposure during the angling simulations. In contrast, offspring from families where one parent was air exposed for an extended time (120 s), were smaller (fork length) at first feeding compared to both the offspring from the control and exercise groups (Table 3.5a, Table S3.2, Figure 3.4). Male parents that had gone through the angling simulation produced offspring that were larger compared to the offspring of female experimental parents. Moreover, fry from the limited air exposed group (60 s) had a proportionally smaller head at first feeding (Table 3.5b, Figure 3.4). Additionally, offspring of parents exposed to the angling simulations exhibited more within-family variability in fork length. Similarly, head length at first feeding was highly variable for fry whose parents were only exercised, but more homogenous for fry whose parents were air exposed for any duration of time (Figure 3.4).

Table 3.5 Summary of General Linear Models (GLM) investigating the effects of adult C&R simulations on the alevins size at first feeding: (a) Fork length and (b) Proportional head length at first feeding. In both cases each of the three C&R simulations are compared to the control, together with the effect of date at first feeding and the sex of the experimental parents.

	d.f.	Estimate	Std. Error	t	p
a. Fork Length at First Feeding					
Intercept		119.393	77.121	1.548	0.13
Exercise	1	-0.035	0.038	-0.930	0.37
Exercise + Air	1	-0.072	0037	-1.922	0.06
Exercise + Extended Air	1	-0.163	0.038	-4.309	<0.001
Sex – Male	1	0.094	0.026	3.560	0.001
Date of First Feeding	1	-0.003	0.002	-1.513	0.14
Residuals	49				
b. Proportional Head Length at First Feeding					
Intercept		0.250	0.002	141.495	<0.001
Exercise	1	-0.001	0.003	-0.423	0.67
Exercise + Air	1	-0.006	0.002	-2.243	0.03
Exercise + Extended Air	1	-0.001	0.002	-0.202	0.84
Residuals	51				

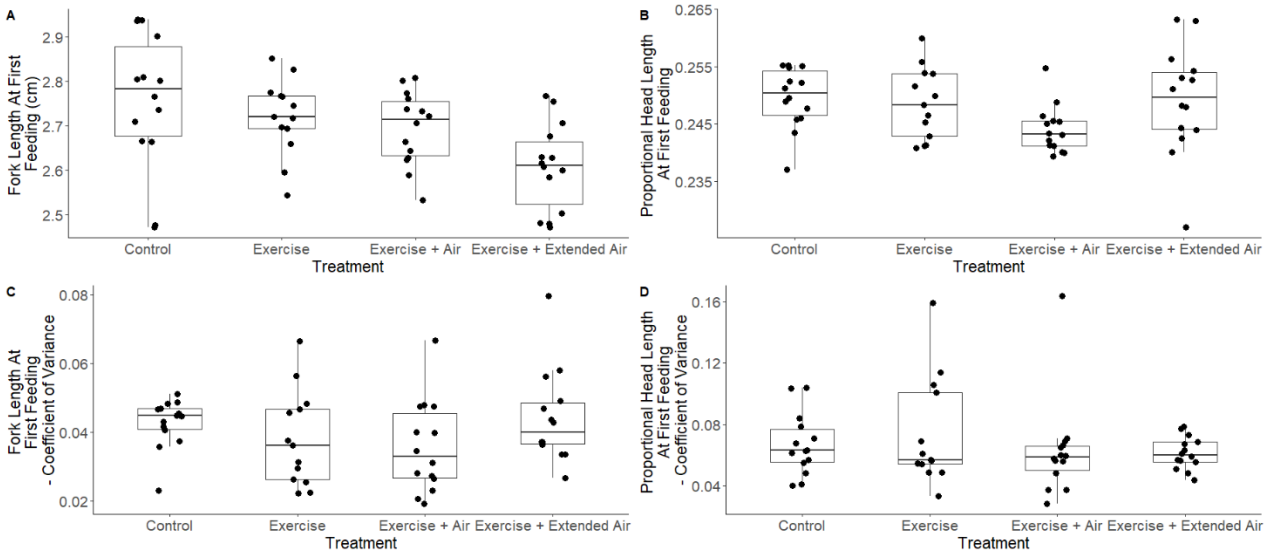

Figure 3.4 Effects of the adult C&R simulations on (a) alevin fork length at first feeding (cm), (b) proportional head length at first feeding, (c) the coefficient of variance for fork length within each family, and (d) the coefficient of variance for proportional head length within each family. Each circular data point represents an experimental family. The boxplot indicates the median, the interquartile range, and maximum and minimum values for each of the treatments (see Table 3.6 for statistical analysis).

3.5.4 Offspring Specific Growth Rates

The offspring from all treatment groups were, on average, the same size three and five months post first feeding (Table 3.6). Furthermore, there was no difference in the proportional head length of the offspring at three months post feeding. However, within-family variability for fork length and proportional head length was greater in familes whose parents were air-exposed for any duration (Figure 3.5).

Table 3.6 Summary of General Linear Models (GLM) investigating the effects of simulated C&R of salmon parents on the size of their offspring (fork length, cm) three and five months after first feeding. Shown are the comparisons of each of the three C&R treatments to the control, together with the effect of the number of offspring present per holding compartment on the date on the measurements.

	d.f.	Estimate	Std. Error	t	p
Intercept		1.147	0.029	40.219	<0.001
Exercise	1	0.017	0.022	0.785	0.43
Exercise + Air	1	0.001	0.022	0.431	0.67
Exercise + Extended Air	1	0.027	0.023	1.202	0.23
Month 5	1	2.529	0.021	117.425	<0.001
Number of Offspring in Compartment	1	0.003	0.001	2.669	0.009
Exercise: Month 5	1	0.009	0.031	0.296	0.77
Exercise + Air: Month 5	1	-0.014	0.030	-0.463	0.64
Exercise + Extended Air: Month 5	1	0.010	0.031	0.327	0.74
Residuals	**47**				

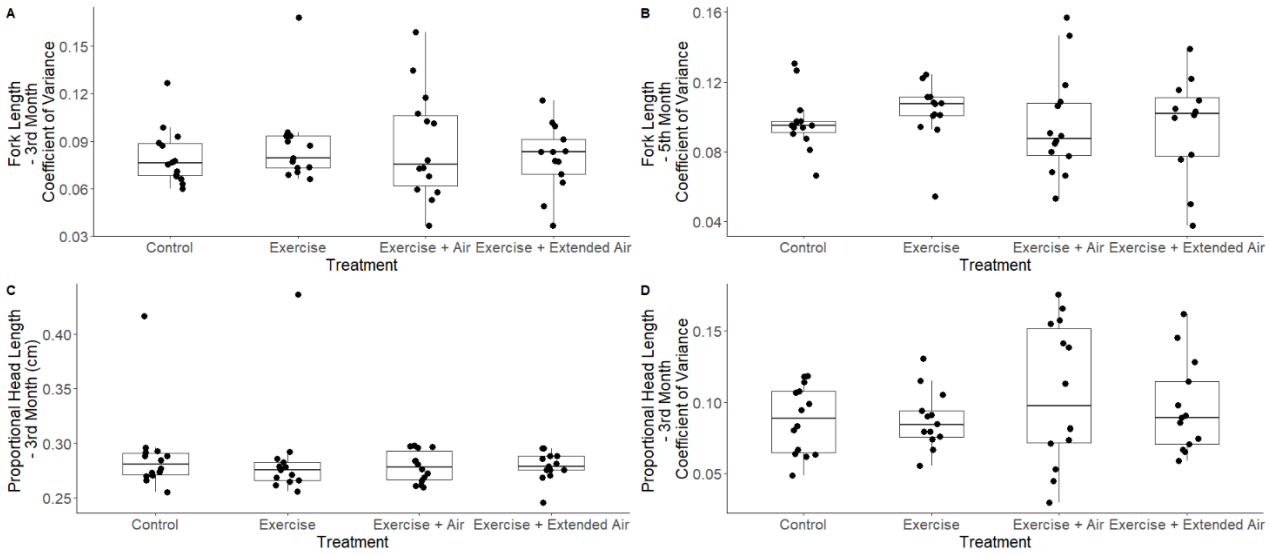

Figure 3.5 Effects of the adult C&R simulations on (a) the within-family Coefficient of Variance for fork length of offspring three months post feeding, (b) the within-family Coefficient of Variance for fork length of offspring five months post feeding, (c) the proportional head length of the offspring at three months post feeding, and (d) the within-family coefficient of variance for proportional head length within each family. The boxplot indicates the median, the interquartile range, and maximum and minimum values for each of the treatments (see Table 3.8 for statistical analysis).

There was no difference in growth rate (fork or head length) over the first three months of feeding among the treatment groups (Table 3.7a). There was however a rapid growth spurt for the offspring whose parents were air exposed between the 3rd and 5th months post first feeding (Figure 3.6; Table 3.7c). Moreover, individuals that started feeding earlier grew at a faster rate for the first three months. What is more, offspring that were smaller at the third month mark had a faster growth rate over the next two months, suggesting a compensatory growth response.

Table 3.7 Summary of General Linear Models (GLM) investigating the effects of simulated C&R of salmon parents on the growth rate of the offspring: (a) SGR 1 Fork length (First Feeding to 3rd month post feeding). Shown are the comparisons of each of the three C&R treatments to the control, together with the effect size and date of first feeding. (b) SGR 1 Absolute head length (First Feeding to 3rd month post feeding). Shown are the comparisons of each of the three C&R treatments to the control, together with the effect of head length at first feeding and date of first feeding. (c) SGR 2 Fork length – 3rd to 5th month post feeding. Shown are the comparisons of each of the three C&R treatments to the control, together with the effect of fork length at 3 months post feeding.

	d.f.	Estimate	Std. Error	t	p
a. SGR 1 Fork Length – First Feeding to 3rd month post feeding					
Intercept		-321.2	80.20	-4.005	<0.001
Exercise	1	0.035	0.040	0.883	0.38
Exercise + Air	1	0.022	0.039	0.562	0.58
Exercise + Extended Air	1	0.086	0.045	1.914	0.06
Fork length at First Feeding	1	-0.201	0.131	-1.536	0.13
Date of First Feeding	1	0.007	0.002	4.018	<0.001
Residuals	47				
b. SGR 1 Head Length – First Feeding to 3rd month post feeding					
Intercept		-673.1	146.6	-4.591	<0.001
Exercise	1	0.012	0.073	0.161	0.87
Exercise + Air	1	0.016	0.077	0.214	0.83
Exercise + Extended Air	1	0.055	0.081	0.678	0.50
Head Length at First Feeding	1	-0.393	0.962	-0.408	0.69

Date of First Feeding	1	0.015	0.003	4.598	<0.001
Residuals	47				

c. SGR 2 Fork Length – 3rd to 5th month post feeding

Intercept		5.723	0.265	21.604	<0.001
Exercise	1	0.031	0.046	0.682	0.50
Exercise + Air	1	0.351	0.044	7.956	<0.001
Exercise + Extended Air	1	-0.008	0.045	-0.188	0.85
Fork length at 3 Months	1	-0.208	0.080	-2.582	0.01
Residuals	48				

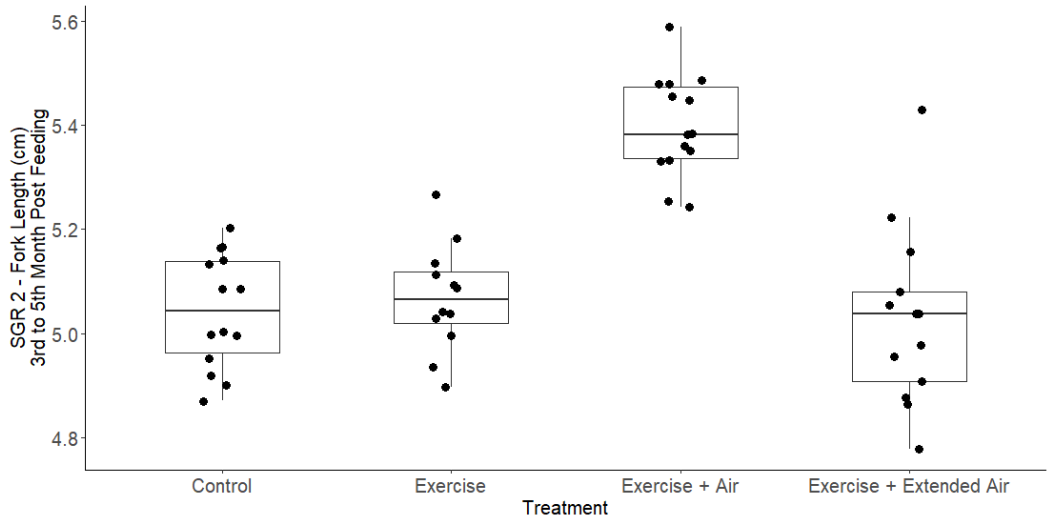

Figure 3.6 Effects of the adult C&R simulations on growth rate of the offspring between the 3rd and 5th month after first feeding (SGR 2 Fork length). The boxplot indicates the median, the interquartile range, and maximum and minimum values for each of the treatments (see Table 3.8 for statistical analysis).

3.5.5 Fry Mortality

Offspring of air exposed parents had a higher percentage total mortality over the first three months of feeding (Table 3.8a, Figure 3.7). Moreover, the variability in percentage mortality across families was greater in the treatment groups compared to the control (Levene's test: $p = 0.01$). Both effects were mainly driven by mortality occurring during the 12-day *Saprolegnia* spp. fungal outbreak, when mortality was greater in families whose parents were air exposed for any duration of time (Table 3.8b, Figure 3.8) and when the among-family variability in mortality was also greater in treated compared to untreated families (Levene's test: $p = 0.001$). After exclusion of the mortality resulting from the fungal outbreak, there was no effect of treatment on residual mortality (Table 3.8c, Figure 3.8).

Table 3.8 Summary of General Linear Models (GLM) investigating the effects of simulated C&R of salmon parents on the offspring's percent mortality within the first three months after first feeding: (a) total mortality (all mortality within the first three months post feeding), (b) mortality caused by the fungus *Saprolegnia* spp. (during the 12-day outbreak in the system), and (c) residual mortality (Offspring mortality within the three months, excluded fungal mortality). Shown are the comparisons of each of the three C&R treatments to the control, along with the percentage increase in fungal spread on the experimental parents' body.

	d.f.	Estimate	Std. Error	t	p
a. Total Mortality					
Intercept		14.658	6.076	2.413	0.02
Exercise	1	9.985	7.962	1.254	0.22
Exercise + Air	1	20.022	8.211	2.438	0.02
Exercise + Extended Air	1	20.263	8.044	2.519	0.02
Fungal Spread - Parent	1	-0.044	0.244	-0.181	0.86
Residuals	47				
b. Mortality due to Fungus *Saprolegnia* spp.					
Intercept		8.515	5.568	1.529	0.13
Exercise	1	7.143	7.297	0.979	0.33
Exercise + Air	1	15.980	7.525	2.124	0.04
Exercise + Extended Air	1	20.621	7.371	2.798	0.007
Fungal Spread - Parent	1	-0.008	0.224	-0.037	0.97
Residuals	47				
c. Residual Mortality					

Intercept		1.518	0.355	4.275	<0.001
Exercise	1	0.626	0.465	1.345	0.19
Exercise + Air	1	0.748	0.480	1.560	0.13
Exercise + Extended Air	1	0.386	0.470	0.821	0.41
Fungal Spread - Parent	1	-0.010	0.014	-0.710	0.48
Residuals	47				

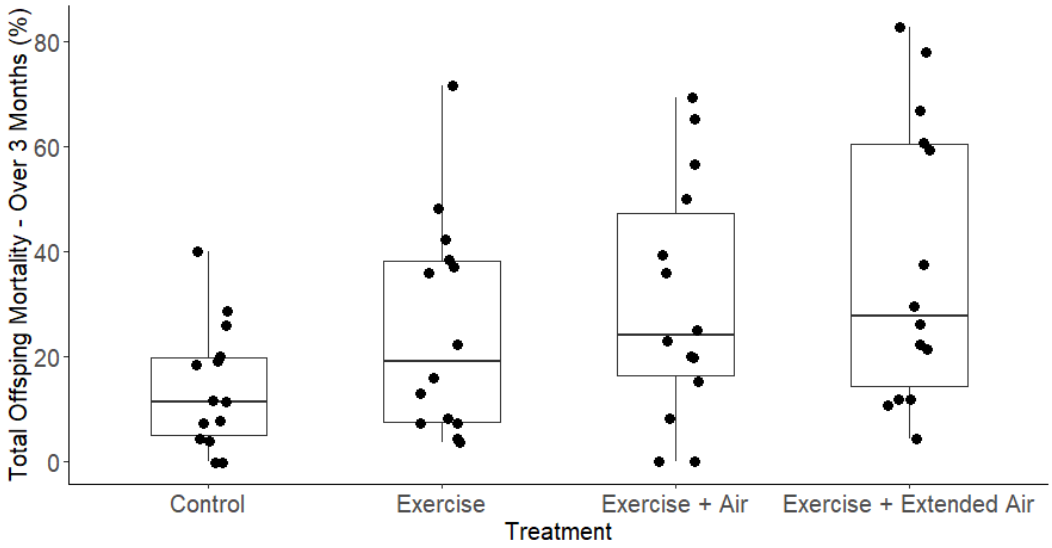

Figure 3.7 Effects of the adult C&R simulations on total offspring % mortality within the first three months post feeding. The boxplot indicates the median, the interquartile range, and maximum and minimum values for each of the treatments. The dotted line represents the 95 % Confidence Interval, and the triangle represents the mean (see Table 3.8 for statistical analysis).

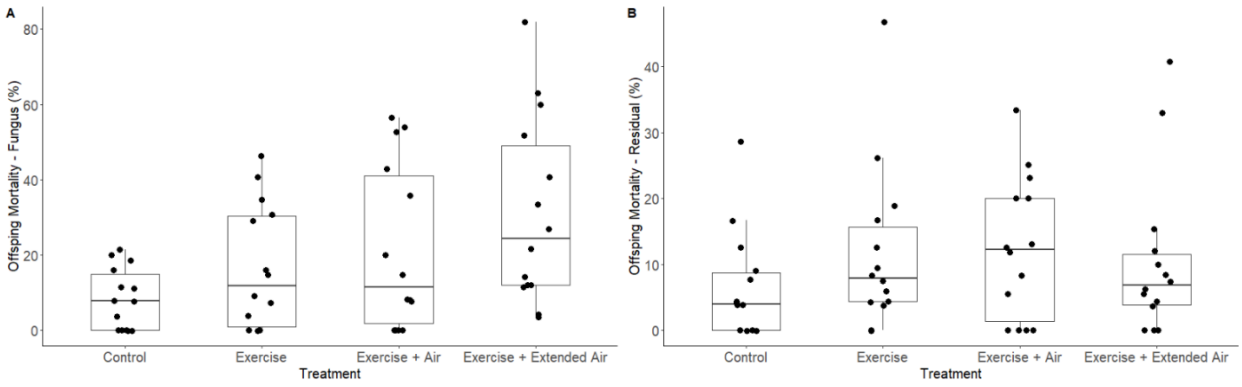

Figure 3.8 Effects of the adult C&R simulations on (a) offspring mortality caused by the 12-day fungal outbreak within the system, and (b) residual mortality (offspring mortality within the first three months post feeding, excluding fungal mortality). The boxplot indicates the median, the interquartile range, and maximum and minimum values for each of the treatments (see Table 3.8 for statistical analysis).

3.6 Discussion

The results here show that simulated catch and release angling of Atlantic salmon immediately (5 – 18 days) before spawning can have unintentional intergenerational effects at the early stages of the offspring's life (summarised in Fig. 3.9). Firstly, there is evidence of higher mortality of eggs from parents (of either sex) that had been air-exposed for an extended period (120 s). In addition, the yolk sac volume of offspring was smaller in the groups where one parent, again irrespective of sex, had been subjected to exercise and air exposure. Intriguingly, however, even though the yolk sac was smaller, the date of first feeding was unaffected by parental treatment. What was affected was the size of the alevins: offspring from the extended air exposure group (120 s) were smaller at first feeding compared to the fish whose parents did not experience any additional disturbance (exercise or air). However, compensatory growth occurred such that by three months post feeding there was no longer any difference in offspring length across treatments. Finally, total offspring mortality within the first three months of feeding was higher in families where one parent had been exposed to air, with the effect primarily arising from a greater vulnerability to the fungus *Saprolegnia* spp., leading to a higher mortality during a 12-day fungal outbreak.

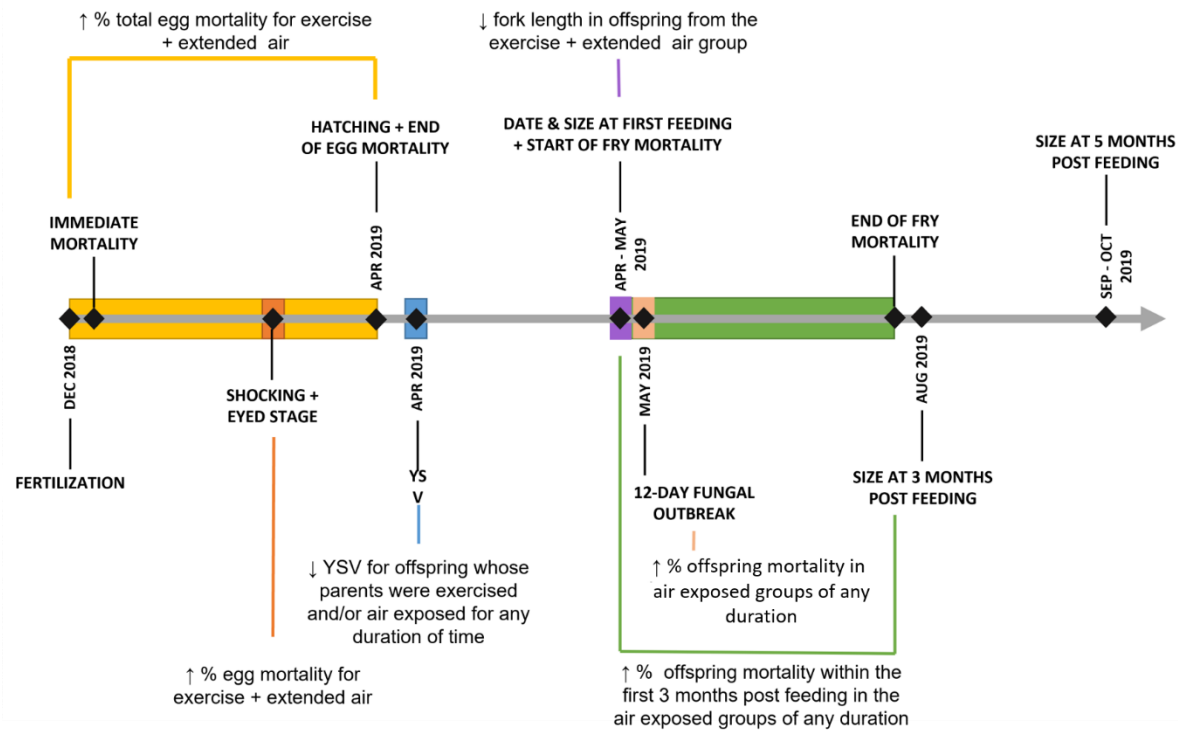

Figure 3.9 A timeline of the main effects that the adult C&R simulations had on the viability, development, and growth of the offspring from fertilization to 5 months post feeding. YSV = yolk sac volume.

The total percentage egg mortality was higher in the families where one of the parents had been exposed to the air for an extended period of time (120 s). Previous investigations on salmonids (Atlantic, chum and sockeye salmon and brown trout) have demonstrated that neither direct or indirect manipulation of egg cortisol affects embryonic survival (Sloman, 2010; Sopinka et al., 2016a; Sopinka et al., 2016b). This was also shown to be true for eggs derived from Atlantic and sockeye salmon subjected to C&R (Booth et al., 1995; Smukall et al., 2019). The main contrast of these studies to mine, was that they did not expose the parent (or the eggs) to any additional stress. The reduction in egg viability in offspring of air-exposed parents in the current investigation was primarily driven by a greater mortality following the shocking challenge at the eyed stage. The shock procedure assists in the identification of any infertile or dying eggs.

Egg mortality was lower in families where the affected parent was trapped towards the end of the collection period. Simulated C&R may have had a different (possibly lesser) impact on egg viability if the adult salmon were caught at an earlier stage of their migration. This is based on evidence that the level of

influence that parental disturbance can have on the offspring is dependent on the developmental stage of the gametes at the time of the disturbance (Schreck et al., 2001; Smukall et al., 2019). For example, if adult fish are exposed to a stressful event during oocyte vitellogenesis, this can have adverse effects on the offspring (Schreck et al., 2001; Faught and Vijayan, 2018). This is due to the transfer to the oocyte at this time of essential hormones, nutrients, lipids, and the pre-cursor of yolk, vitellogenin, all of which are necessary for proper embryonic development (Schreck et al., 2001; Blount et al., 2016; Faught and Vijayan, 2018). It is important to note that mothers are not the only ones that can affect the development of the zygote. Fathers can also potentially adjust their offspring's phenotype to prepare them for a harsh environment (defence mechanisms) and protect them during early development, by transferring several epigenetic compounds, such as RNAs and proteins via sperm during fertilization (Immler, 2018). Conversely, stress to the father during the period prior to mating could impair these processes: it is noteworthy that the observed effects of the simulated C&R protocols on offspring mortality and viability in the present study were independent of whether the stressor was applied to the mother or the father. An increased among family variability in egg mortality was also detected in the treatment groups compared to the control. Since the study was based on wild fish, and I had no information regarding their life history up to point of capture, this increased variability in egg mortality may be an outcome of some individuals having experienced a stressful event (such as real C&R, or a close encounter with a predator) prior to the investigation, such that a further stressful event causes a larger response. Another possibility is that the stressor protocols, although being of standardised duration, had differing impacts on individual fish due to variation in their physiological state, energy reserves and stamina. Not taking this into consideration could disproportionally influence the physiology of the experimental fish, and consequently their offspring. For example, the pre-determined exercise duration used on the fish could overly exhaust one individual, which would force it to use a greater portion of the non-reusable resource it contains. This in turn could reduce resources that the fish could invest in its offspring. In contrast, a fish with a higher natural endurance and determination would require a longer fight time to get landed. Using the same duration of exercise for all fish is therefore not representative of what the fish would experience in true catch and release angling. The variation in egg mortality could also arise from some

individuals being more vulnerable to the effects of stress than others. One thing this investigation has not considered, which could have affected the outcome, is the personality and phenotype of individual salmon (Lennox et al., 2017; Claireaux et al., 2018; Koeck et al., 2019). For example, by collecting experimental fish using a trap, I have omitted the critical decision that wild salmon have to make as to whether or not to take the angler's lure, and have therefore grouped all individuals into one situation (Koeck et al., 2019). This could be considered both as a benefit and drawback to this investigation. The benefit is that the experimental population was not pre-biased, however the drawback is that some of the individual fish sampled might never have been caught by angling in the first place.

Yolk sac volume was diminished in the offspring of parents of either sex that were subjected to disturbance. This has previously been observed in female salmonids where their plasma cortisol level was elevated in the days prior to spawning (Eriksen et al., 2006; Eriksen et al., 2007; Andersson et al., 2011; Sopinka et al., 2014). The size of the yolk sac is of significance for the subsequent development of the offspring (McCormick et al., 1998). Salmonid research has demonstrated that zygotes cannot produce their own essential developmental hormones and are as such dependent on the yolk sac reserves that the parents incorporate during oogenesis (McCormick et al., 1998; Eriksen et al., 2007; Andersson et al., 2011). The yolk sac also contains energy that is needed during embryonic development and over the critical period of transition to exogenous food, and so a larger yolk sac increases the probability of survival during periods of starvation after hatching (McCormick et al., 1998; Eriksen et al., 2006). The yolk sac volume can also reflect maternal investment towards the offspring (McCormick et al., 1998; Eriksen et al., 2007) and provides an indication of endocrine condition of the mother during spawning, by mirroring her plasma cortisol (Eriksen et al., 2013). Andersson et al., (2011) suggested that maternal adjustment of yolk sac volume can represent alternative reproductive strategies, corresponding to proactive and reactive mechanisms for managing stress. Offspring from the proactive reproductive strategy originate from low stress parents, who invest in relatively large yolk sacs which provide the offspring with the energy reserves to be more aggressive and establish the most optimal feeding territories (Andersson et al., 2011). In contrast, offspring from the reactive reproductive strategy stem from high stress parents

that produce relatively small yolk sacs, so that the resulting offspring need to adopt more energy-conserving behaviours when emerging from the nest (Andersson et al., 2011). I also found that there was reduced within-family variability in yolk sac volume when one of the parents had been exposed to air. This might diminish the family's ability to match an unpredictable environment, although within-family variability in parental provisioning is not considered a viable bet-hedging strategy in salmonids (Einum & Fleming 2004).

There was no detectable difference observed for date of first feeding, even with the presence of diminished yolk sacs in families of parents exposed to simulated C&R. A possible explanation is that parents have developed mechanisms to buffer some of the detrimental effects caused by stress (Schreck et al., 2001). However, adults experiencing extended air exposure (120 s) during their late upstream migration to their spawning grounds produced offspring that were smaller in length at first feeding. The constrained development of the affected offspring could be a result of smaller nutritional reserves (in the smaller yolk sac), epigenetic processes caused by altered hormonal balance within the eggs, or relocation of nutritional resources to areas within the egg in need of repair and maintenance. Eriksen et al. (2006) found similar effects when farmed female Atlantic salmon were given cortisol implants 6 days prior to stripping, which acted as a proxy of increased environmental stress. As a result, the offspring yolk sac volume was reduced, and their fork length was shorter both at hatching and first feeding. Likewise, at four months post fertilization, Pacific salmon eggs that were exposed to high concentration of cortisol produced fry with an overall smaller body size, whereas a low dosage of cortisol seemed to have no effect (Capelle et al., 2017). Moreover, the current investigation revealed that offspring were smaller at first feeding if it was the mother that was stressed compared to if the stressor was applied to the father. This is logical since females provide the nutrients necessary for the embryo to develop and grow (Warriner et al., 2020).

By the third month post feeding, there was no longer any difference in the average fork length across treatment groups. It is possible that this could arise from size-selective mortality, with smaller fish being more likely to have died off by this point, leaving behind the larger individuals within each family, but it is important to note that the fish were fed to excess throughout this period, so reducing the likelihood of small fish dying through lack of food. Under natural conditions, where

food may be limited, there could be more exaggerated differences in survival between fish of different sizes earlier in development (Einum & Fleming 1999). In addition, smaller individuals in the wild would be more likely to be captured by predators.

Total offspring mortality was overall low, but nevertheless higher in the families whose parents were air exposed. This was mainly a result of the offspring mortality during the short-term *Saprolegnia* spp. outbreak in the system. This vulnerability could be problematic in the wild since *Saprolegnia* spp. is an opportunistic fungus that is near-ubiquitous in aquatic ecosystems and infects damaged tissue (Olsen et al., 2010). Once established, it spreads rapidly across the skin, fins, and gills by secreting a cotton like mycelium coat that is often lethal to fish (Olsen et al., 2010). The apparent greater vulnerability of the offspring of stressed parents could arise from a reduced immunocompetence, partly arising from investment in growth to compensate for the poorer maternal investment in the egg: European starlings *Sturnus vulgaris* exposed to GCs as a proxy of maternal stress also exhibited weakened immunocompetence, possibly through either direct suppression of the phytohemagglutinin response, or indirectly through the trade-off between compensatory growth and immune response (Love et al., 2005). Several other papers highlight this compromise between accelerated growth and increased risk of disease, however the research tends to concentrate on mammals and birds rather than fish (Metcalfe and Monaghan, 2001; Veru et al., 2014; Taylor et al., 2016). Moreover, it has been shown that females that experience oxidative stress during egg development can develop protein impairment in specific immune proteins, i.e. immunoglobulins, which can leave the passive immunity of their offspring compromised (Blount et al., 2016). Another area within this field that needs further in-depth research is how prenatal parental stress affects the development and the susceptibility of the offspring's immune response (Veru et al., 2014; Taylor et al., 2016). The evidence up to this point suggests that parental stress introduced during specific developmental periods can inhibit stem cell proliferation, and thus affect offspring lymphocyte numbers (Veru et al., 2014).

These results indicate that C&R of the parents, and especially air exposure, shortly before spawning could potentially have some adverse intergenerational effects at the early developmental stages of Atlantic salmon. To understand the influences at the scale of a population however, we must first identify and acknowledge the

effects at the individual level. Moreover, the conditions under which the adults are angled, as well as the environment in which the offspring will live are important and should be taken into consideration. Lastly, it important to note that all these effects should be examined further for both parents equally, since this investigation clearly reveals that stressing the father prior to spawning can have as big of an effect on the next generation as can stressing the mother.

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3.8 Supplementary Information

Table S3.1 Summary of all final General Linear Models (GLM) used to investigate the effects that the adult angling simulations had on the offspring.

Variable of Interest	Model used
Total Egg Mortality	lm(log((Total Mortality)+0.01) ~ Treatment + Date of Trapping + Days Elapsed + Parental Fungal Spread + Parental Sex)
Immediate Egg Mortality	lm(log((Immediate Mortality)+1) ~ Treatment + Date of Trapping + Days Elapsed + Parental Fungal Spread)
Egg Mortality Prior to Eye Stage	lm(log((Prior to Eye Stage) ~ Treatment + Date of Trapping + Days Elapsed + Parental Fungal Spread)
Mortality Shock	lm(log((Shock)+0.01) ~ Treatment + Days Elapsed + Parental Fungus Pre-Treatment + Parental Sex + Egg Mortality Prior to Eye Stage)
Yolk Sac Volume	lm((Average YSV) ~ Treatment + Period Between Fertilization and Preservation)
Date of First Feeding	lm(Date Of First Feeding ~ Treatment + Date Of Fertilization +Percentage Fungal Spread Parents + YSV)
Fork Length at First Feeding	lm(Fork Length At First Feeding ~ Treatment + Parental Sex + Date Of First Feeding)
Proportional Head Length at First Feeding	lm(Proportional Head Length At First Feeding - Fork Length ~ Treatment)
Three and five months	lm(Fork Length ~ Treatment * Months + Number of Offspring in Compartment At Time Of Measurement)
SGR 1 Fork Length	lm(SGR 1 FL ~ Treatment + Size At First Feeding + Date Of First Feeding)
SGR 1 Head Length	lm(SGR 1 HL ~ Treatment + Head Length At First Feeding + Date Of First Feeding)
SGR 2 Fork Length	lm(SGR 2 FL ~ Treatment + Size at 3 months)
Total Mortality	lm(Total Mortality Offspring ~ Treatment + Fungal Spread - Parent)

Mortality due to Fungus Saprolegnia spp.	lm(Fungal Mortality Offspring ~ Treatment + Fungal Spread - Parent)
Residual Mortality	lm(log((Residual Mortality)+1) ~ Treatment + Fungal Spread - Parent)

Table S3.2 Summary of Tukey Multiple Comparisons of Means output (95 % family-wise confidence level) for the effects that the parental C&R simulations have on the offspring's fork length at first feeding.

	Lwr.	**Upr.**	**p**
Exercise – Control	-0.1388534	0.06177875	0.74
Exercise + Air – Control	-0.1621185	0.03476320	0.32
Exercise + Extended Air – Control	-0.2493037	-0.05242204	0.001
Exercise + Air – Exercise	-0.1254564	0.07517575	0.91
Exercise + Extended Air – Exercise	-0.2126416	-0.01200949	0.02
Exercise + Extended Air – Exercise + Air	-0.1856261	0.01125560	0.10

Chapter 4 – Simulated catch-and-release angling of adult wild Atlantic salmon (*Salmo salar*), decreases the activity and exploration of a novel environment in offspring and increases aggression.

4.1 Summary

There has been a recent realisation of the importance of understanding parental effects and epigenetic inheritance, especially under context-specific scenarios, since these could influence the phenotypes of the next generation, and so affect their fitness. Yet, there are still various key unknowns in this area, such as how stress experienced by parents prior to breeding can affect the behaviour of the next generation. This study examines how stressors, meant to simulate catch and release (C&R) angling, experienced by wild Atlantic salmon shortly before spawning affected the boldness (risk-taking behaviour), activity, exploration, and aggression of their offspring. Adults of both sexes were collected from the river Blackwater, N. Scotland, using a permanent fish trap that intercepted fish on their upstream spawning migration. They then experienced one of four disturbance treatments that comprised of exercise and air exposure of different durations. They were then mated (using IVF) with an unstressed mate, and the offspring reared in controlled conditions prior to testing for behavioural traits when 3-4 months old. The results revealed that activity and exploration of a novel environment was reduced in offspring whose experimental parent was exercised and then exposed in air for an extended period, although exploration rate in the same group was positively related to fish size. Furthermore, exploration was also lower in offspring from the exercise group. Lastly, offspring of a parent subjected to either exercise or exercise + extended air exposure displayed higher levels of aggression compared to offspring of control parents, while performing more lateral rather than direct attacks. These results suggest that C&R angling of wild Atlantic salmon close to the time of spawning could affect the behaviour of the offspring during the early stage of life when they are dispersing and competing for feeding territories, which could influence their chances of survival.

4.2 Introduction

There is growing evidence that the physiological state and previous experience of the parents can influence the phenotypes of the next generation, without altering their genetic make-up (Eriksen et al., 2007; Burton and Metcalfe, 2014; Lennox et al. 2015; Stringwell et al., 2015; He et al., 2016; Bautista and Burggren, 2019). This type of phenotypic alteration is known as epigenetic inheritance/ programming and can be induced by either or both parents (maternal, paternal, or parental effects; Jensen et al., 2014; Immler, 2018; Bautista and Burggren, 2019; Warriner et al., 2020).

Parental effects caused by stress experienced by the parents prior to reproduction have largely been regarded as having negative consequences for offspring, through the rise of maladaptive traits that cause a reduction in their overall fitness (Eriksen et al., 2013; Haussmann and Heidinger, 2015; Bautista and Burggren, 2019; Lehto and Tinghitella, 2019). However, a very important aspect of epigenetic inheritance that was recently reconsidered is the indirect transfer of information and preparation of the offspring to cope with the environmental stressors they will potentially experience in their lifetime (Immler, 2018; Thayer et al., 2018; Bautista and Burggren, 2019; Warriner et al., 2020). This could be achieved via two, non-mutually exclusive ecological processes: (1) transgenerational phenotypic plasticity (TPP), which is the ability of offspring to phenotypically respond to environmental stimuli that the parents experienced; and (2) environmental matching, which is an anticipatory parental effect whereby parents alter the phenotype of their offspring so as to improve offspring performance in the quality of environment that the parents themselves experienced, since this is an indication of what the offspring may themselves face (Jensen et al., 2014; Sopinka et al., 2014; Bautista and Burggren, 2019; Warriner et al., 2020). The field of epigenetics still has many fundamental unknowns (Immler, 2018), and as such needs further investigation so as to be able to assess the importance that distinct experiences of adults can have on the next generation.

There is an array of examples where parental effects have been shown to affect the physiology, behaviour, and development of offspring in response to a range of biotic and abiotic environmental stimuli (Eriksen et al., 2006; Eriksen et al., 2007;

Jensen et al., 2014; Sopinka et al., 2014; Atherton and McCormick, 2020). The greatest epigenetic effects are expected to arise from the mother, since she provides a proportionally higher quantity of resources to the offspring compared to the father (Sopinka et al., 2014; Redfern et al., 2017). An example of this association between mother and offspring phenotype comes from studies showing that female Atlantic salmon given implants containing the glucocorticoid stress hormone cortisol produced offspring with a reduced survival rate, increased frequency of deformities, shorter fork lengths and reduced yolk sacs sizes (Eriksen et al., 2006; Eriksen et al., 2007; Eriksen et al., 2013). Moreover, offspring from the same cortisol-treated females had a higher locomotory activity (Eriksen et al., 2013). Equivalent findings were also observed with eggs directly exposed to cortisol prior to fertilization (simulating the effect of a stress response by the mother), where the offspring exhibited a higher oxygen consumption during embryonic development as well as increased aggression post hatching (Sloman, 2010).

It is important to note that the extent of the effects on the offspring can depend on the intensity, duration and timing of the stressor experienced by the parent, as well as the sensitivity of both the species and individual fish (Colson et al., 2015; Taylor et al., 2016). Adult wild Atlantic salmon are deemed particularly resistant to stress during their reproductive stage however, they still experience different levels of stress (exhaustion and/or air exposure) from catch & release (C&R) angling events during their upstream migration to their natal breeding grounds (Olsen et al., 2010; Lennox, 2018). C&R angling has been shown to have various effects on the survival, behaviour, physiology, and reproduction of the captured fish (Jensen et al., 2010; Gale et al., 2011; Richard et al., 2014; Immler, et al., 2018; Twardek et al., 2018; Roth et al., 2019; Smukall et al., 2019; Chapter 2). However, there is still little known regarding the consequences of C&R angling for the offspring of adult fish that were captured shortly before spawning.

The typical response of fish experiencing a stressor is activation of the HPI (hypothalamic-pituitary-interrenal) axis, resulting in the increased synthesis of adrenal and non-adrenal hormones (i.e. corticosteroids and testosterone). These circulate in the bloodstream and can potentially affect developing gametes and hence alter the phenotype of the offspring (Stringwell et al., 2015; Ghio et al., 2016; Sopinka et al. 2016a; Taylor et al. 2016). There have been several studies

that have examined the effect of maternal stress on the initial stages of offspring development in fish (Eriksen et al., 2006; Eriksen et al., 2007; Allen et al., 2008; Eriksen et al., 2015; Thayer et al., 2018), but very few have explored offspring performance and characteristics at subsequent life stages (Eriksen et al., 2007; Andersson et al., 2011); moreover, there have been no previous investigations of the effect of paternal stress on offspring.

Here I investigate the effects that C&R angling of Atlantic salmon can have on the behaviour (risk-taking, activity, exploration, and aggression) of their offspring during the early stages of juvenile life, when the young fish would be competing for feeding territories in their natal stream. I predicted that the offspring whose parents (both males and females) experienced the greatest cumulative disturbance shortly before spawning would demonstrate the greatest change in their behaviour, compared to the offspring from unaffected parents. The hypothesis was tested experimentally by subjecting adult wild Atlantic salmon to different levels of exercise and air exposure (as a means of simulating the stressors experienced during C&R) shortly before spawning, and then monitoring the behaviour of their offspring reared under controlled conditions. The level of infection from the fungus *Saprolegnia* spp. on the experimental parent was also taken into account, since *Saprolegnia* is a widespread opportunistic fungus that naturally affects adult salmon in the period leading up to spawning (Olsen et al., 2010; Arlinghaus et al., 2013; Havn et al., 2015; Smukall et al., 2019) and could act as an additional (non-experimental) stressor. Activities like C&R could leave an individual vulnerable to such an infection (Olsen et al., 2010; Wedemeyer and Wydoski, 2008; Smukall et al., 2019), forcing the fish to use a portion of their resources into fighting off the infection rather than reproduction (Tufts et al., 2000; Olsen et al., 2010; Lennox, 2018). Additionally, the size of the yolk sac was also taken into account, since offspring with smaller yolk sacs tend to be smaller in size and are more aggressive compared to their conspecifics (Andersson et al., 2011; Larsen et al., 2015). They also transition from endogenous to exogenous feeding earlier, therefore they emerge sooner from the nest and need to establish and defend a feeding territory promptly (Armstrong and Nislow, 2006; Larsen et al., 2015). However, early emerging individuals are more vulnerable against predation due to their smaller size and increased exposure (Armstrong and Nislow, 2006).

4.3 Methods

4.3.1 Salmon Collection and Catch & Release Simulation

Adult wild Atlantic salmon were caught between November and December 2018 on their upstream spawning migration, using a permanent fish trap installed on the river Blackwater, Scotland (see chapter 2). They were then transferred (in water-filled bags to avoid any air exposure) to holding tanks supplied with a continuous turnover of water from the river, and were left undisturbed for a period of 24-48 h to give them time to recover from the trapping and transfer. Male and female salmon (15 fish per treatment per sex, n = 120 in total) were then randomly assigned to one of four pre-determined C&R simulation treatments. Females were chosen with the stipulation that they were still hard (i.e. had not yet released their eggs into the body cavity) at the time of the treatment. The treatments consisted of different levels of exercise and air exposure (Struthers et al., 2018; Smukall et al., 2019) 5 – 18 days prior to spawning (Table 4.1; Chapter 2). Exercise was conducted in an arena (diameter: 4 m, height: 1.5 m, water depth: 0.18 m) where fish were manually chased for 210 s, being kept moving by gentle hand-tapping on the tail or sides whenever they ceased swimming. Air exposure (0, 60 or 120 s) was achieved by holding the fish out of water in a knotless hand-net. Control fish were also transferred between the pre- and post-treatment holding tanks so as to keep all procedures constant across groups, while keeping the disturbance that the control fish experienced to a minimum (e.g. by transferring the fish in individual water-filled plastic bags so as to prevent air exposure). The water temperature in the arena and holding tanks was 6 ± 1.5 ^{o}C. The simulations were intended to reproduce the stressors that fish would experience during an actual angling event. It should be noted however, that they do not include the physical damage to the mouth that a fish would normally sustain from a hook. Moreover, the investigation was conducted after the end of the permitted angling season in Scotland, although angling during this period is allowed for brood stock hatchery purposes and for recreational purposes in other countries.

Table 4.1 Summary of the four treatment groups used in the experiment and the cumulative levels of acute disturbance they represent, indicated by the number of asterisks; see Chapter 2 for full description of treatments.

Treatment	Exercise	Air exposure	Cumulative disturbance
Control	No	No	
Exercise	210 s	No	*
Exercise + Air Exposure	210 s	60 s	**
Exercise + Extended Air Exposure	210 s	120 s	***

4.3.2 Artificial Fertilization

Artificial fertilization was conducted by crossing experimental (60 females, 52 males) and non-experimental salmon on a one-to-one ratio. This produced a total of 112 crossings (see chapter 3 for details). The gametes of each pair were carefully mixed together and soaked in water for about 60 min, to allow the eggs to swell and the shell to harden. After being stripped, the adults were released back into the loch from where they were collected (upstream from the permanent trap). The fertilized eggs were later incubated at the Scottish and Southern Energy (SSE) hatchery in Contin, in individual family trays.

4.3.3 Fungal Infection of the Parents

Photos (Sony Cyber-shot DSC-WX100 camera) of both sides of the body of the experimental adults were taken on the date of trapping and again immediately before the artificial fertilization (see chapter 2). This allowed estimation of the total percentage area of the fish that was covered by the fungus *Saprolegnia* spp. before the C&R simulation, and the increase in the fungal area on the body of the fish between this time and the time of mating. The calculations were performed using the software ImageJ (version 1.51r). The sequence in which the photos were analysed by the reviewer was random and blind to treatment group.

4.3.4 Offspring Transport and Maintenance

Due to space constraints, only a subset of families could be kept segregated through to the juvenile stage. Therefore, once they had reached the alevin stage

(hatched embryos with an attached yolk sac), 56 families (7 per treatment per sex of experimental parent) out of the original 112 families were randomly selected to be transported (April 9th, 2019) and housed for further investigation at the University of Glasgow. Upon arrival in Glasgow, each family was randomly (Number generator random; version 2.0) assigned to its own compartment (dimensions: 19 x 13 cm; water depth: 15.5 cm) within a single recirculating stream system (water flow = 0.99 L/s). The offspring (25 individuals per family) were kept at a low water temperature (7 ^{o}C) in the dark. Once their yolk sac was exhausted and they started to actively feed, the water temperature was steadily increased over the following two months (May – June) to 12 ^{o}C, to simulate natural conditions. The photoperiod was also changed to 12L:12D (8:00 – 20:00). The salmon were fed with commercial salmon feed starter crumb pellets from the time of first feeding (28/4/19 – 16/5/19, depending on family) until July 2nd, 2019, and thereafter with small bloodworms. They were fed 3x a day (at 9:00, 13:00 and 17:00 h) from first feeding until June 9th, 2019, then 2x a day (at 9:00 and 17:00) from June 10th to July 1st, 2019, and finally 1x a day (at 12:00) from that point on. All families were fed to excess in their individual compartments, and excess food and faeces were removed daily by siphon.

4.3.5 Yolk Sac Volume

On the same date that the families of offspring were transported to Glasgow, an additional 336 alevins (n = 6 per family per sex of parental experimental fish) were euthanized with an overdose of anaesthetic (clove oil) and preserved in absolute ethanol. These individuals were later used to estimate the average yolk sac volume (YSV) of the offspring per family (see chapter 3). This was accomplished by taking a photo (Sony Cyber-shot DSC-WX100) of the offspring from a set distance, alongside a known size reference, and then calculating (to the nearest 0.001 mm) the length and height of the yolk sac using ImageJ software. The volume was later estimated using the formula for a prolate spheroid (Ching et al., 2012; Baron-Aguilar et al., 2013; Sulaeman, 2017; Thomas et al., 2020) as indicated below, where 'h' and 'l' are the maximum height and length of the yolk sac respectively:

$$YSV = \left(\frac{\pi}{6}\right) \times l \times (h)^2$$

4.3.6 Offspring Behaviour

The behavioural traits (boldness, activity, exploration, and aggression) of the offspring from each family were examined by exposing each selected fish to a sequence of trials. These trials were run between July and August 2019 in 2 identical translucent plastic arenas (dimension: 61 x 37.5 x 38 cm) with a water depth of 3.5 cm and a water temperature of 13^{o} C (Figure 4.1a). Equal number of individuals from each family were assigned to be tested in each arena, to minimise any confounding arena-effects. All experimental fish were fasted for 24 h prior to the trials so that they would no longer be digesting food (affecting their available aerobic scope) at the time of the trial. The sequence in which the families were tested was randomly assigned. All test fish (maximum of 8 offspring per family; see later for details of which families had fewer than 8 surviving representatives) from each family (54 families; Control: 14 families, Exercise: 12 families, Exercise + Air: 14 families and Exercise + Extended Air: 14 families) were removed from their compartments on the day of the trials, using a hand-net, and placed in individual transparent containers that were connected to a larger tank to maintain water circulation. This allowed for transport of the fish without any excess air exposure or handling, which kept stress level to a minimum. All fish from a given family were tested on the same day due to the difficulty of segregating tested from non-tested individuals for more than a few hours (since they were too small and fragile to tag at this point), and so as to avoid fasting a family multiple times. The behaviours in each trial were recorded using a video camera (Panasonic HC-VX870 4K) and analysed at a later time, so as not to affect the behaviour of the fish due to the presence of an observer. To avoid issues of pseudoreplication the behaviours were analysed using the mean value of all the individuals within each family, rather than individually. At the end of the trials the fork length (Electronic Calliper Waterproof IP67, 0-150 mm; to nearest 0.01 mm) and mass (Ohaus E01140 Explorer Analytical Balance; to the nearest 0.1 mg) of the fish that went through the trials were recorded and averaged for each family.

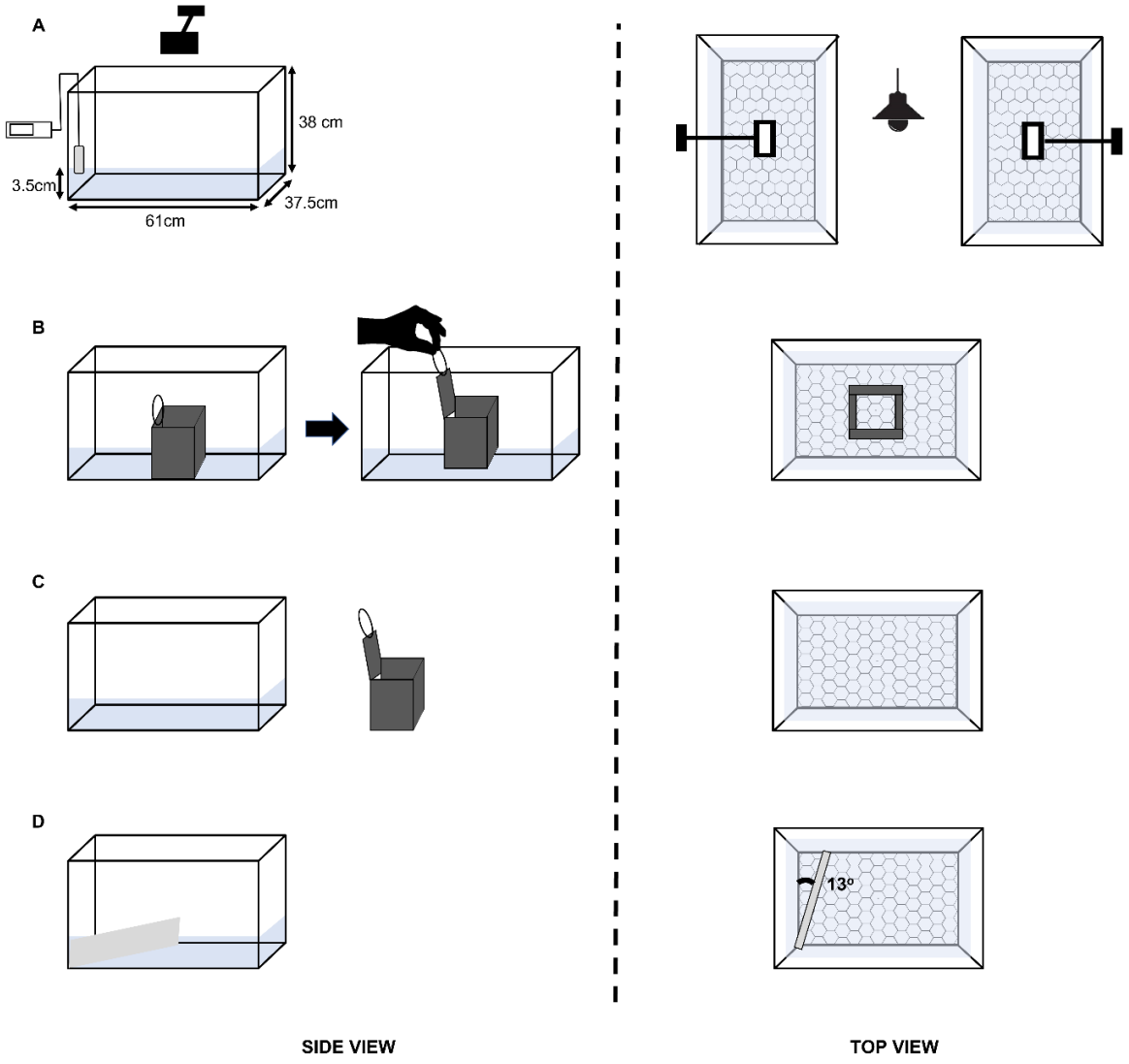

Figure 4.1 Depiction of the arenas in which the sequence of behavioural tests were conducted, as seen from the side (left diagrams) and from above (right). a. General features of the two arenas, showing their dimensions, the water depth, the position of the temperature probe and light, and the position of the overhead cameras. The arenas were constructed from translucent plastic sheeting. b. The set-up of the arena for the emergence (risk taking) test. The side walls and door of the start-box (shown in dark grey) were made of opaque plastic, with the top being left open. The box was placed in the middle of the arena, with the door always facing the left side of the arena. The door was raised by hand at the start of the test. c. The activity and exploration trials were conducted in the arena once the start-box was removed. The hexagonal grid marked on the bottom of the arena was used to calculate the percentage area of the novel environment that was explored by the fish. d. A mirror (37.5 x 7.5 cm) was placed at an angle of 13º

to the end wall of the arena at the start of the last part of the experiment, in order to investigate aggression.

4.3.6.1 Risk-taking trait

Offspring risk-taking traits were quantified using an emergence test (Figure 4.1b). The fish were individually acclimated in the start-box (12 x 12 cm; opaque walls with no ceiling or floor) for 10 min prior to the start of the trial. One of the walls formed a sliding door, which was then opened by hand to allow the fish to exit the starter box and enter the arena, which was an unknown and unfamiliar environment. An emergence time (in seconds) was recorded as the point when the pectoral fins of the fish first passed the door threshold. If the offspring was still inside the box after 180 s, the box was removed and a maximum score for emergence time (180 s) was given to the individual. The fish's emergence score was then converted to a percentage of the maximum time of 180. The greater the percentage, the more time it took the offspring to emerge into an unknown environment and thus the lower their level of risk-taking.

Seven individuals from 5 families escaped from the emergence box during the acclimation period prior to the start of the trial and were therefore not used in the analysis. In addition, 7 families had fewer than 8 individuals running through the trials due to limiting numbers of surviving fish (1 family = 7 individuals, 2 families = 6 individuals, 1 family = 5 individuals, 1 family = 4 individuals, and 2 families = 2 individuals). The final number of fish that provided data for testing risk-taking traits was 401.

4.3.6.2 Activity and Exploration

The second part of the trials, which lasted an additional 180 s, began immediately the emergence test was completed (either by the fish exiting the start-box box or time running out). If a fish was still inside the start-box when time ran out, the box (which had no base) was lifted, so exposing the fish to the arena. Offspring activity and exploration of a novel environment (the arena) were simultaneously video recorded using the same method as above (Figure 4.1c). Activity was defined as the percentage of the total trial time (180 s) that the fish was moving within the arena, and exploration was quantified as the total percentage floor area explored within the same timeframe. The bottom of the arena was lined with a

hexagonal grid, where each complete hexagon represented 1.28 % of the total floor area (the half hexagons at the edge of the arena represented a percentage area of 0.64 %). Each hexagon was considered as explored once the pectoral fins of the fish passed over its boundary, and the percentage area explored was calculated as the percentage of these grid squares that were entered during the 180 s.

The videos of 5 individuals from 4 families were corrupted and could not be used in the analysis. In addition, as with the measurement of risk-taking traits, 7 families had less than 8 fish running through the trials due to limiting numbers. The final number of fish that contributed data for the analyses of activity and exploration behaviour was therefore 403.

4.3.6.3 Aggression

Offspring aggression was then recorded using the video camera to film the behaviour of the fish when it encountered its own reflection in a mirror (Figure 4.1d). The mirror (37.5 x 7.5 cm) was lowered into place so that it rested at an angle of 13 º to the end wall that was closest to the fish at the end of the activity and exploration phase of the trials. This position ensured that the fish would immediately see its reflection in the mirror. Aggression over the next 180 s was then quantified in three ways: a. number of frontal (forward) attacks, b. number of lateral (sideways) attacks, and c. total number of attacks (which was the sum of frontal and lateral attacks) on the mirror. The frontal and lateral attacks were also expressed as a percentage of the total number of attacks. Due to technical issues not all video recordings lasted the full 180 s, therefore the median family recording time across all treatment groups was 174 s (with the lower quartile = 172 s and the upper quartile = 178 s).

The videos of 8 individuals from 4 families lost their focus and could not be used in the analysis. In addition, as with the two previous measurements 7 of the families had a few fish missing from the start of the trials. The final number of fish used for the data analysis of aggression was 400.

4.4 Statistics

General Linear Models (GLMs) were run in R (Version Ri386 3.4.4) to examine whether the C&R simulation of the parents influenced key behavioural traits in the offspring. GLMs were used to examine whether the C&R simulations on the parents affected the offspring's risk-taking traits (how fast they emerged from a refuge into a novel environment), exploration of a novel environment (what percentage of the novel environment they explored), activity (how active they were during the exploration phase of the experiment), and their level of aggression (the total number of attacks against their mirror reflection, and the percentage of those attacks that were frontal and that were lateral). The main explanatory variable in the models was the C&R simulation that the experimental parent experienced (treatment). All initial models also included the experimental parent's sex (since each offspring was the result of a mating between a fish subjected to the experiment and a non-experimental mate), the experimental parent's initial % body area covered by the fungus *Saprolegnia* spp. on the date of trapping, and the % increase in the spread of fungus by the time of mating. Moreover, all initial models included the mass and length of the offspring on the date of the trials (taken as the average for the family), the date the trial was conducted, and the average yolk sac volume of the family at the time of alevin collection. The interaction between treatment and experimental parent's sex was included in all models, as were the 2-way interactions between parental treatment and size of the offspring (length or mass), and parental treatment and the fungal infection (initial and spread) of the parents. The model for frontal and lateral attacks also included the total number of attacks and total recorded time in the arena as explanatory variables.

The values for aggression (total number of attacks, frontal, and lateral attacks) underwent logarithmic transformation in order to normalise the data. The models with the lowest AIC value were selected and assessed through residual fit plots for linearity, normality of residuals, and homogeneity of variance. The significance in the final models was checked using p-values (with $p = 0.05$ taken as the threshold for significance). If a variable of interest was found to be significant, then the variable was investigated further using Tukey multiple comparison of means. Levene's tests for homogeneity of variance was run for all final models, as to

investigate the variance across treatments. A summary of the final models can be seen in the supplementary information (Table S4.1).

4.5 Results

4.5.1 Risk-taking traits

There was no difference among parental treatments in the time it took the offspring to enter a novel environment (Table 4.2; Figure 4.2). There was however, reduced variability in emergence time in offspring from parents that experienced either exercise and/or air exposure of any duration, both among families (Figure 4.2a; Levene's test: $p = 0.005$) and within a given family (Figure 4.2b).

Table 4.2 General Linear Model (GLM) investigating the effects of pre-spawning stressors associated with C&R simulation of the parents on the risk-taking traits of the offspring (the time it took the offspring to emerge from a refuge into an unknown environment as a percentage of the total time). Shown is the final model (with the lowest AIC), with the comparisons of each of the three C&R treatments to the control, together with the effect of the average family body mass of the offspring at the time of the trial, their yolk sac volume (family mean) on the date of transport to the University of Glasgow and the percentage increase of the body covered by the fungus *Saprolegnia* spp. on the parents between the date of trapping and the artificial fertilization.

	d.f	**Estimate**	**Std. Error**	**t**	**p**
Intercept		30.248	14.169	2.135	0.04
Exercise	1	-0.616	7.035	-0.088	0.93
Exercise + Air	1	-11.621	7.577	-1.534	0.13
Exercise + Extended Air	1	-3.417	7.506	-0.455	0.65
Mass	1	36.113	20.142	1.793	0.08
Fungal Spread – Parents	1	-0.150	0.208	-0.717	0.48
Yolk Sac Volume	1	-178.311	167.288	-1.066	0.29
Residuals	41				

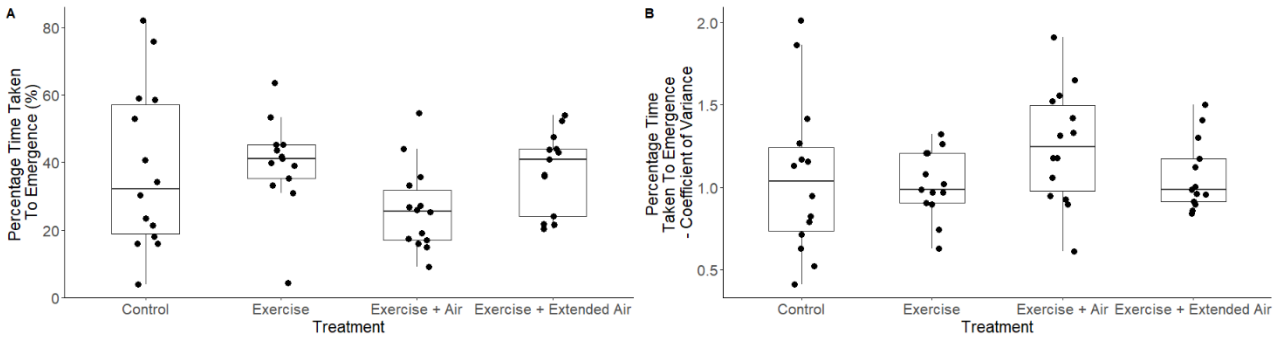

Figure 4.2 Effects of the C&R simulation of the parents on (a) the percentage emergence time of the offspring, which was the time it took them to emerge into an unknown environment as a percentage of the total trial time (high score means that it took the offspring a long time to emerge into an unknown environment, and thus have low tendency to take risks), and (b) the coefficient of variance for the percentage emergence time within each family. Each circular data point represents an experimental family. The boxplot indicates the median, the interquartile range, and maximum and minimum values for each of the treatments (see Table 4.2 for statistical analysis).

4.5.2 Activity and Exploration of a Novel Environment

Offspring from the exercise and exercise + extended air group had a lower overall activity compared to those from the control group (Table 4.3). Moreover, offspring that were larger (measured as mean body mass of the family) at the time of testing were less active (Table 4.3).

In addition, offspring of parents that were in the exercise + extended air treatment group explored less of the novel environment compared to the offspring of the control parents, as did those whose experimental parent had a greater increase in the spread of the fungus *Saprolegnia* spp. on their body between the date of trapping and the artificial fertilization (Table 4.4). Fish in the exercise + extended air group produced offspring that explored more of the novel environment as the average mass of the family increased – i.e. bigger fish explored more (Table 4.4, Figure 4.3). Offspring from parents in the other three treatment groups expressed the opposite trend (Figure 4.3).

Table 4.3 General Linear Model (GLM) investigating the effects of pre-spawning stressors associated with C&R simulation of the parents on the activity of the offspring (the percentage of the total trial time that the fish were actively moving). Shown is the final model (with the lowest AIC), with the comparisons of each of the three C&R treatments to the control, together with the effects of the mass of the offspring at the time of the trial, the sex of the experimental parent, the yolk sac volume of the offspring on the date of transport to the University of Glasgow and the percentage increase of the body covered by the fungus *Saprolegnia* spp. on the parents between the date of trapping and the artificial fertilization.

	d.f.	Estimate	Std. Error	t	p
Intercept		75.267	9.971	7.548	<0.001
Exercise	1	-12.117	4.959	-2.444	0.02
Exercise + Air	1	-9.464	5.354	-1.768	0.08
Exercise + Extended Air	1	-17.034	5.363	-3.176	0.003
Parental Sex – Male	1	6.163	4.413	1.397	0.17
Mass	1	-38.921	14.075	-2.765	0.009
Fungal Spread - Parent	1	-0.195	0.179	-1.093	0.28
Yolk Sac Volume	1	-140.863	122.214	-1.153	0.26
Residual	40				

Table 4.4 General Linear Model (GLM) investigating the effects of pre-spawning stressors associated with C&R simulation of the parents on the exploration of a novel environment by the offspring (the percentage area of a novel environment explored). Shown is the final model (with the lowest AIC), with the comparisons of each of the three C&R treatments to the control, together with the effects of the mass of the offspring at the time of the trial, the sex of the experimental parent, and the percentage increase of the body covered by the fungus *Saprolegnia* spp. on the parents between the date of trapping and the artificial fertilization.

	d.f.	Estimate	Std. Error	t	p
Intercept		37.682	6.949	5.423	<0.001
Exercise	1	-4.757	9.500	-0.501	0.62
Exercise + Air	1	0.254	9.540	0.027	0.98
Exercise + Extended Air	1	-23.698	9.939	-2.384	0.02
Mass	1	-14.812	12.256	-1.209	0.23
Fungal Spread – Parent	1	-0.178	0.079	-2.253	0.03
Parental Sex – Male	1	2.946	1.952	1.509	0.14
Exercise: Mass	1	6.659	16.403	0.406	0.69
Exercise + Air: Mass	1	7.524	17.331	0.434	0.67
Exercise + Extended Air: Mass	1	36.359	17.506	2.094	0.04
Residuals	40				

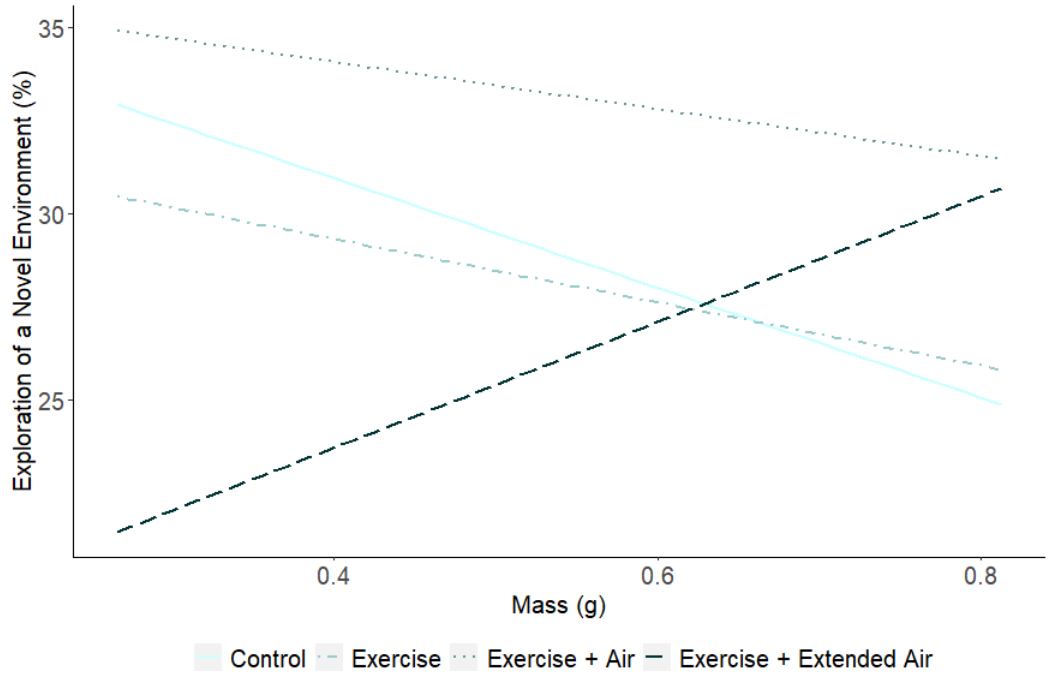

Figure 4.3 Effects of the C&R simulation of the parents on the relationship between the body size (mass, g) of their offspring at the time of testing and the exploration of a novel environment (expressed as the percentage of that environment that was explored during the trial).

4.5.3 Aggression

The offspring from the families whose parents experienced either exercise, or exercise + air, performed more attacks per unit time on their mirror reflection compared to the offspring from the control group (Table 4.5; Figure 4.4). They also showed more among-family variability in the number of attacks performed (Levene's test: $p = 0.046$). Moreover, fish from families which were on average larger at the time of testing (longer mean fork length) performed fewer attacks than did those from families of smaller individuals. When the total number of attacks was broken down into frontal and lateral attacks, the data illustrated that the treatment effect on overall aggression was driven by a difference in the number of lateral attacks: offspring from the exercise, and exercise + air treatment groups conducted a higher percentage of lateral attacks than did offspring from the control group (Table 4.5; Figure 4.4). Moreover, as the length of the fish increased, the total number of frontal attacks they conducted was reduced. Lastly, a higher percentage of the attacks by offspring from the families that weighed more on average were lateral rather than frontal attacks.

Table 4.5 The final General Linear Models (GLM) investigating the effects of pre-spawning stressors associated with C&R simulation of the parents on aggression by the offspring. (a) The total number of attacks the offspring performed on the mirror; shown are the comparisons of each of the three C&R treatments to the control, together with the effects of the average length (cm) of offspring per family at the time of the trial. (b) The number of frontal attacks on the mirror as a percentage of the total number of attacks. Shown are the comparisons of each of the three C&R treatments to the control, together with the effects of the average length (cm) of offspring per family at the time of the trial, and the average total recorded time the trials lasted (s) for each family. (c) The number of lateral attacks on the mirror as a percentage of the total number of attacks. Shown are the comparisons of each of the three C&R treatments to the control, together with the effects of the average mass (g) of the offspring per family at the time of the trial, the total recorded time the trial lasted, and the date the trial was conducted.

	d.f.	Estimate	Std. Error	t	p
a. Total Attacks					
Intercept		3.051	0.232	13.247	<0.001
Exercise	1	0.129	0.042	3.069	0.004
Exercise + Air	1	0.100	0.042	2.404	0.02
Exercise + Extended Air	1	0.069	0.042	1.623	0.11
Length	1	-0.017	0.006	-2.922	0.005
Residuals	49				
b. Direct Attacks					
Intercept		2.895	0.644	4.491	<0.001
Exercise	1	1.464	0.715	2.049	0.046
Exercise + Air	1	1.624	0.652	2.490	0.02
Exercise + Extended Air	1	0.013	0.719	0.018	0.99
Length	1	0.027	0.012	2.303	0.03

Total Time	1	0.005	0.003	1.717	0.09
Exercise: Length	1	-0.037	0.018	-2.066	0.04
Exercise + Air: Length	1	-0.042	0.017	-2.509	0.02
Exercise + Extended Air: Length	1	0.000	0.018	0.015	0.99
Residuals	45				

c. Lateral Attacks

Intercept		-780.4	361.8	-2.157	0.04
Exercise	1	0.551	0.171	3.215	0.002
Exercise + Air	1	0.422	0.162	2.611	0.01
Exercise + Extended Air	1	0.274	0.166	1.656	0.10
Total Time	1	-0.012	0.009	-1.207	0.23
Mass	1	-2.786	0.659	-4.229	<0.001
Date of the Trial	1	0.018	0.008	2.173	0.03
Residuals	47				

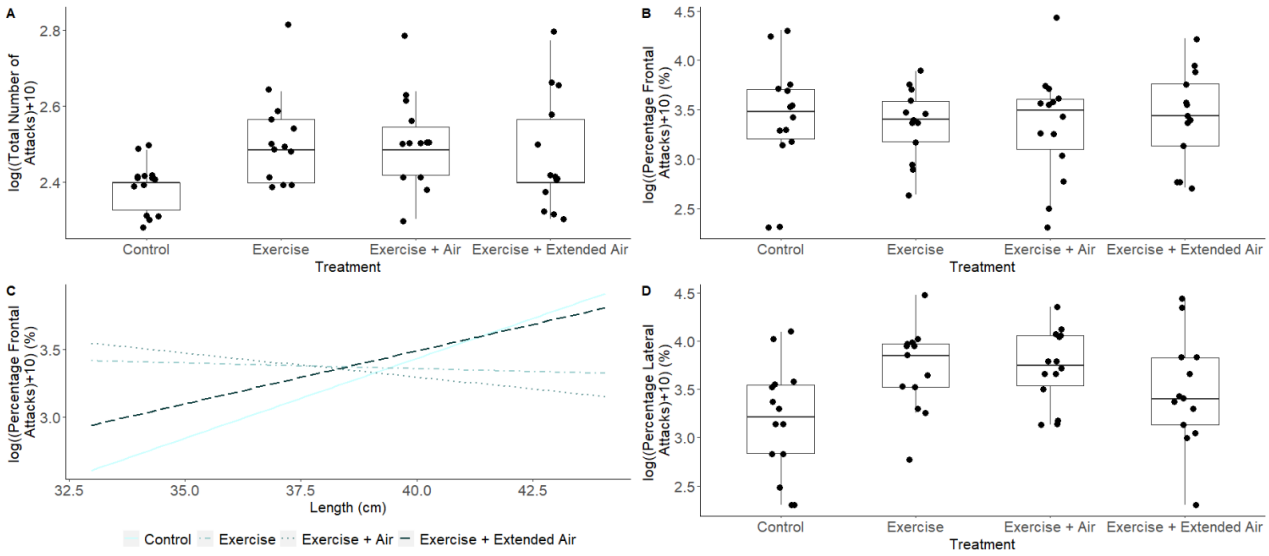

Figure 4.4 Effects of the C&R simulation of the parents on (a) the total number of attacks per trial on the mirror, (b) the number of frontal attacks on the mirror, as a percentage of the total number of attacks, (c) the relationship between the average length (cm) of the fish per family on the date of the trial and frontal attacks on the mirror, as a percentage of the total number of attacks, and (d) lateral attacks (sideways) on the mirror, as a percentage of the total number of attacks. Each circular data point represents an experimental family. The boxplot indicates the median, the interquartile range, and maximum and minimum values for each of the treatments (see Table 4.5 for statistical analysis).

4.6 Discussion

The results reveal that exposing adult Atlantic salmon to stressors intended to simulate the experience of catch and release (C&R) angling in the days leading up to spawning can have several effects on the behaviour of their offspring during early life; moreover, these effects may influence the ability of offspring to obtain and hold a feeding territory. Firstly, even though the stressors associated with C&R did not affect the time it took the offspring to emerge into a novel environment, it did reduce the variability in that emergence time. Moreover, offspring whose parents were either exercised, or exercised and then air exposed for an extended period of time (120 s), were less active compared to the offspring of unaffected parents. In addition, parents from the extended air group produced offspring that explored less of the novel environment, although the exploration

rate was greater in families that had exhibited faster growth in body mass. Lastly, offspring from the exercise and the exercise + air group were more aggressive, performing more attacks on a mirror image, compared to offspring whose parents had not experienced any stress.

The risk-taking behaviour (time it took the offspring to emerge from a refuge into a novel environment) did not differ across parental treatments. This result is analogous to a previous study on Brook trout (*Salvelinus fontinalis*) in which females with elevated cortisol levels, either through ingestion in food or as a result of physical disturbance, produced offspring that showed no difference in the latency to emerge into a novel environment (Ghio et al., 2016). In contrast, female largemouth bass (*Micropterus salmoides*) that were intraperitoneally injected with cortisol produced offspring that, when tested as young-of-year juveniles, took longer to emerge from their shelter (Redfern et al., 2017). An individual's position on the boldness-shy continuum is expected to be associated with its foraging and predator avoidance, with increased shyness resulting in reduced foraging and enhanced predator avoidance (Andersen et al., 2018; Wang et al., 2021). Hence, modifications to the boldness trait could potentially result in alterations to the structure of ecosystems as a result of changes in prey or predator size distributions and shifts in biomass (Andersen et al., 2018; Wang et al., 2021). Not seeing a difference in boldness in the current investigation may have been due to the fact that our disturbance treatments on the experimental parents may have had no effect on the risk-taking attribute of the offspring, or that changes in this personality trait are non-detectible in the short term, so any changes in this would only be detectable once a threshold is surpassed (Wang et al., 2021). Furthermore, the wild salmon population that was used in this investigation has been restocked back into the Conon through use of artificial IVF matings by the Cromarty Firth Fisheries Board for at least 12 generations. Therefore, another possible explanation for not observing any effects on the risk-taking behaviour of the offspring could be that the restocking and hatchery rearing procedure that the fish have being going through may already have selected for a specific shift in this boldness-shy continuum (Wang et al., 2021)

Simulated C&R on the parents did not seem to affect the boldness of the offspring, but it does appear to have induced other significant behavioural modifications. Both activity and overall exploration of a novel environment were lower in

offspring whose parents experienced exercise or exercise + extended air exposure. Interestingly however, when fry size was taken into consideration, the offspring of the exercise + extended air parental treatment showed increased exploration the larger they were at the time of testing. In contrast, offspring from the three other treatment groups showed the opposite effect of body mass. Other studies of salmonids that have simulated maternal stressors by using cortisol manipulation, either by cortisol implants in females prior to spawning, or by directly bathing eggs in cortisol, also found reduced locomotor activity in offspring (Eriksen at al., 2006; Burton et al., 2011; Sopinka et al. 2016b). Nevertheless, this trend is not consistent for all studies. For example, an acute confinement experiment conducted by Eriksen et al. (2015) found that fry from females with elevated cortisol levels displayed higher activity levels. Therefore, results from such measurements/experiments appear to be highly context specific, and can change with offspring age and size (Sopinka et al., 2016b). Furthermore, research at the intergenerational level that is focused on investigating the effects of stress has most often been conducted by treating the eggs with exogenous hormones (i.e. GCs), as it is the simplest and most pragmatic way of manipulating stress levels (Redfern at al., 2017). This approach, however, eliminates any compensatory buffering that the mother might have performed to try to shield her offspring and minimize the adverse effects; it also excludes other routes by which parental experiences can be transmitted to offspring (e.g. through epigenetic marks and microRNA molecules in gametes; Redfern at al., 2017).

These behavioural modifications may be adaptive, if the stressed parents are preparing their offspring for an unpredictable, high-risk environment. The reduction in activity and exploration observed in the offspring may lessen the odds of being spotted and eaten by a predator (note that C&R angling can be viewed as escape from a predatory attack). This interpretation is reinforced by the results of Redfern et al. (2017), who showed that offspring from cortisol-treated female largemouth bass (*Micropterus salmoides*) spent a longer time being static/inactive after being exposed to a predator, compared to offspring from non-treated females. However, as the size of the fish grows, the risk of predation decreases (both because they are less at risk from gape-limited predators and because their escape speed is faster), and so they may benefit from increasing their exploration of an unknown environment in search of optimal resources and shelter.

Higher levels of aggression were expressed by the offspring belonging to the disturbance groups, and more specifically in the exercise and exercise + air treatment groups. The same treatments also performed a higher percentage of lateral attacks compared to fish in the control group. Sloman (2010) obtained very similar results when testing the effects of exposing brown trout (*Salmo trutta*) eggs to cortisol. The resulting juveniles displayed both a higher level of aggression towards the mirror and conducted more lateral attacks in comparison to control juveniles (Sloman, 2010). Likewise, mothers experiencing a mild stressor prior to reproduction can also produce offspring that exhibit increased aggression, as shown by Eaton et al. (2015) using female guppies, *Poecilia reticulata*. There is also evidence that parental stress may alter testosterone levels within the eggs of the offspring during development, which can have significant fitness consequences (Andersson et al., 2004; Guibert et al., 2013). Higher levels of aggression, either through increased levels of cortisol or testosterone, can provide the offspring with a competitive ability against conspecifics, which might be especially beneficial when emerging into an unpredictable environment (Royle et al., 2001; Sloman, 2010; Burton et al., 2011; Eaton et al., 2015; Ahmed et al., 2016). However, offspring experiencing increased testosterone during the developmental stage may also exhibit several other characteristics that may be deleterious, including reduced skeletal growth and immunosuppression (Andersson et al., 2004; Burton et al., 2011; Guibert et al., 2013), several of which traits which were observed in the previous chapter (Chapter 3).

These results indicate that simulated C&R of Atlantic salmon parents, 5 - 18 days prior spawning could have effects on the behaviour of the offspring during the early stages of life when they are establishing feeding territories. Mortality is high and competition intense during this period, since they tend to have a very short dispersal distance from the spawning grounds and so occur at high densities during the first summer of life (Einum et al., 2011; Brunsdon et al., 2017). Even though a lot of research effort has been spent identifying the effects of stressors on the adults, there are still many gaps in knowledge on how the same stressors affect the next generation of offspring (Richard et al., 2013; Redfern at al., 2017). This is especially important in the context of anthropogenic stressors, since the way the offspring react to these is what will determine if a population will continue to survive in a changing world.

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4.8 Supplementary Information

Table S4.1 Summary of all final General Linear Models (GLM) used to investigate the effects that the adult C&R simulations had on the offspring's behaviour.

Variable of Interest	Model used
Risk-taking Traits	lm(Percentage Emergence ~ Treatment + Mass + Fungal Spread – Parent + YSV)
Activity	lm(Percentage Activity ~ Treatment + Parental Sex + Mass + Fungal Spread – Parent + YSV)
Exploration of Novel Environment	lm(Exploration ~ Treatment * Mass + Fungal Spread – Parent + Parental Sex)
Total Attacks	lm(log((Total Attacks)+10) ~ Treatment + Length)
Direct Attacks	lm(log((Percentage Forward Attacks)+10) ~ Treatment * Length + Total Time)
Sneak Attack	lm(log(Percentage Side Attacks+10) ~ Treatment + Total Time + Mass + Date of the Trial)

Chapter 5 - Simulated C&R Affects the Pairwise Dominance of Offspring

5.1 Summary

Variability in metabolic rate is of ecological importance, as it is associated with traits that affect the life history and success of an individual. One such positive association is between the metabolic rate of an individual animal and its dominance status. Individual juvenile salmonid fish that are dominant in an environment tend to obtain the most profitable feeding territories, and so may grow faster. This investigation studies how simulated catch and release (C&R) angling of adult Atlantic salmon shortly before spawning affects the pairwise dominance and metabolic traits (SMR, MMR and AS) of their offspring. Wild adult salmon collected from the river Blackwater, N. Scotland, were subject to a disturbance protocol meant to simulate two of the main stressors encountered by fish during angling, namely exercise and exposure to air. To achieve this, equal numbers of male and female adults underwent one of four treatments that comprised different levels of exercise and air exposure. The experimental parent was then mated with an unstressed counterpart, and the offspring were allowed to develop under controlled conditions. Measurements of metabolic rate and dominance status in pairwise contests for feeding territories were then collected at 3-4 and 4-5 months after first feeding respectively. Results showed that offspring from parents that were exercised but that did not experience air exposure showed a lower maximum metabolic rate and aerobic scope compared to control fish. Moreover, offspring of control parents tended to be dominant over offspring of disturbed parents on the first 2 days of the feeding trials, but not on the third. No difference in dominance status was found between the offspring from the Exercise and Exercise + Extended Air Exposure treatments. These outcomes suggest that offspring of fish that have not experienced C&R angling close to spawning may have an advantage when it comes to establishing the most profitable feeding territories in the wild.

5.2 Introduction

Variation among individuals in their metabolic rate is often linked to variation in their behaviour and/or physiology (i.e. activity, aggression, territoriality, social

interactions, oxidative stress; Hoogenboom et al., 2013; Dijkstra et al., 2015; Eliason and Farrell, 2016). This variability is thus of ecological relevance as it affects the life history of individuals. Many species, including salmonids, demonstrate a strong positive association between individual metabolic rate and dominance status (Burton et al., 2011b; Hoogenboom et al., 2013; Mathot et al., 2015; Metcalfe et al., 2016; Sanchez-Gonzales and Nicieza, 2021). Most of the research in this area has been done by examining the ability of individuals to obtain food and establish feeding territories and then relating their probability of success to their metabolic rate relative to that of their competitors (Metcalfe et al., 2016). Being dominant can lead to the establishment of a better feeding territory and acquisition of shelter, and thus may improve survival (Reid et al., 2011; Sanchez-Gonzales and Nicieza, 2021). Gilmour et al., (2005) showed that dominant juvenile rainbow trout (*Oncorhynhus mykiss*) exhibit higher aggression toward subordinate conspecifics, and acquire the largest quantity of food (Gilmour et al., 2005). In a resource-limiting environment a higher standard metabolic rate (SMR, the minimal level of oxygen consumption while at rest) can be either advantageous, since it can assist individuals to obtain the best territories, or detrimental as it can be energetically costly when food availability is restricted (Reid et al., 2011; Hoogenboom et al., 2013; Metcalfe et al., 2016). The cost-benefit ratio of a given metabolic rate is thus context-dependent.

The metabolic rate of an animal is partly influenced by parental (i.e. non-genetic) effects, including those associated with stress (Burton et al. 2013). There is evidence that exposing fish eggs directly or through the mother with exogenous stress hormones and stressors can affect the physiology and behaviour of offspring. Brown trout eggs exposed to cortisol for a total of 3 h prior to fertilization exhibited higher rates of oxygen consumption while at the developmental egg stage (Sloman, 2010). Furthermore, Eaton et al., (2015) showed that female guppies (*Poecilia reticulata*) subjected to a mild stressor produced offspring that were more aggressive.

The consequences of any changes in average levels of aggression or dominance as a result of parental stress are not always clear. Displaying elevated aggression, particularly when emerging into a novel or unpredictable environment similar to that experienced by first-feeding fish, can be advantageous as it offers a

competitive edge for obtaining territories and resources (Sopinka et al., 2014; Dijkstra et al., 2015). Exhibiting higher aggression, however, can come at the cost of higher metabolic expenditure, as there can be a positive association between SMR and the level of aggression that an individual will express (Reid et al.,2011; Dijkstra et al., 2015). Nonetheless, several investigations of juvenile salmonids (rainbow and brown trout) have indicated that fish with cortisol levels higher than control individuals are not only less aggressive, but also socially subordinate with a higher probability of losing competitive encounters (Sloman et al., 2001; Overli et al., 2002; DiBattista et al., 2005; Schjolden et al., 2009; Burton et al., 2011a). Furthermore, the swimming performance can also be altered in offspring whose mothers experience a stressor such as being chased (Sopinka et al., 2014). For instance, sockeye salmon (*Oncorhynchus nerka*) fry originating from eggs laid by disturbed mothers (chased twice a day with a net) can recover from burst swimming activity faster but can only swim for short durations of time (Sopinka et al. 2014). These influences indicate that parental stress can affect offspring phenotype through several different mechanisms. In fish, there is limited knowledge about the subtle, long-term effects that parental stress can cause on the relationship between behaviour and physiology (Sloman, 2010). This is particularly true in semelparous individuals that are less able to postpone reproduction even when conditions are harsh (Sopinka et al., 2014). Combining various effects that arise from parental exposure to stressors in a natural or semi-natural setting can provide a more complete picture of how the biotic and abiotic factors can have a cumulative effect on future generations (Sopinka et al., 2014).

Adult salmon can experience several anthropogenic stressors during their journey from the ocean to the spawning grounds. Anthropogenic stressors have been shown in various species to affect the phenotype of the offspring (Eriksen et al., 2006; Eriksen et al., 2007; Schreck, 2010; McGhee et al., 2012; Eriksen et al., 2013; Madaro et al., 2015; Sopinka, 2015; Atherton and McCormick, 2020). Here I investigate how parental pre-spawning stress from a catch and release (C&R) simulation of wild Atlantic salmon can affect the performance (metabolic rate and dominance) of the offspring. In doing so, I measured the SMR, maximum metabolic rate (MMR – the greatest possible utilisation of oxygen by an organism in an environment at a specific temperature) and aerobic scope (AS – the capacity/ range through which an organism can increase its metabolic rate; AS = MMR – SMR)

of the offspring of the experimentally treated parents. Dominance status was measured through several sets of feeding trials involving competition for resources. Both metabolic rate and dominance were compared among offspring of parents that had experienced differing level of stress associated with C&R. Based on the results of Sloman (2010) and Eaton et al. (2015), I predicted that offspring from disturbed experimental parents (male or female) would have a higher metabolic rate (SMR, MMR and AS) than offspring of non-disturbed (control) parents. Additionally, given the commonly observed positive association between metabolic rate and dominance status (Mathot et al., 2015; Metcalfe et al., 2016; Sanchez-Gonzales and Nicieza, 2021), I predicted that dominance status would be positively correlated with the level of disturbance that the experimental parent of the offspring experienced during the C&R simulation.

5.3 Methods

5.3.1 Adult Collection and Treatments

Wild Atlantic salmon were collected from the river Blackwater, N. Scotland during the spawning migration between the months November to December 2018. Collection was achieved using a fish trap set-up by the Cromarty Firth Fisheries Board. Both female and male adult salmon [n = 120; 15 fish per treatment (x4) per sex (x2)] were assigned at random to one out of four experimental treatments, which were designed to simulate the stressors that a fish would experience during a normal catch and release (C&R) angling event. The treatments comprised of different intensities of exercise and air exposure (Struthers et al., 2018; Smukall et al., 2019; Table 5.1; Figure 5.1; Chapter 2) aimed at replicating the exhaustion caused initially by fighting the rod, potentially followed by exposure in air while the angler removes the hook and photographs or examines the fish, but excluding any stress or physical damage that might be caused by the hook itself. Females were selected based on the condition that they had not released their eggs in the body cavity prior to the start of the C&R simulation. All experimental fish, male and female, were left 24 – 48 h undisturbed in their tanks (diameter: 4 m, height: 1.5 m, water depth: 0.18 m; water temperature: 6 ± 1.5 ^{o}C – directly supplied from the river Blackwater) to recover from the stress of confinement and handling while being retrieved from the trap. To further minimize stress and handling, all

fish transfers were conducted by carrying fish individually in water-filled plastic bags. Furthermore, the pre-determined treatments were applied to the fish 5 – 18 days prior to spawning, with the time of spawning being determined by a female having released eggs into the body cavity. It should be noted that the study took place after the end of the normal angling season in Scotland, although angling during this period is still permitted for brood stock hatchery purposes in Scotland, and for recreational activities in other areas.

External artificial fertilization was performed on a one-to-one ratio of experimental (60 females, 52 males) to non-experimental fish. The final number of crossings conducted was 112 (see chapter 3 for details). The gametes were carefully mixed and placed in water for about 60 mins, for the eggs to swell and the shell to harden. After having been stripped, the adults were released back into the loch, upstream from the trap. The hardened eggs were then incubated, in individual family trays, at the Scottish and Southern Energy (SSE) hatchery in Contin.

Table 5.1 Summary of the four C&R simulations and their cumulative levels of disturbance (indicated by the number of asterisks); see Chapter 2 for full description of treatments.

Treatment	Exercise	Air exposure	Cumulative disturbance
Control	No	No	
Exercise	210 s	No	*
Exercise + Air Exposure	210 s	60 s	**
Exercise + Extended Air Exposure	210 s	120 s	***

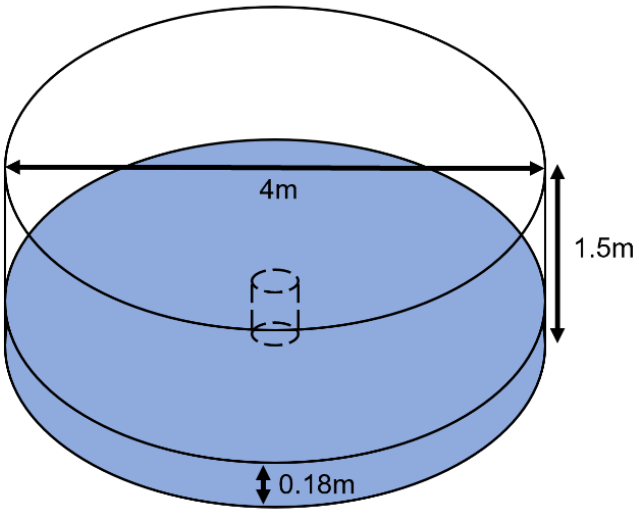

Figure 5.1 Diagram of the arena used to conduct the C&R simulations on the adult Atlantic salmon. Presented are the dimensions of the arena used for the exercise protocol, where fish were physically chased for 120 s (0 s for controls). Fish were encouraged to continuously swim by gently tapping their tail or sides by hand. Air exposure (0, 60 or 120 s) was then conducted by manually lifting fish out of the water using a knotless hand-net, after the exercise protocol. Even though control fish were neither exercised or air-exposed, they were still transferred (in water-filled bags) between pre- and post- holding tanks. Water (6 ± 1.5 ^{o}C) to the arena was supplied by the river Blackwater.

5.3.2 Offspring Transport and Maintenance

On April 9th, 2019, a total of 1,400 alevins (25 individuals per family; hatched offspring with a yolk sac present) from 56 families (7 per treatment per sex) randomly (Number generator random; version 2.0) selected families were transported to the University of Glasgow out of the initial 112 families. The subset of families was selected due to the limited availability of space at the University facilities. Here the offspring were housed in a single recirculating stream system (water flow = 0.988 L/s) in the dark at a low water temperature (7 ^{o}C), with each family being randomly allocated to a single compartment (dimensions: 19 x 13 cm; water depth: 15.5 cm). When the offspring began active feeding and their yolk sacs were depleted, the temperature of the water was gradually raised to 12 ^{o}C over a period of 2 months (May – June). The temperature and rate of increase was set to replicate natural stream conditions. Moreover, the photoperiod was switched to a 12L:12D (8:00 – 20:00) cycle. Furthermore, from first feeding

(28/4/19 – 16/5/19, depending on family) up to July 2nd, the offspring were fed with commercial salmon starter crumb pellets, and then with small bloodworms. Feeding was scheduled 3x per day (9:00, 13:00 and 17:00) until June 9th, and thereafter 2x a day (9:00 and 17:00) until July 1st. Lastly, from July 2nd and onwards the offspring were fed 1x a day, at noon. All compartments were fed to excess, with the remaining food and faeces cleaned daily by siphon.

5.3.3 Maximum and Standard Metabolic Rate, and Aerobic Scope

Between July 16th and August 18th, 2019, when the fish were approximately 3 months old, the metabolic rates of representatives from each family (2 – 8 per family) were measured at the end of day when the behavioural tests were performed (see chapter 4). The maximum (MMR) and standard (SMR) metabolic rate of the offspring were estimated from the rate of oxygen uptake using an intermittent flow-through respirometry (Masterflex L/S, Cole parmer, model 7534-06). All fish were fasted for 24 hrs prior to measurements. The metabolic assays were conducted on a total of 408 fish (Control: 112; Exercise: 98; Exercise + Air: 108; Exercise + Extended Air: 90), but data from 19 of these (Control: 3; Exercise + Air: 14; Exercise + Extended Air: 2) were excluded from the analysis due to technical issues during measurements of oxygen uptake. The assays were performed in 16 identical glass chambers (18 mL), submerged in an aerated, temperature-controlled water bath (80 × 40 × 29 cm; 92.8 L; 13 ^{o}C ± 0.5), with the water being recirculated using peristaltic pumps and gas impermeable tygon tubing (11.3 ± 3.3 mL; volume of tubing in the recirculation circuit was included in the calculation of final respirometer volume). Bacterial oxygen consumption was minimized by passing the water through a UV filter sterilizer, and by fully bleaching the system once (and rinsing with fresh water 3x) the morning before each trial. Additionally, bacterial oxygen consumption was measured before and after each trial for a duration of 1 cycle (2 min flush: 8 min closed). Oxygen uptake per chamber was measured every 2 s using a fibre optic sensor that was placed inside the probe flow-through cells and was connected to a four-channel Firesting O2 system (PyroScience). The probes were calibrated using a two-point system (0 % and 100 % saturation) at the start of the experiment in July, as well as once during the first week of August. The 100 % calibration was conducted using highly aerated water, while the 0 % calibration was set using a solution of sodium sulphite in sodium tetraborate. Furthermore, a single-point calibration (100 % saturation)

was performed on the same sensors on a daily basis before the start of the trials. The flow cycle was operated by a flush pump connected to a timer that was set to 2 min flushing and an 8 min closed phase. During flushing the water used was from a fully aerated water bath kept at 100 % saturation, which returned the chambers to normoxic conditions. To achieve MMR the fish were exercised (continuous swim by gently tapping the tail of the fish by hand) to exhaustion (3 mins) in a circular bucket (water depth = 2.5 ± 0.5 cm), and then immediately (< 10 s) transferred into the glass chambers, without any additional air exposure, to measure their oxygen consumption rate (mg O_2 h^{-1}). MMR was calculated in Excel using the first flow cycle. This was achieved by running rolling regression slopes every 1 s, after a 30 s wait period at the start, and a 3 min exclusion period at the end. Each rolling regression lasted 2 min. The line with steepest slope was used for the MMR. The fish were then left in the respirometer overnight (18 h 6 min ± 1 h 26 min; 108 ± 6 slopes) to allow the measurement of the SMR (mg O_2 h^{-1}) (Killen et al., 2017), which was estimated using the lowest 10^{th} percentile of recordings. SMR was calculated in R (Version Ri386 3.4.4) using a script and the package FishResp. Oxygen saturation did not fall below 65 % during the first cycle (where the MMR was measured), or below 80 % after the first cycle (measurements used for the SMR). Once all the fish were in the chambers, a black tarp, which was not removed until the next day, was placed on top of the respirometer to shield them from any external disturbance (so that measurements were conducted in the dark). No additional barriers were placed between the fish while in the chambers. The difference between absolute MMR and SMR was used to calculate the absolute aerobic scope (AAS; the boundaries within which aerobic activities can take place) of the individuals. The R^2 was above 95 %, except on 3 occasions when it was above 90 %. The fork length (Electronic Calliper Waterproof IP67, 0-150 mm; to nearest 0.01 mm) and mass (Ohaus E01140 Explorer Analytical Balance; to the nearest 0.1 mg) of the fish were measured after respirometry. Mass (average: 0.549 g; range: 0.132 – 1.659 g) of the fish was used as an explanatory variable for both the MMR and SMR during the General linear models run in R (see statistics). Furthermore, the mass of the fish was subtracted from the volume of the respirometer when calculating the O_2 uptake. Following recommended practice (Killen et al., 2021), a checklist of the essential respirometry criteria (Table S5.2) and the log of the MMR, SMR, AS and mass of individual fish (Table S5.3) can be found at the supplementary information.

5.3.4 Dominance Trials

Fish (n = 255; Control: 96, Exercise: 82, Exercise + Extended Air: 77) were randomly selected from 40 families (Control: 14; Exercise: 13; Exercise + Extended Air: 13) and anesthetized using benzocaine, weighed (mass: 1.182 ± 0.769 g), measured (fork length: 46.085 ± 11.415 mm), and given their own individual visible implant elastomer tag (coloured ink-like tags that are injected under the fish's skin, but which are still visible to the observer). This process took place between August 22nd – 26th, 2019, when the fish were approximately 14 weeks old. Then after a seven-day recovery period, the dominance status of the fish was tested by placing them in pairs in arenas (dimensions: 19 x 13 cm; water depth: 15.5 cm) situated in a recirculating stream system (water flow = 0.988 L/s; Figure 5.2), that had a glass panel on one side (for behavioural observations). The size of the arenas was only sufficient for one fish to hold a territory (based on the relationship between juvenile salmon size and territory size (Grant & Kramer 1990), so inducing the two fish to compete for ownership. Each control (A) fish was paired against one individual of each of the 'Exercise' (B) or 'Exercise + Extended Air' (D) families (i.e. A1 vs B1/ A1 vs B2/ …. / A1 vs B7 and then A1 vs D1/ A1 vs D2 ….. A1 vs D7). The matching pairs and series between Control vs Exercise (A vs B) & Control vs Exercise + Extended Air (A vs D) was randomly and equally mixed so that the control group didn't have an unfair advantage during the second set of matches. During the experiment, a total of 211 dominance trials (Control vs Exercise: 83; Control vs Exercise + Extended Air: 77; Exercise vs Exercise + Extended Air: 51) took place between September 2nd and October 4th.

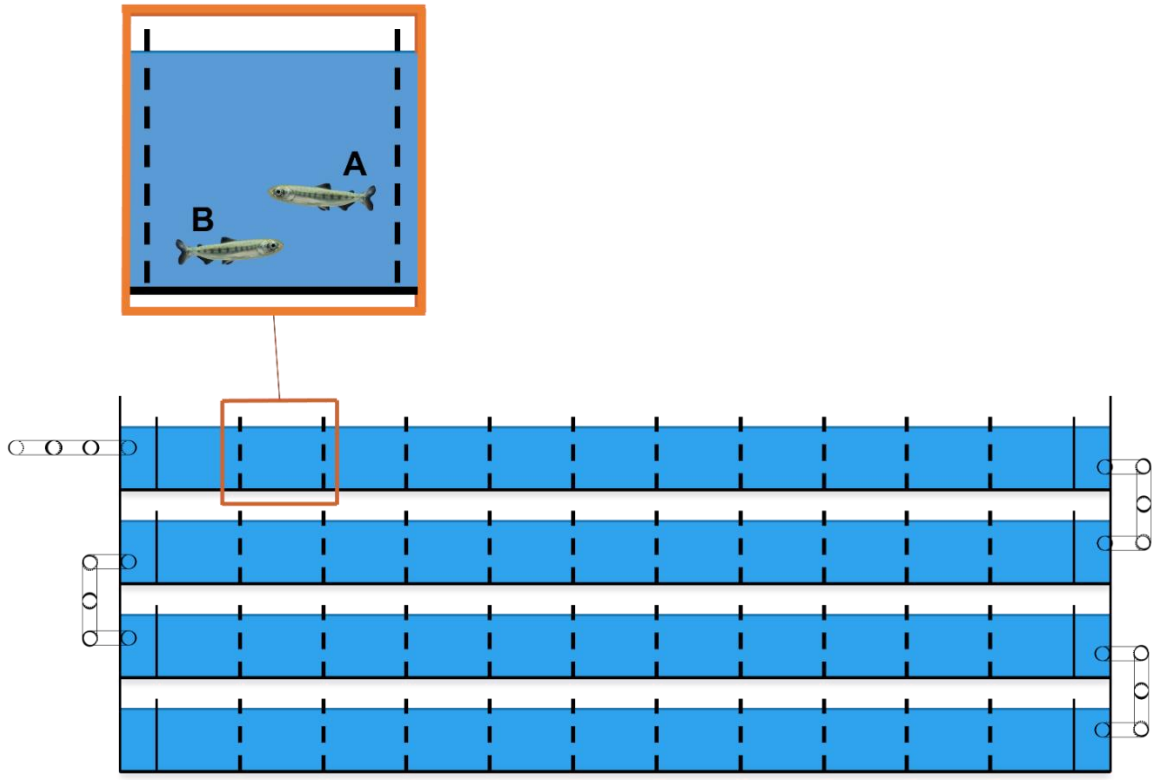

Figure 5.2 Diagram of the arena set up used to conduct the dominance trials. Each arena held 2 fish, 1 fish from each treatment (depending on the treatments running at the time). The possible combinations were Control vs Exercise, Control vs Exercise + Extended Air, and Exercise vs Exercise + Extended Air.

On the first day (day 0) of the trials, the fish were placed within their assigned arenas. Then between day 1-3 the dominance trials were run. Following previous studies, dominance was calculated by combining scores for both position in the arena and ability to obtain food (Harwood et al., 2003; Metcalfe et al., 2003). Each feeding score was based on introducing a single bloodworm at the upstream end of each arena using forceps, and recording which fish took the food item. This was repeated a total of 21 times over a three-day period (7 trials per day, with a 30 min interval between each feed). Each pair of fish was then scored on their initial position within the arena (right before the introduction of each food item) as well as their ability to obtain the food item (Table 5.2), in a similar manner to that used by Harwood et al. (2003). Dominance was assigned at the end of each day to the fish that obtained at least a 5-point difference in score. If the score difference between the two fish was less than 5 points, then no dominance classification was assigned to the pair (i.e. the relationship was defined as inconclusive). Once the 3-day trial had ended, an overall dominance outcome was

also assigned to the pair. Similar to before, this was achieved by adding all the points over the whole trial period, and the individual with at least a 5-point difference was assigned as dominant. If the difference in score was less than 5 points, then no dominance was assigned. At the end of each day, all fish occupying the experimental arenas were fed to excess using bloodworms so as to prevent changes in nutritional state or feeding motivation over the course of a trial from affecting the outcome of contests. Once a set of experimental trials was completed (day 4), the fish were re-weighed and their fork length was measured. The fish were then returned to their original compartments (family compartment).

Table 5.2 Summary of the procedure used for the dominance trials and the reward point system. The feeding procedure was repeated 21 times over a three-day period (7 times per day, with a 30 min interval between each feed).

		Action	Description	Points
Day 0	Place fish in the arena			N/A
Day 1 – 3	Dominance trials	Holding the most profitable position in the arena	The nearest central position downstream of where the bloodworm was released	+ 1
		Obtaining uncontested food item	Obtaining the bloodworm without the other fish challenging for it	+ 1
		Obtaining contested food item	Obtaining the bloodworm when the other fish challenged for it	+ 2
Day 4	Measure fish and return to their initial compartment (family compartment)			N/A

5.4 Statistics

General Linear Models (GLMs) were run in R (Version Ri386 3.4.4) to examine whether the C&R simulation of the parents affected physiological traits in the

offspring. GLMs were used to examine whether the C&R simulations on the parents affected the offspring's maximum metabolic rate (MMR), standard metabolic rate (SMR), and aerobic scope (AS). The main explanatory variable in the models was the C&R simulation that the experimental parent experienced (treatment), and the logarithmic transformation of offspring mass (log(Mass)). Moreover, all models included the experimental parents' sex, and the Julian date that the respirometry was conducted as possible explanatory variables.

Dominance amongst the Control, Exercise, and Exercise + Extended Air groups was analysed by running logistic regressions in R. In order to avoid pseudoreplication only one fish was used from each pair in each logistic regression. Thus the outcome for (a randomly selected) half of the pairs from each competition (i.e. Control vs Exercise) were considered from the perspective of one of the competitors (i.e. Control) and the other half from the other competitor (i.e. Exercise). Separate logistic regressions were run on the data from each observation day (Day 1, Day 2, and Day 3) as well as using the data on overall dominance over the whole 3-day period. These analyses were run separately for contests between Control vs Exercise fish, Control vs Exercise + Extended Air, and Exercise vs Exercise + Extended Air. The main explanatory variable in the models was the C&R simulation that the experimental parent experienced (treatment). However, all models also included as possible explanatory variables the experimental parents' sex and the offspring's length. The significance of the variables in the final models was established using p-values (with $p = 0.05$ taken as the threshold for significance). If a categorical variable was found to be significant, the categories were investigated further using a Tukey multiple comparison of means. Homogeneity of variance across treatments was determined by running a Levene's tests for all final models. A summary of all the final models can be found in the supplementary information (Table S5.1).

5.5 Results

5.5.1 MMR, SMR and AS

In all three metabolic measurements, there was a positive relationship between metabolic rate and log fish mass. After controlling for this, the SMR of offspring

was unaffected by parental treatment (Table 5.3; Figure 5.3). However, both the MMR and AS in the offspring whose parents had been only exercised was lower compared to that of control offspring (Table 5.3; Figure 5.3). Furthermore, offspring whose affected parent was the father had a lower SMR, MMR, and AS compared to those whose affected parent was the mother (Table 5.3).

Table 5.3 General Linear Model (GLM) investigating the effects of pre-spawning stressor associated with C&R angling simulations of the parents on (a) the standard metabolic rate (SMR), (b) maximum metabolic rate (MMR) and (c) Aerobic scope (AS) of the offspring, each time correcting for offspring mass (log(Mass)). Shown are the comparisons of each of the three C&R treatments to the control, together with the effects of the sex of the experimental parent (i.e. whether it was the mother or father that was subjected to the experimental treatment).

	d.f.	Estimate	Std. Error	t	p
a. Standard Metabolic Rate (SMR)					
Intercept		0.179	0.003	62.394	<0.001
Exercise	1	-0.002	0.003	-0.556	0.58
Exercise + Air	1	-0.001	0.003	-0.333	0.74
Exercise + Extended Air	1	0.006	0.003	0.195	0.85
log(Mass)	1	0.095	0.003	36.411	<0.001
Parental Sex – Male	1	-0.008	0.002	-3.669	<0.001
Residuals	**376**				
b. Maximum Metabolic Rate (MMR)					
Intercept		0.572	0.010	59.286	<0.001
Exercise	1	-0.021	0.010	-2.165	0.03
Exercise + Air	1	0.006	0.010	0.623	0.53
Exercise + Extended Air	1	-0.009	0.010	-0.872	0.38

log(Mass)	1	0.310	0.009	35.332	<0.001
Parental Sex – Male	1	-0.028	0.007	-3.978	<0.001
Residuals	**376**				
c. Aerobic Scope (AS)					
Intercept		0.393	0.009	44.940	<0.001
Exercise	1	-0.019	0.009	-2.207	0.03
Exercise + Air	1	0.007	0.009	0.797	0.42
Exercise + Extended Air	1	-0.009	0.009	-1.027	0.31
log(Mass)	1	0.215	0.008	27.037	<0.001
Parental Sex – Male	1	-0.021	0.006	-3.186	0.002
Residuals	**376**				

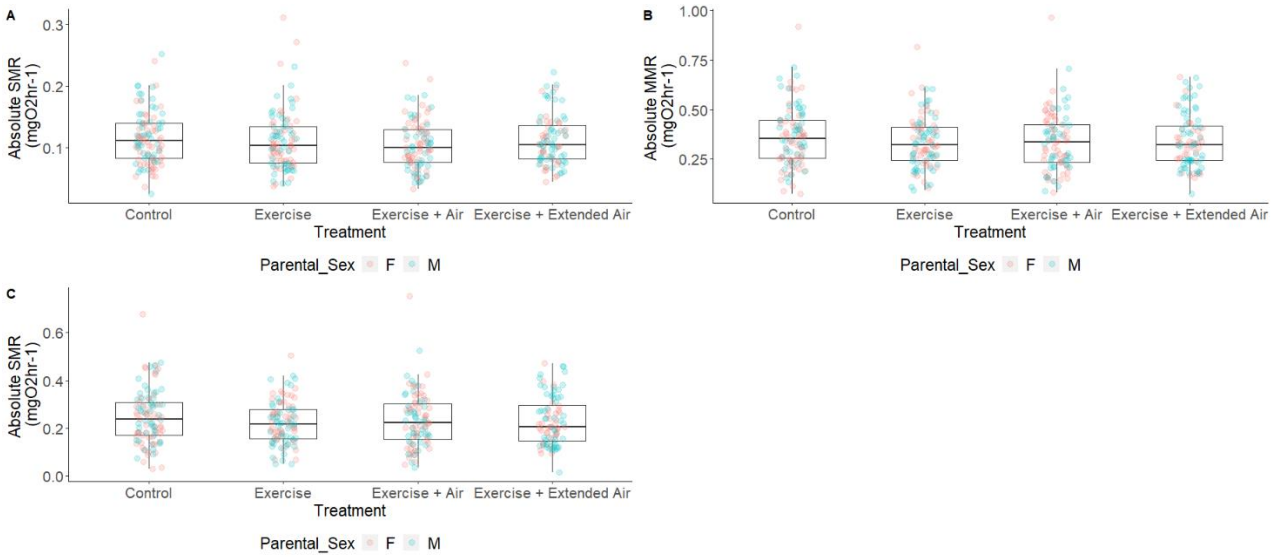

Figure 5.3 Effects of the C&R simulation of the parents on (a) the absolute standard metabolic rate (SMR) (b) the absolute maximum metabolic rate (MMR), (c) the absolute aerobic scope (AS) (see Table 5.3 and Table 5.4 for statistical analysis). The blue shade represents offspring from male experimental parents and the red shade represents offspring from female experimental parents.

5.5.2 Dominance Trials

5.5.2.1 Control vs Exercise

Offspring from the control treatment were on average dominant over the offspring from the exercise treatment during the overall 3-day trial (Table 5.4; Figure 5.5). When the dominance interactions were broken down per day, it was observed that fish from the control group were dominant during day 1 and 2, but there was no clear trend for dominance on day 3 (Table 5.4; Figure 5.4).

Table 5.4 Logistic regressions investigating the effects of pre-spawning stressors associated with C&R angling simulations of the parents on the dominance interactions amongst their offspring. This analysis only considers contests between offspring from the control and exercise group. Presented are the dominance results for each observation day of the experiment (Day 1, Day 2, and Day 3), and as an overall result over the 3-day period.

	d.f.	Estimate	Std. Error	z	p
Day 1					
Intercept		-1.913	2.751	-0.696	0.49
Competitors: Exercise vs Control	1	-1.284	0.561	-2.288	0.02
Control Fish Length	1	-0.015	0.045	-0.333	0.74
Exercise Fish Length	1	0.063	0.040	1.552	0.12
Residuals	**57**				
Day 2					
Intercept		1.326	2.535	0.523	0.60
Competitors: Exercise vs Control	1	-1.026	0.515	-1.991	0.047
Control Fish Length	1	-0.004	0.041	-0.089	0.93
Exercise Fish Length	1	-0.016	0.035	-0.471	0.64
Residuals	**66**				
Day 3					
Intercept		0.888	2.548	0.349	0.73
Competitors: Exercise vs Control	1	-0.701	0.503	-1.393	0.16
Control Fish Length	1	-0.033	0.042	-0.804	0.42

Exercise Fish Length	1	0.013	0.034	0.394	0.69
Residuals	**68**				
Overall (Day 1 – Day 3)					
Intercept		0.704	2.514	0.280	0.78
Competitors: Exercise vs Control	1	-1.020	0.513	-1.987	0.047
Control Fish Length	1	-0.012	0.041	-0.302	0.76
Exercise Fish Length	1	0.005	0.035	0.131	0.90
Residuals	**65**				

5.5.2.2 Control vs Exercise + Extended Air

As with the previous comparison, offspring from the control group were on average dominant over those whose parents experienced both exercise and extended air exposure during the overall 3-day trial (Table 5.5; Figure 5.5). When dominance was examined per day, it was revealed that offspring from the control parents were dominant during days 1 and 2, but no clear dominance was observed on day 3 (Table 5.5; Figure 5.4).

Table 5.5 Logistic regressions investigating the effects of pre-spawning stressors associated with C&R angling simulations of the parents on the dominance interactions amongst their offspring. This analysis only considers contests between offspring from the control and exercise + extended air group. Presented are the dominance results for each observation day of the experiment (Day 1, Day 2, and Day 3), and as an overall result over the 3-day period.

	d.f.	Estimate	Std. Error	z	p
Day 1					
Intercept		-0.032	3.352	-0.010	0.99
Competitors: Exercise + Extended Air vs Control	1	-1.227	0.618	-1.987	0.047
Control Fish Length	1	0.029	0.056	0.522	0.60
Exercise + Extended Air Fish Length	1	-0.008	0.051	-0.161	0.87
Residuals	**48**				
Day 2					
Intercept		0.208	3.022	0.069	0.95
Competitors: Exercise + Extended Air vs Control	1	-1.257	0.563	-2.235	0.03
Control Fish Length	1	0.014	0.046	0.292	0.77
Exercise + Extended Air Fish Length	1	-0.001	0.048	-0.012	0.99
Residuals	**54**				
Day 3					
Intercept		-2.019	2.747	-0.735	0.46

Competitors: Exercise + Extended Air vs Control	1	-0.663	0.523	-1.266	0.21
Control Fish Length	1	0.052	0.045	1.171	0.24
Exercise + Extended Air Fish Length	1	0.001	0.048	0.024	0.98
Residuals	**59**				
Overall (Day 1 – Day 3)					
Intercept		-0.646	3.016	-0.214	0.83
Competitors: + Extended Air vs Control	1	-1.241	0.560	-2.217	0.03
Control Fish Length	1	0.035	0.048	0.719	0.47
Exercise + Extended Air Fish Length	1	-0.004	0.047	-0.094	0.93
Residuals	**54**				

5.5.2.3 Exercise vs Exercise + Extended Air

Parental treatment had no effect on the overall dominance between offspring from the exercise versus exercise + extended air groups (Table 5.6; Figure 5.5). Moreover, when dominance was broken down into individual observation days, there was no difference in dominance between the two treatment groups on any day (Table 5.6; Figure 5.4).

Table 5.6 Logistic regressions investigating the effects of pre-spawning stressors associated with C&R angling simulations of the parents on the dominance interactions amongst their offspring. This analysis only considers contests between offspring from the exercise and exercise + extended air group. Presented are the dominance results per day of observation (Day 1, Day 2, and Day 3), and as an overall result over the 3-day period. The experimental parents' sex was also included as an explanatory variable.

	d.f.	Estimate	Std. Error	z	p
Day 1					
Intercept		0.990	3.788	0.261	0.79
Competitors: Exercise vs Exercise + Extended Air	1	1.216	0.647	1.880	0.06
Exercise Fish Length	1	-0.009	0.045	-0.199	0.84
Exercise + Extended Air Fish Length	1	-0.029	0.053	-0.540	0.59
Residuals	**43**				
Day 2					
Intercept		-0.144	3.943	-0.037	0.97
Competitors: Exercise vs Exercise + Extended Air	1	0.988	0.697	1.417	0.16
Exercise Fish Length	1	0.040	0.056	0.718	0.47
Exercise + Extended Air Fish Length	1	-0.053	0.058	-0.913	0.36
Residuals	**39**				
Day 3					
Intercept		-2.122	3.791	-0.560	0.58

Competitors: Exercise vs Exercise + Extended Air	1	0.569	0.699	0.814	0.42
Exercise Fish Length	1	0.047	0.050	0.943	0.35
Exercise + Extended Air Fish Length	1	-0.027	0.054	-0.491	0.62
Residuals	**38**				

Overall (Day 1 – Day 3)

Intercept		-0.284	3.913	-0.073	0.94
Competitors: Exercise vs Exercise + Extended Air	1	0.911	0.696	1.309	0.19
Exercise Fish Length	1	0.031	0.054	0.580	0.56
Exercise + Extended Air Fish Length	1	-0.042	0.056	-0.751	0.45
Residuals	**40**				

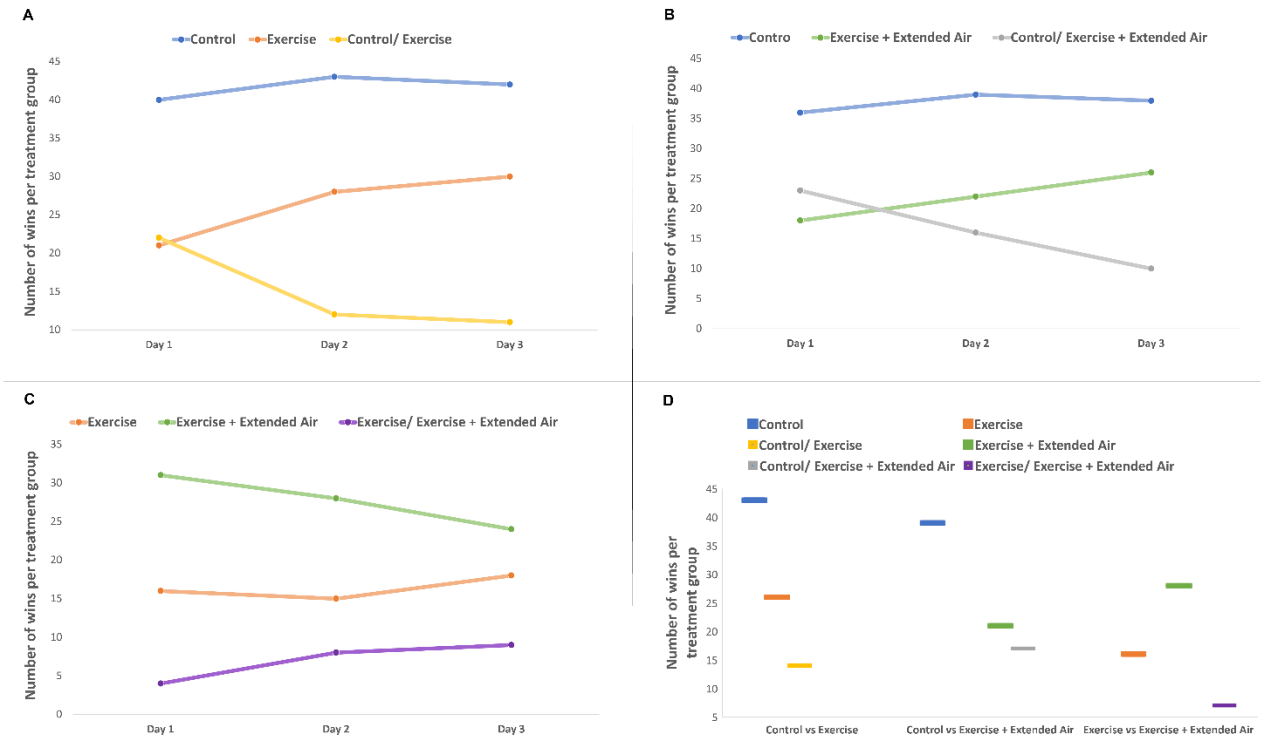

Figure 5.4 Changes in the offspring dominance across the duration of the trials. A. Trials of Control vs Exercise offspring, B. Trials of Control vs Exercise + Extended Air, C. Trials of Exercise vs Exercise + Extended Air, and D. Dominance over the 3-day period for all combinations of trials. Control: The dominant offspring was from the control group, Exercise: The dominant offspring was from the exercise group, Exercise + Extended Air: The dominant offspring from the exercise + extended air group. Control/Exercise: There was no clear dominance between the control and exercise group, Control/Exercise + Extended Air: There was no clear dominance between the control and exercise + extended air group, and Exercise/ Exercise + Extended Air: There was no clear dominance between the exercise and exercise + extended air group.

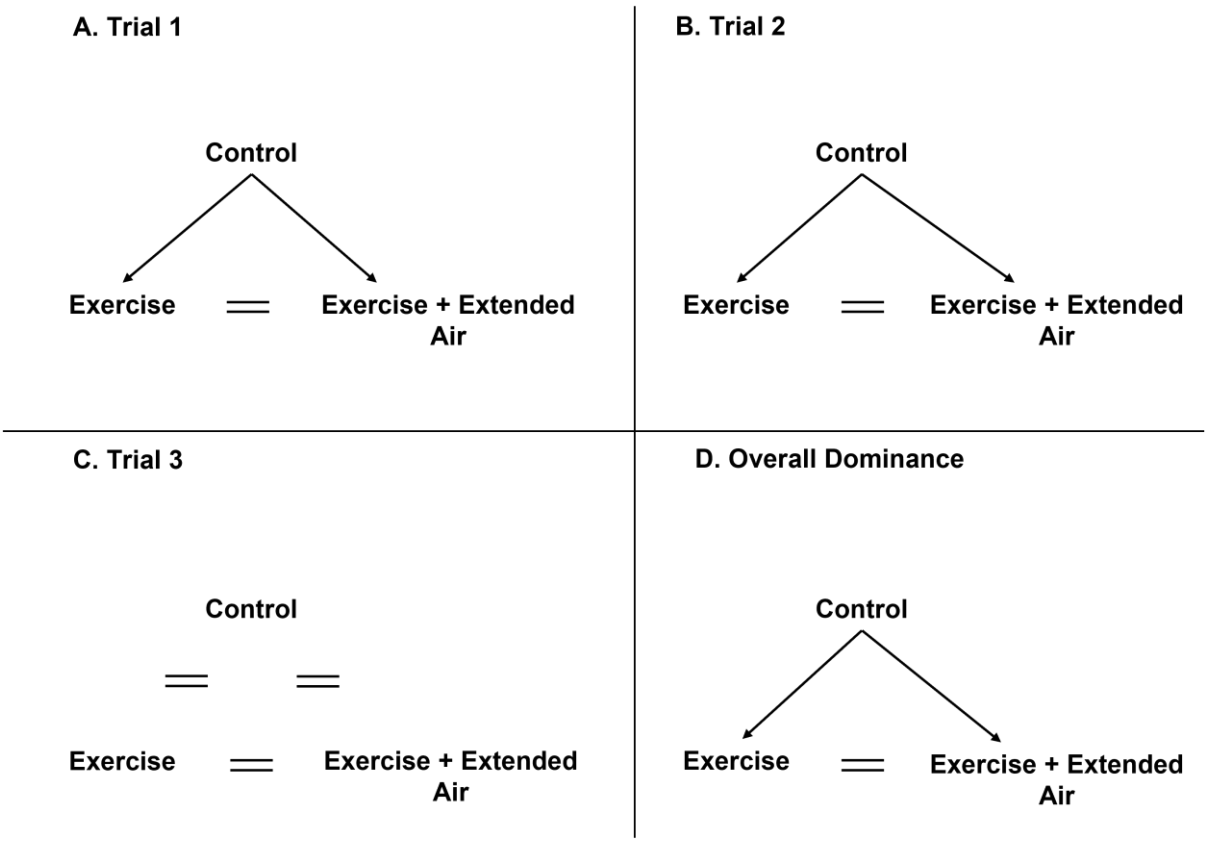

Figure 5.5 Summary of the results from the dominance trials. A. Trial 1 (day 1) of the 3-day trial, B. Trial 2 of the 3-day trial, C. Trial 3 of the 3-day trial and D. Overall dominance over the 3-days. Arrows indicates the direction of dominance, while the equal sign indicates no clear dominance between the two categories of fish.

5.6 Discussion

The results suggest that overall, the metabolic rate of offspring was mostly unaffected by whether parents were exposed to disturbances similar to what they would normally experience during a C&R angling event. However, while the SMR (standard metabolic rate) of the offspring was unaltered by parental treatment, offspring whose parents were exposed to exercise (but not air exposure) had both a lower MMR (maximum metabolic rate) and lower AS (aerobic scope). Moreover, there was an effect of the parental treatments on offspring dominance status, with a significant tendency for offspring from the 'Control' group to be dominant over offspring from either the 'Exercise' or the 'Exercise + Extended Air' groups.

Lastly, there was no clear difference in dominance between offspring from these latter two treatment groups.

The metabolic phenotype (Metcalfe et al., 2016) of Atlantic salmon offspring was largely unaffected by the parental treatment. The SMR, which is the minimum amount of energy required by an organism to stay alive at a specific temperature (Metcalfe et al., 2016), was similar across treatments. This suggests that it is not affected by parental stress, a similar finding to that of Burton et al. (2011b). In contrast to this findings, Sloman (2010) showed that increased cortisol concentrations within the eggs of brown trout led to an increase in the SMR of the offspring prior to hatching. Moreover, a study using brown trout found that the SMR of individual fish was linked to their dispersal after hatching (Sanchez-Gonzalez and Nicieza, 2021). Thus individuals with a higher metabolic rate (and larger body size) stayed closer to the site of origin in the upper section of a stream, rather than migrating further downstream (Sanchez-Gonzalez and Nicieza, 2021). It has also been proposed that rainbow trout with relatively higher SMRs are more likely to migrate early to the marine environment, as the freshwater ecosystem is energetically more limiting for them (Sloat and Reeves, 2014).

In the current study the MMR and AS were lower in offspring whose experimental parent was exercised. The AS indicates the amount of oxygen available to perform any activity above the basic demands that an organism requires to stay alive (for example reproduction, migration, growth and feeding; Auer et al., 2015; Eliason and Farrell, 2016; Durtsche et al., 2021). Reducing the aerobic scope may be a way for the organisms to combat a harsh unpredictable environment, with an unstable food source (Auer et al., 2015; Durtsche et al., 2021). Having a lower AS means that they require fewer resources (food) to maintain their metabolic rate and thus survive. Furthermore, since AS is positively linked to traits such as activity (Halsey et al., 2018; Hollins et al., 2018), having a lower AS could lower the overall motility of the animal, making them less vulnerable to predators as well as angling (Killen et al., 2015; Redfern et al. 2017; Hollins et al., 2018). However, there is some evidence that having a high AS is advantageous in an environment rich in food since it is positively related to fitness traits such as competitive dominance, activity and boldness (Auer et al., 2015; Eliason and Farrell, 2016). This was shown in juvenile brown trout where individuals with a higher AS and ready access to food displayed higher growth (Auer et al., 2015).

Finally, offspring from wild semelparous females exposed to a recurrent chase protocol produced offspring that performed shorter burst swimming activities (Sopinka et al., 2014). Being able to recover from burst swimming swiftly and quickly is critical in salmonids, both as juveniles (avoid predators) and adults (migration; Eliason and Farrell, 2016), but it requires a high AS, so it may be the case that AS was also reduced in offspring of chased females in that study.

There was a clear reduction in the dominance status of offspring whose parents were either exercised, or exercised and then exposed to air for an extended period of time, in comparison with controls. It is important to note that a link between metabolic rate and dominance was not examined directly in the current study, since different individuals were used for the dominance trials and the investigation of metabolism. However, there is an extensive literature showing that social dominance in salmonids is often associated with a higher rate of metabolism (Metcalfe et al. 2016), and so it may be presumed that there was also likely to be an association in the present study. The link means that dominant fish have a higher cost of living as well as an ability to obtain optimal territories, so that the benefits are context-dependent (Gilmour et al., 2005; Schjolden et al., 2009; Reid et al., 2011; Hoogenboom et al., 2013). Dominance may thus only improve fitness in the presence of a predictable high food environment (Reid et al., 2011; Hoogenboom et al., 2013).

Interestingly, the relationships between parental treatment and dominance in the current investigation showed a dynamic trend: control offspring were dominant over the disturbed treatments on day 1 and 2 of the trials, but by day 3 there was no clear dominance across treatments. Under laboratory conditions this might yield some interesting shifts in hierarchy and could potentially lead to changes in territory establishment as individuals become familiar with each other and the environment. However, in a natural setting offspring from stressed parents might not have this opportunity since they are likely to be quickly displaced from territories (which was not possible in the confines of a laboratory arena), making it difficult for them to re-establish themselves into the hierarchy. These individuals would get evicted from the most optimal habitats, which would not only leave them short of food, but also expose them to a higher predation risk as they disperse further into unknown and possible unfavourable habitats.

These results reveal that parental stress similar to that which salmon would experience from being caught by an angler could, if incurred close to the time of spawning, influence the potential ability of their offspring to acquire a feeding territory. The majority of the knowledge accumulated on parental stress, and its behavioural and physiological consequences for offspring, have been collected using laboratory-based research and domesticated animals (Sopinka et al., 2014). Since wild populations tend to live in unpredictable environments with harsher conditions than those in the laboratory, and since most of the affected traits are habitat-dependent, a new holistic approach that includes both wild populations and more realistic testing environments must be designed and incorporated into such investigations. This will offer a better understanding on how parental stress can result in adjustment of an offspring's phenotype, for better or worse, based on the habitat that they themselves experience.

5.6 References

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5.7 Supplementary information

Table S5.1 Summary of all final General Linear Models (GLMs) and Logistic regressions used to investigate the effects that the adult C&R simulations had on the offspring's physiology (GLMs) and dominance (Logistic regressions).

Variable of Interest		Model used
Physiology		
Absolute MMR		lm(MMR Abs ~ Treatment + log(Mass) + Parental Sex)
Absolute SMR		lm(SMR Abs ~ Treatment + log(Mass) + Parental Sex)
Absolute AS		lm(AS Abs ~ Treatment + log(Mass) + Parental Sex)
Dominance Trials	**Trial**	
Control vs Exercise		
	Day 1	glm(Trial 1 Dominance ~ Competitors + Control Fish Length + Exercise Fish Length, data = Offspring Dominance A vs B, family = "binomial")
	Day 2	glm(Trial 2 Dominance ~ Competitors + Control Fish Length + Exercise Fish Length, data = Offspring Dominance A vs B, family = "binomial")
	Day 3	glm(Trial 3 Dominance ~ Competitors + Control Fish Length + Exercise Fish Length, data = Offspring Dominance A vs B, family = "binomial")
	Overall (Day 1 – 3)	glm(Overall Dominance ~ Competitors + Control Fish Length + Exercise Fish Length, data = Offspring Dominance A vs B, family = "binomial")
Control vs Exercise + Extended Air		

	Day 1	glm(Trial 1 Dominance ~ Competitors + Control Fish Length + Exercise and Extended Air Fish Length, data = Offspring Dominance A vs D, family = "binomial")
	Day 2	glm(Trial 2 Dominance ~ Competitors + Control Fish Length + Exercise and Extended Air Fish Length, data = Offspring Dominance A vs D, family = "binomial")
	Day 3	glm(Trial 3 Dominance ~ Competitors + Control Fish Length + Exercise and Extended Air Fish Length, data = Offspring Dominance A vs D, family = "binomial")
	Overall (Day 1 – 3)	glm(Overall Dominance ~ Competitors + Control Fish Length + Exercise and Extended Air Fish Length, data = Offspring Dominance A vs D, family = "binomial")
Exercise vs Exercise + Extended Air		
	Day 1	glm(Trial 1 Dominance ~ Competitors + Exercise Fish Length + Exercise and Extended Air Fish Length, data = Offspring Dominance B vs D, family = "binomial")
	Day 2	glm(Trial 2 Dominance ~ Competitors + Exercise Fish Length + Exercise and Extended Air Fish Length, data = Offspring Dominance B vs D, family = "binomial")
	Day 3	glm(Trial 3 Dominance ~ Competitors + Exercise Fish Length + Exercise and Extended Air Fish Length, data = Offspring Dominance B vs D, family = "binomial")
	Overall (Day 1 – 3)	glm(Overall Dominance ~ Competitors + Exercise Fish Length + Exercise and Extended Air Fish Length, data = Offspring Dominance B vs D, family = "binomial")

Table S5.2 The checklist of essential criteria for the aquatic intermittent-flow respirometry.

	Criterion and Category	Response	Value (where required)	Units
	EQUIPMENT, MATERIALS, AND SETUP			
1	Body mass of animals at time of respirometry		0.549; range: 0.132 – 1.659	g
2	Volume of empty respirometers		18	mL
3	How chamber mixing was achieved	Water being recirculated using peristaltic pumps		
4	Ratio of net respirometer volume (plus any associated tubing in mixing circuit) to animal body mass		28.75 (range: 26.05-32.64): 0.549 (range: 0.132 – 1.659)	mL : g
5	Material of tubing used in any mixing circuit	PVC tubing		
6	Volume of tubing in any mixing circuit		11.3 ± 3.3	mL
7	Confirm volume of tubing in any mixing circuit was included in calculations of oxygen uptake	Yes, it was included		
8	Material of respirometer (e.g. glass, acrylic, etc.)	Glass		
9	Type of oxygen probe and data recording	Fiber optic sensors		
10	Sampling frequency of water dissolved oxygen		2	s
11	Describe placement of oxygen probe (in mixing circuit or directly in chamber)	Sensor placed inside probe holders		

12	Flow rate during flushing and recirculation, or confirm that chamber returned to normoxia during flushing	Chambers returned to normoxic conditions during flushing		
13	Timing of flush/closed cycles		2/ 8	min
14	Wait (delay) time excluded from closed measurement cycles	30 wait period and 180 min exclusion period at the end	30/ 180	s
15	Frequency and method of probe calibration (for both 0 and 100 % calibrations)	Probes calibrated using a two-point system at the start of the experiment in July, and again during the first week of August. A single-point calibration was performed on the same sensors on a daily basis before the start of the trials.		
16	State whether software temperature compensation was used during recording of water oxygen concentration	Yes, temperature compensation was used		
	MEASUREMENT CONDITIONS			
17	Temperature during respirometry		13 ± 0.5	^{o}C
18	How temperature was controlled	Temperature-controlled water bath		
19	Photoperiod during respirometry	12L:12D	8:00 – 20:00	hr

20	If (and how) ambient water bath was cleaned and aerated during measurement of oxygen uptake (e.g. filtration, periodic or continuous water changes)	The passing water went through a UV filter sterilizer, and system was fully bleaching 3 times the morning before each trial.		
21	Total volume of ambient water bath and any associated reservoirs		92.8	L
22	Minimum water oxygen dissolved oxygen reached during closed phases	Did not fall below 65 % during the first cycle (where MMR was measured) and 80 % after the first cycle (measurements used for SMR)		
23	State whether chambers were visually shielded from external disturbance	Once all the fish were in the chambers, a black tarp, which was not removed until the next day, was placed on top of the respirometer.		
24	How many animals were measured during a given respirometry trial (i.e. how many animals were in the same water bath)		16	
25	If multiple animals were measured simultaneously, state whether they were able to see each other during measurements	There was no shield between the fish		
26	Duration of animal fasting before placement in respirometer		24	h
27	Duration of all trials combined (number of days to measure all animals in the study)	July 16th and August 18^{th,} 2019		

28	Acclimation time to the laboratory (or time since capture for field studies) before respirometry measurements		3 – 4	months
	BACKGROUND RESPIRATION			
29	Whether background microbial respiration was measured and accounted for, and if so, method used (e.g. parallel measures with empty respirometry chamber, measurements before and after for all chambers while empty, both)	Background microbial respiration was measured and account for using the FishResp script in r		
30	If background respiration was measured at beginning and/or end, state how many slopes and for what duration	Yes – one complete cycle	2 flush/ 8 closed	min
31	How changes in background respiration were modelled over time (e.g. linear, exponential, parallel measures)			
32	Level of background respiration (e.g. as a percentage of SMR)			
33	Method and frequency of system cleaning (e.g. system bleached between each trial, UV lamp)	Bleaching (1x) and rinsing 3x before each trial and water filtration through a UV lamp		
	STANDARD OR ROUTINE METABOLIC RATE			
34	Acclimation time after transfer to chamber, or alternatively, time to reach beginning of metabolic rate measurements after introduction to chamber	After the first cycle, which was used for the MMR	10	min

35	Time period, within a trial, over which oxygen uptake was measured (e.g. number of hours)		18 h 6 min ± 1 h 26 min; 108 ± 6 slopes	h ± min
36	Value taken as SMR/RMR (e.g. quantile, mean of lowest 10 percent, mean of all values)	lowest 10 percentile		
37	Total number of slopes measured and used to derive metabolic rate (e.g. how much data were used to calculate quantiles)		108 ± 6 slopes	slopes
38	Whether any time periods were removed from calculations of SMR/RMR (e.g. data during acclimation, periods of high activity [e.g. daytime])	30 s wait period at the start, and a 180 s exclusion period at the end		s
39	r^2 threshold for slopes used for SMR/RMR (or mean)		90 – 95	%
40	Proportion of data removed due to being outliers below r-squared threshold	None		
	MAXIMUM METABOLIC RATE			
41	When MMR was measured in relation to SMR (i.e. before or after)	MMR measured first and then SMR		
42	Method used (e.g. critical swimming speed respirometry, swim to exhaustion in swim tunnel, or chase to exhaustion)	Exhaustive exercise (Chase)	180	s
43	Value taken as MMR (e.g. the highest rate of oxygen uptake value after transfer, average of highest values)	First cycle after transfer to chambers	2 flush/ 8 closed	min
44	If MMR measured post-exhaustion, length of activity challenge or chase (e.g. 2 min, until exhaustion, etc.)		180	S

45	If MMR measured post-exhaustion, state whether further air-exposure was added after exercise	No further air exposure		
46	If MMR measured post-exhaustion, time until transfer to chamber after exhaustion or time to start of oxygen uptake recording		<10	s
47	Duration of slopes used to calculate MMR (e.g. 1 min, 5 min, etc.)		8	min
48	Slope estimation method for MMR (e.g. rolling regression, sequential discrete time frames)	running rolling regression slopes every 1 s		
49	How absolute aerobic scope and/or factorial aerobic scope is calculated (i.e. using raw SMR and MMR, allometrically mass-adjusted SMR and MMR, or allometrically mass-adjusting aerobic scope itself)	Subtracting the absolute MMR minus the absolute SMR		
	DATA HANDLING AND STATISTICS			
50	Sample size		255	Fish
51	How oxygen uptake rates were calculated (software or script, equation, units, etc.)	Script in R		
52	Confirm that volume (mass) of animal was subtracted from respirometer volume when calculating oxygen uptake rates	Yes		
53	State whether analyses accounted for variation in body mass and describe any allometric mass-corrections or adjustments	Yes. Mass was used as a variable in the model. Mass underwent logarithmic transformation.		

Table S5.3 Summary of the fish metabolic data. Included are the date of the respirometry, the experimental parents treatment and sex, the offspring's fork length and mass, and the SMR, MMR and AS.

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
16/07/2019	C	252	F	1	1.1	42.68	0.664	-0.1778	0.2384	-0.6228	0.4009	-0.3970	0.1626	-0.7890
16/07/2019	C	252	F	2	1.2	38.54	0.434	-0.3625	0.0887	-1.0522	0.3430	-0.4647	0.2543	-0.5947
16/07/2019	C	252	F	3	1.3	46.54	0.822	-0.0851	0.1641	-0.7848	0.4680	-0.3298	0.3038	-0.5174
16/07/2019	C	252	F	4	1.4	38.77	0.449	-0.3478	0.1080	-0.9666	0.2467	-0.6078	0.1387	-0.8579
16/07/2019	C	252	F	5	2.1	39.46	0.525	-0.2798	0.1231	-0.9098	0.3986	-0.3995	0.2755	-0.5599
16/07/2019	C	252	F	6	2.2	36.63	0.356	-0.4486	0.1143	-0.9419	0.2922	-0.5344	0.1778	-0.7500
16/07/2019	C	252	F	7	2.3	40.8	0.507	-0.2950	0.1078	-0.9676	0.3918	-0.4069	0.2840	-0.5466
16/07/2019	C	252	F	8	2.4	35.12	0.318	-0.4976	0.1525	-0.8168	0.4965	-0.3041	0.3440	-0.4635
16/07/2019	B	221	M	1	3.1	39.8	0.428	-0.3686	0.1144	-0.9415	0.5224	-0.2820	0.4080	-0.3894
16/07/2019	B	221	M	2	3.2	37.57	0.434	-0.3625	0.1198	-0.9214	0.3450	-0.4622	0.2252	-0.6475
17/07/2019	D	228	M	1	1.1	32.97	0.3	-0.5229	0.0684	-1.1646	0.1817	-0.7407	0.1132	-0.9460
17/07/2019	D	228	M	2	1.2	38.99	0.416	-0.3809	0.1049	-0.9794	0.4448	-0.3519	0.3399	-0.4687
17/07/2019	D	228	M	3	1.3	40.2	0.486	-0.3134	0.1508	-0.8216	0.3248	-0.4884	0.1740	-0.7595

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
17/07/2019	D	228	M	4	1.4	39.55	0.535	-0.2716	0.1028	-0.9880	0.2356	-0.6279	0.1328	-0.8769
17/07/2019	D	228	M	5	2.1	37.28	0.412	-0.3851	0.0786	-1.1047	0.2395	-0.6208	0.1609	-0.7935
17/07/2019	D	228	M	6	2.2	36.1	0.357	-0.4473	0.0836	-1.0777	0.2763	-0.5586	0.1927	-0.7151
17/07/2019	D	228	M	7	2.3	39	0.433	-0.3635	0.0818	-1.0874	0.4140	-0.3830	0.3322	-0.4786
17/07/2019	D	228	M	8	2.4	39.1	0.502	-0.2993	0.1069	-0.9711	0.3868	-0.4125	0.2799	-0.5530
17/07/2019	B	254	F	1	3.1	34.25	0.308	-0.5114	0.0658	-1.1816	0.2506	-0.6010	0.1848	-0.7333
17/07/2019	B	254	F	2	3.2	35.84	0.358	-0.4461	0.0954	-1.0204	0.3850	-0.4146	0.2896	-0.5382
17/07/2019	B	254	F	3	3.3	37.61	0.416	-0.3809	0.0912	-1.0398	0.3470	-0.4597	0.2557	-0.5922
17/07/2019	B	254	F	4	3.4	36.69	0.279	-0.5544	0.0710	-1.1490	0.4366	-0.3599	0.3656	-0.4369
17/07/2019	B	254	F	5	4.1	34.69	0.334	-0.4763	0.0791	-1.1018	0.2461	-0.6090	0.1669	-0.7774
17/07/2019	B	254	F	6	4.2	35.6	0.281	-0.5513	0.0693	-1.1590	0.2142	-0.6692	0.1449	-0.8391
17/07/2019	B	254	F	7	4.3	35.1	0.358	-0.4461	0.0798	-1.0981	0.2286	-0.6410	0.1488	-0.8274
17/07/2019	B	254	F	8	4.4	37.88	0.466	-0.3316	0.0862	-1.0647	0.2975	-0.5265	0.2113	-0.6750
18/07/2019	D	206	M	1	1.1	35.06	0.38	-0.4202	0.0864	-1.0637	0.2055	-0.6872	0.1192	-0.9239
18/07/2019	D	206	M	2	1.2	37.63	0.429	-0.3675	0.1030	-0.9872	0.2225	-0.6527	0.1195	-0.9226

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
18/07/2019	D	206	M	3	1.3	36.73	0.401	-0.3969	0.0983	-1.0073	0.2049	-0.6885	0.1066	-0.9724
18/07/2019	D	206	M	5	2.1	36.29	0.41	-0.3872	0.0978	-1.0098	0.2470	-0.6073	0.1493	-0.8261
18/07/2019	D	206	M	6	2.2	38.67	0.454	-0.3429	0.0808	-1.0926	0.4899	-0.3099	0.4091	-0.3881
18/07/2019	D	206	M	7	2.3	39.32	0.499	-0.3019	0.0884	-1.0536	0.2427	-0.6150	0.1543	-0.8117
18/07/2019	D	206	M	8	2.4	39.85	0.52	-0.2840	0.1191	-0.9241	0.3582	-0.4459	0.2391	-0.6214
18/07/2019	B	250	F	1	3.1	28.57	0.168	-0.7747	0.0378	-1.4229	0.1056	-0.9764	0.0678	-1.1686
18/07/2019	B	250	F	2	3.2	33.36	0.313	-0.5045	0.0759	-1.1200	0.2148	-0.6680	0.1389	-0.8573
18/07/2019	B	250	F	3	3.3	35.57	0.402	-0.3958	0.0789	-1.1027	0.2701	-0.5685	0.1911	-0.7187
18/07/2019	B	250	F	4	3.4	43.03	0.591	-0.2284	0.1041	-0.9824	0.4115	-0.3857	0.3073	-0.5124
18/07/2019	B	250	F	5	4.1	33.79	0.282	-0.5498	0.0794	-1.1003	0.2735	-0.5630	0.1941	-0.7119
18/07/2019	B	250	F	6	4.2	33.01	0.272	-0.5654	0.0499	-1.3015	0.1642	-0.7847	0.1142	-0.9422
18/07/2019	B	250	F	7	4.3	33.07	0.26	-0.5850	0.0412	-1.3854	0.1719	-0.7648	0.1307	-0.8837
18/07/2019	B	250	F	8	4.4	41.74	0.58	-0.2366	0.1073	-0.9696	0.3572	-0.4471	0.2499	-0.6022
19/07/2019	C	203	F	1	1.1	28.33	0.132	-0.8794	0.0329	-1.4833	0.0794	-1.1001	0.0465	-1.3321
19/07/2019	C	203	F	2	1.2	31.84	0.226	-0.6459	0.0504	-1.2979	0.1461	-0.8352	0.0958	-1.0188

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
19/07/2019	C	203	F	3	1.3	34.35	0.305	-0.5157	0.0540	-1.2680	0.1670	-0.7774	0.1130	-0.9469
19/07/2019	C	203	F	4	1.4	36.9	0.402	-0.3958	0.0760	-1.1189	0.1824	-0.7390	0.1064	-0.9732
19/07/2019	C	203	F	5	2.1	32.29	0.258	-0.5884	0.0444	-1.3528	0.1511	-0.8208	0.1067	-0.9719
19/07/2019	C	203	F	6	2.2	34.15	0.273	-0.5638	0.0604	-1.2189	0.1731	-0.7616	0.1127	-0.9480
19/07/2019	C	219	M	1	2.3	37.45	0.366	-0.4365	0.0701	-1.1540	0.2374	-0.6246	0.1672	-0.7767
19/07/2019	C	219	M	2	2.4	34.47	0.276	-0.5591	0.0730	-1.1367	0.2030	-0.6924	0.1300	-0.8859
19/07/2019	C	219	M	3	3.1	36.29	0.356	-0.4486	0.0585	-1.2328	0.2360	-0.6272	0.1775	-0.7509
19/07/2019	C	219	M	4	3.2	37.75	0.449	-0.3478	0.0799	-1.0973	0.2564	-0.5911	0.1765	-0.7533
19/07/2019	C	219	M	5	3.3	34.54	0.302	-0.5200	0.0437	-1.3600	0.2078	-0.6824	0.1641	-0.7848
19/07/2019	C	219	M	6	3.4	35.64	0.323	-0.4908	0.0460	-1.3373	0.2491	-0.6037	0.2031	-0.6923
19/07/2019	D	259	F	1	4.1	31.53	0.226	-0.6459	0.0623	-1.2056	0.1545	-0.8111	0.0922	-1.0353
19/07/2019	D	259	F	2	4.2	33.97	0.278	-0.5560	0.0525	-1.2802	0.1801	-0.7445	0.1276	-0.8940
19/07/2019	D	259	F	3	4.3	33.03	0.275	-0.5607	0.0443	-1.3536	0.1809	-0.7426	0.1366	-0.8646
19/07/2019	D	259	F	4	4.4	37.18	0.358	-0.4461	0.0553	-1.2571	0.1773	-0.7514	0.1219	-0.9139
22/07/2019	C	255	F	1	1.1	30.17	0.205	-0.6882	0.0521	-1.2835	0.1433	-0.8438	0.0912	-1.0399

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
22/07/2019	C	255	F	2	1.2	35.01	0.34	-0.4685	0.0712	-1.1473	0.2214	-0.6548	0.1502	-0.8234
22/07/2019	C	255	F	3	1.3	37.77	0.367	-0.4353	0.0942	-1.0259	0.2319	-0.6346	0.1377	-0.8610
22/07/2019	C	255	F	4	1.4	40.39	0.495	-0.3054	0.1058	-0.9755	0.2691	-0.5700	0.1633	-0.7869
22/07/2019	C	255	F	5	2.1	30.64	0.248	-0.6055	0.0803	-1.0951	0.1383	-0.8592	0.0580	-1.2368
22/07/2019	C	255	F	6	2.2	33.89	0.303	-0.5186	0.0972	-1.0124	0.2099	-0.6780	0.1127	-0.9480
22/07/2019	C	255	F	7	2.3	35.77	0.346	-0.4609	0.0992	-1.0034	0.2312	-0.6359	0.1320	-0.8794
22/07/2019	C	255	F	8	2.4	39.57	0.495	-0.3054	0.1004	-0.9984	0.3432	-0.4645	0.2428	-0.6148
22/07/2019	A	260	F	1	3.1	35.61	0.358	-0.4461	0.0715	-1.1458	0.2492	-0.6034	0.1778	-0.7502
22/07/2019	A	260	F	2	3.2	35.41	0.33	-0.4815	0.0676	-1.1701	0.2364	-0.6264	0.1688	-0.7727
22/07/2019	A	260	F	3	3.3	38.96	0.476	-0.3224	0.1195	-0.9225	0.2550	-0.5934	0.1355	-0.8680
22/07/2019	A	260	F	4	3.4	41.42	0.584	-0.2336	0.1014	-0.9942	0.3993	-0.3987	0.2979	-0.5259
22/07/2019	A	260	F	5	4.1	33.15	0.282	-0.5498	0.0946	-1.0242	0.1905	-0.7201	0.0959	-1.0181
22/07/2019	A	260	F	6	4.2	34.81	0.334	-0.4763	0.0535	-1.2716	0.1964	-0.7068	0.1429	-0.8450
22/07/2019	A	260	F	7	4.3	36.76	0.392	-0.4067	0.0790	-1.1026	0.2606	-0.5840	0.1817	-0.7407
22/07/2019	A	260	F	8	4.4	40.95	0.538	-0.2692	0.1092	-0.9619	0.3757	-0.4252	0.2665	-0.5743

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
23/07/2019	A	231	M	1	1.1	32.98	0.271	-0.5670	0.0646	-1.1898	0.1962	-0.7072	0.1316	-0.8806
23/07/2019	A	231	M	2	1.2	33.05	0.261	-0.5834	0.0636	-1.1965	0.1352	-0.8689	0.0716	-1.1449
23/07/2019	A	231	M	3	1.3	37.52	0.42	-0.3768	0.0846	-1.0728	0.2760	-0.5591	0.1914	-0.7179
23/07/2019	A	231	M	4	1.4	43.15	0.659	-0.1811	0.1324	-0.8782	0.3667	-0.4357	0.2344	-0.6301
23/07/2019	A	231	M	5	2.1	35.93	0.356	-0.4486	0.0908	-1.0419	0.2305	-0.6373	0.1397	-0.8547
23/07/2019	A	231	M	6	2.2	34.53	0.298	-0.5258	0.0746	-1.1273	0.2102	-0.6775	0.1356	-0.8679
23/07/2019	A	231	M	7	2.3	39.69	0.505	-0.2967	0.0893	-1.0490	0.3089	-0.5101	0.2196	-0.6584
23/07/2019	A	231	M	8	2.4	40.59	0.57	-0.2441	0.1038	-0.9840	0.3996	-0.3984	0.2958	-0.5289
23/07/2019	D	235	F	1	3.1	35.8	0.373	-0.4283	0.0868	-1.0616	0.3209	-0.4937	0.2341	-0.6306
23/07/2019	D	235	F	2	3.2	41.42	0.594	-0.2262	0.1181	-0.9277	0.3928	-0.4058	0.2747	-0.5611
23/07/2019	D	235	F	3	3.3	38.9	0.45	-0.3468	0.0938	-1.0278	0.2713	-0.5665	0.1775	-0.7507
23/07/2019	D	235	F	4	3.4	48.08	1.004	0.0017	0.1979	-0.7036	0.5936	-0.2265	0.3957	-0.4027
23/07/2019	D	235	F	5	4.1	37.35	0.447	-0.3497	0.1019	-0.9920	0.3060	-0.5142	0.2042	-0.6900
23/07/2019	D	258	F	1	4.2	38.58	0.455	-0.3420	0.0923	-1.0349	0.3336	-0.4768	0.2413	-0.6174
23/07/2019	D	258	F	2	4.3	40.67	0.533	-0.2733	0.1062	-0.9739	0.3080	-0.5114	0.2018	-0.6950

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
24/07/2019	C	218	M	1	1.1	33.95	0.248	-0.6055	0.0519	-1.2848	0.1568	-0.8045	0.1049	-0.9790
24/07/2019	C	218	M	2	1.2	37.81	0.423	-0.3737	0.0917	-1.0375	0.2687	-0.5707	0.1770	-0.7520
24/07/2019	C	218	M	3	1.3	41.55	0.56	-0.2518	0.1073	-0.9693	0.3505	-0.4554	0.2431	-0.6141
24/07/2019	C	218	M	4	1.4	48.71	1.034	0.0145	0.1822	-0.7395	0.5606	-0.2514	0.3784	-0.4220
24/07/2019	C	218	M	5	2.1	36.28	0.37	-0.4318	0.0795	-1.0997	0.2109	-0.6760	0.1314	-0.8814
24/07/2019	C	218	M	6	2.2	36.59	0.406	-0.3915	0.0934	-1.0298	0.2659	-0.5752	0.1726	-0.7630
24/07/2019	C	218	M	7	2.3	40.4	0.577	-0.2388	0.1027	-0.9883	0.3926	-0.4060	0.2899	-0.5378
24/07/2019	C	218	M	8	2.4	44.58	0.722	-0.1415	0.1225	-0.9120	0.5058	-0.2960	0.3834	-0.4164
24/07/2019	D	230	M	1	3.1	32.14	0.308	-0.5114	0.0620	-1.2076	0.1765	-0.7532	0.1145	-0.9412
24/07/2019	D	230	M	2	3.2	38.86	0.512	-0.2907	0.0958	-1.0185	0.3150	-0.5017	0.2192	-0.6592
24/07/2019	D	230	M	3	3.3	37.13	0.479	-0.3197	0.0890	-1.0506	0.3213	-0.4931	0.2323	-0.6340
24/07/2019	D	230	M	4	3.4	43.16	0.73	-0.1367	0.1448	-0.8392	0.5044	-0.2972	0.3596	-0.4442
24/07/2019	D	230	M	5	4.1	38.81	0.553	-0.2573	0.1243	-0.9054	0.3288	-0.4831	0.2044	-0.6894
24/07/2019	D	230	M	6	4.2	33.38	0.333	-0.4776	0.0724	-1.1402	0.1977	-0.7039	0.1253	-0.9019
24/07/2019	D	230	M	7	4.3	43.36	0.717	-0.1445	0.1513	-0.8202	0.4795	-0.3192	0.3282	-0.4839

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
24/07/2019	D	230	M	8	4.4	44.44	0.774	-0.1113	0.1469	-0.8330	0.5189	-0.2849	0.3720	-0.4294
28/07/2019	B	222	M	1	1.1	31	0.271	-0.5670	0.0457	-1.3404	0.1087	-0.9636	0.0631	-1.2002
28/07/2019	B	222	M	2	1.2	35.1	0.31	-0.5086	0.0622	-1.2063	0.2022	-0.6942	0.1400	-0.8538
28/07/2019	B	222	M	3	1.3	36.59	0.411	-0.3862	0.0634	-1.1977	0.2236	-0.6506	0.1601	-0.7955
28/07/2019	B	222	M	4	1.4	45.91	0.772	-0.1124	0.1347	-0.8706	0.4609	-0.3364	0.3261	-0.4866
28/07/2019	B	222	M	5	2.1	33.06	0.235	-0.6289	0.0434	-1.3625	0.1180	-0.9280	0.0746	-1.1271
28/07/2019	B	222	M	6	2.2	37.32	0.408	-0.3893	0.0750	-1.1250	0.2585	-0.5876	0.1835	-0.7364
28/07/2019	B	222	M	7	2.3	36.26	0.41	-0.3872	0.0708	-1.1498	0.2751	-0.5605	0.2043	-0.6898
28/07/2019	B	222	M	8	2.4	39.83	0.543	-0.2652	0.1014	-0.9940	0.3949	-0.4036	0.2935	-0.5324
28/07/2019	B	237	F	1	3.1	37.4	0.402	-0.3958	0.0704	-1.1523	0.2990	-0.5243	0.2286	-0.6409
28/07/2019	B	237	F	2	3.2	38.32	0.444	-0.3526	0.0924	-1.0343	0.2923	-0.5341	0.1999	-0.6991
28/07/2019	B	237	F	3	3.3	42.72	0.68	-0.1675	0.1237	-0.9076	0.4533	-0.3436	0.3296	-0.4821
28/07/2019	B	237	F	4	3.4	46.05	0.819	-0.0867	0.1377	-0.8611	0.4933	-0.3069	0.3556	-0.4490
28/07/2019	B	237	F	5	4.1	36.39	0.39	-0.4089	0.1291	-0.8891	0.2949	-0.5303	0.1658	-0.7805
28/07/2019	B	237	F	6	4.2	38.33	0.523	-0.2815	0.0925	-1.0340	0.3354	-0.4745	0.2429	-0.6146

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
28/07/2019	B	237	F	7	4.3	43.06	0.699	-0.1555	0.1124	-0.9494	0.4193	-0.3774	0.3070	-0.5129
28/07/2019	B	237	F	8	4.4	43.73	0.675	-0.1707	0.0996	-1.0017	0.3926	-0.4060	0.2930	-0.5331
29/07/2019	B	217	M	1	1.1	33.91	0.333	-0.4776	0.0692	-1.1597	0.1913	-0.7184	0.1220	-0.9136
29/07/2019	B	217	M	2	1.2	35.43	0.359	-0.4449	0.1008	-0.9964	0.1908	-0.7194	0.0900	-1.0458
29/07/2019	B	217	M	3	1.3	43.97	0.765	-0.1163	0.1517	-0.8191	0.3749	-0.4261	0.2232	-0.6513
29/07/2019	B	217	M	4	1.4	47.21	1.13	0.0531	0.1743	-0.7588	0.4362	-0.3603	0.2619	-0.5818
29/07/2019	B	217	M	5	2.1	37.07	0.459	-0.3382	0.0959	-1.0182	0.2410	-0.6180	0.1451	-0.8384
29/07/2019	B	217	M	6	2.2	37.53	0.456	-0.3410	0.0836	-1.0778	0.2414	-0.6173	0.1578	-0.8020
29/07/2019	B	217	M	7	2.3	42.75	0.658	-0.1818	0.1352	-0.8691	0.3803	-0.4199	0.2451	-0.6107
29/07/2019	B	217	M	8	2.4	44.7	0.813	-0.0899	0.1447	-0.8394	0.4232	-0.3735	0.2784	-0.5553
29/07/2019	A	226	M	1	3.1	35.63	0.365	-0.4377	0.0829	-1.0817	0.2278	-0.6425	0.1449	-0.8388
29/07/2019	A	226	M	2	3.2	43.7	0.719	-0.1433	0.1424	-0.8466	0.3960	-0.4023	0.2536	-0.5958
29/07/2019	A	226	M	3	3.3	42.01	0.714	-0.1463	0.1422	-0.8471	0.4630	-0.3344	0.3208	-0.4937
29/07/2019	A	226	M	4	3.4	48.14	0.989	-0.0048	0.1991	-0.7009	0.6729	-0.1720	0.4738	-0.3244
29/07/2019	A	226	M	5	4.1	38.72	0.486	-0.3134	0.1057	-0.9759	0.3143	-0.5027	0.2086	-0.6807

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
29/07/2019	A	226	M	6	4.2	40.81	0.566	-0.2472	0.1038	-0.9840	0.4089	-0.3884	0.3051	-0.5156
29/07/2019	A	226	M	7	4.3	43.03	0.719	-0.1433	0.1553	-0.8088	0.4458	-0.3508	0.2905	-0.5368
29/07/2019	A	226	M	8	4.4	45.85	0.91	-0.0410	0.1874	-0.7273	0.5138	-0.2892	0.3264	-0.4862
30/07/2019	A	240	F	1	1.1	28.62	0.205	-0.6882	0.0371	-1.4305	0.0722	-1.1414	0.0351	-1.4546
30/07/2019	A	240	F	2	1.2	31.79	0.272	-0.5654	0.0740	-1.1306	0.1738	-0.7600	0.0997	-1.0011
30/07/2019	A	240	F	3	1.3	40.54	0.593	-0.2269	0.1404	-0.8528	0.3787	-0.4217	0.2384	-0.6227
30/07/2019	A	240	F	4	1.4	38.19	0.4736	-0.3246	0.1131	-0.9467	0.2950	-0.5302	0.1819	-0.7402
30/07/2019	A	240	F	5	2.1	33.32	0.32	-0.4949	0.0656	-1.1834	0.1828	-0.7379	0.1173	-0.9308
30/07/2019	A	240	F	6	2.2	35.65	0.414	-0.3830	0.0767	-1.1155	0.2149	-0.6677	0.1383	-0.8593
30/07/2019	A	240	F	7	2.3	37.2	0.437	-0.3595	0.0804	-1.0946	0.2447	-0.6114	0.1643	-0.7844
30/07/2019	A	240	F	8	2.4	37.8	0.476	-0.3224	0.1042	-0.9822	0.5301	-0.2756	0.4260	-0.3706
30/07/2019	D	244	F	1	3.1	34.44	0.324	-0.4895	0.0684	-1.1652	0.2510	-0.6004	0.1826	-0.7385
30/07/2019	D	244	F	2	3.2	37.08	0.407	-0.3904	0.1027	-0.9885	0.3011	-0.5213	0.1984	-0.7024
30/07/2019	D	244	F	3	3.3	43.27	0.729	-0.1373	0.1414	-0.8495	0.4337	-0.3628	0.2923	-0.5342
30/07/2019	D	244	F	4	3.4	43.86	0.658	-0.1818	0.1278	-0.8933	0.4317	-0.3648	0.3039	-0.5173

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
30/07/2019	D	244	F	5	4.1	32.47	0.266	-0.5751	0.0730	-1.1368	0.1663	-0.7790	0.0934	-1.0299
30/07/2019	D	244	F	6	4.2	35.95	0.406	-0.3915	0.0808	-1.0924	0.2397	-0.6203	0.1589	-0.7989
30/07/2019	D	244	F	7	4.3	38.1	0.439	-0.3575	0.0919	-1.0368	0.2816	-0.5503	0.1897	-0.7219
30/07/2019	D	244	F	8	4.4	42.95	0.703	-0.1530	0.1302	-0.8854	0.4051	-0.3924	0.2749	-0.5608
31/07/2019	A	201	M	1	1.1	33.82	0.352	-0.4535	0.0639	-1.1944	0.1552	-0.8091	0.0913	-1.0396
31/07/2019	A	201	M	2	1.2	40.16	0.586	-0.2321	0.1155	-0.9373	0.2861	-0.5435	0.1706	-0.7681
31/07/2019	A	201	M	3	1.3	43.3	0.665	-0.1772	0.1207	-0.9184	0.3421	-0.4658	0.2214	-0.6547
31/07/2019	A	201	M	4	1.4	47.45	0.947	-0.0237	0.1880	-0.7259	0.4781	-0.3205	0.2901	-0.5374
31/07/2019	A	201	M	5	2.1	36.76	0.468	-0.3298	0.0878	-1.0565	0.2675	-0.5726	0.1797	-0.7454
31/07/2019	A	201	M	6	2.2	43.89	0.78	-0.1079	0.1309	-0.8831	0.4300	-0.3666	0.2991	-0.5242
31/07/2019	A	201	M	7	2.3	44.4	0.756	-0.1215	0.1399	-0.8543	0.3427	-0.4651	0.2029	-0.6928
31/07/2019	A	201	M	8	2.4	46.85	0.931	-0.0311	0.1779	-0.7498	0.6181	-0.2089	0.4403	-0.3563
31/07/2019	A	215	M	1	3.1	37.05	0.413	-0.3840	0.0888	-1.0517	0.2373	-0.6247	0.1485	-0.8282
31/07/2019	A	215	M	2	3.2	41.21	0.581	-0.2358	0.1207	-0.9182	0.4706	-0.3273	0.3499	-0.4561
31/07/2019	A	215	M	3	3.3	45.54	0.871	-0.0600	0.1652	-0.7821	0.5100	-0.2924	0.3448	-0.4624

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
31/07/2019	A	215	M	4	3.4	46.77	0.893	-0.0491	0.1675	-0.7761	0.5319	-0.2742	0.3645	-0.4384
31/07/2019	A	215	M	5	4.1	36.63	0.445	-0.3516	0.1398	-0.8545	0.3462	-0.4607	0.2064	-0.6853
31/07/2019	A	215	M	6	4.2	40.18	0.577	-0.2388	0.1107	-0.9560	0.3744	-0.4267	0.2637	-0.5789
31/07/2019	A	215	M	7	4.3	46.66	0.949	-0.0227	0.1798	-0.7453	0.5397	-0.2678	0.3600	-0.4438
31/07/2019	A	215	M	8	4.4	46.2	0.791	-0.1018	0.1548	-0.8103	0.4827	-0.3163	0.3279	-0.4842
01/08/2019	B	211	M	1	1.1	31.35	0.261	-0.5834	0.0426	-1.3702	0.0927	-1.0328	0.0501	-1.3003
01/08/2019	B	211	M	2	1.2	35.24	0.382	-0.4179	0.0738	-1.1318	0.2054	-0.6873	0.1316	-0.8808
01/08/2019	B	211	M	3	1.3	42.9	0.671	-0.1733	0.1149	-0.9397	0.3605	-0.4431	0.2456	-0.6098
01/08/2019	B	211	M	4	1.4	44.42	0.838	-0.0768	0.1536	-0.8137	0.4045	-0.3931	0.2509	-0.6005
01/08/2019	B	211	M	5	2.1	32.21	0.275	-0.5607	0.0619	-1.2081	0.1802	-0.7443	0.1182	-0.9272
01/08/2019	B	211	M	6	2.2	35.43	0.38	-0.4202	0.0758	-1.1205	0.1984	-0.7024	0.1227	-0.9113
01/08/2019	B	211	M	7	2.3	42.47	0.654	-0.1844	0.1266	-0.8976	0.2794	-0.5537	0.1529	-0.8157
01/08/2019	B	211	M	8	2.4	45.62	0.813	-0.0899	0.1604	-0.7947	0.5496	-0.2599	0.3892	-0.4098
01/08/2019	B	253	F	1	3.1	31.17	0.54	-0.2676	0.0666	-1.1767	0.1346	-0.8708	0.0681	-1.1671
01/08/2019	B	253	F	2	3.2	40.11	0.513	-0.2899	0.0870	-1.0605	0.3169	-0.4990	0.2299	-0.6384

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
01/08/2019	B	253	F	3	3.3	41.41	0.625	-0.2041	0.1035	-0.9853	0.2914	-0.5355	0.1880	-0.7259
01/08/2019	B	253	F	4	3.4	46.14	0.883	-0.0540	0.1419	-0.8481	0.4116	-0.3855	0.2697	-0.5691
01/08/2019	B	253	F	5	4.1	37.4	0.467	-0.3307	0.1008	-0.9966	0.2087	-0.6806	0.1079	-0.9670
01/08/2019	B	253	F	6	4.2	37.63	0.454	-0.3429	0.0935	-1.0291	0.2699	-0.5688	0.1764	-0.7536
01/08/2019	B	253	F	7	4.3	44.49	0.718	-0.1439	0.1352	-0.8691	0.3937	-0.4048	0.2585	-0.5875
01/08/2019	B	253	F	8	4.4	41.1	0.56	-0.2518	0.1139	-0.9433	0.3149	-0.5019	0.2009	-0.6970
02/08/2019	D	207	M	1	1.1	36.65	0.442	-0.3546	0.0597	-1.2239	0.0728	-1.1376	0.0131	-1.8818
02/08/2019	D	207	M	2	1.2	38.19	0.49	-0.3098	0.0790	-1.1026	0.3182	-0.4972	0.2393	-0.6211
02/08/2019	D	207	M	3	1.3	41.4	0.587	-0.2314	0.0854	-1.0685	0.3298	-0.4817	0.2444	-0.6119
02/08/2019	D	207	M	4	1.4	49.23	1.101	0.0418	0.1840	-0.7352	0.6203	-0.2074	0.4363	-0.3602
02/08/2019	D	207	M	5	2.1	36.23	0.42	-0.3768	0.0577	-1.2389	0.1787	-0.7479	0.1210	-0.9173
02/08/2019	D	207	M	6	2.2	45.19	0.756	-0.1215	0.1120	-0.9506	0.4224	-0.3742	0.3104	-0.5081
02/08/2019	D	207	M	7	2.3	45.63	0.816	-0.0883	0.1260	-0.8995	0.4978	-0.3029	0.3718	-0.4297
02/08/2019	D	207	M	8	2.4	49.56	1.124	0.0508	0.1805	-0.7435	0.6384	-0.1949	0.4579	-0.3392
02/08/2019	C	249	F	1	3.1	33.87	0.34	-0.4685	0.0616	-1.2108	0.2685	-0.5711	0.2069	-0.6842

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
02/08/2019	C	249	F	2	3.2	35.29	0.402	-0.3958	0.0650	-1.1872	0.2677	-0.5724	0.2027	-0.6932
02/08/2019	C	249	F	3	3.3	42.34	0.7	-0.1549	0.1156	-0.9370	0.4246	-0.3720	0.3090	-0.5100
02/08/2019	C	249	F	4	3.4	42.93	0.73	-0.1367	0.1355	-0.8680	0.4817	-0.3173	0.3461	-0.4608
02/08/2019	C	249	F	6	4.2	36.68	0.457	-0.3401	0.0844	-1.0739	0.2647	-0.5772	0.1804	-0.7439
02/08/2019	C	249	F	7	4.3	39.28	0.515	-0.2882	0.0940	-1.0271	0.3576	-0.4465	0.2637	-0.5789
02/08/2019	C	249	F	8	4.4	42.57	0.69	-0.1612	0.1247	-0.9041	0.4417	-0.3548	0.3170	-0.4989
05/08/2019	B	229	M	1	1.1	35.02	0.377	-0.4237	0.0642	-1.1926	0.1869	-0.7285	0.1227	-0.9112
05/08/2019	B	229	M	2	1.2	37.37	0.491	-0.3089	0.0941	-1.0265	0.2621	-0.5816	0.1680	-0.7747
05/08/2019	B	229	M	3	1.3	40.84	0.62	-0.2076	0.1209	-0.9176	0.2771	-0.5574	0.1562	-0.8064
05/08/2019	B	229	M	4	1.4	45.01	0.856	-0.0675	0.1648	-0.7830	0.4275	-0.3691	0.2627	-0.5806
05/08/2019	B	229	M	5	2.1	36.04	0.377	-0.4237	0.0570	-1.2438	0.1907	-0.7197	0.1336	-0.8741
05/08/2019	B	229	M	6	2.2	37.67	0.504	-0.2976	0.0982	-1.0077	0.3268	-0.4857	0.2286	-0.6409
05/08/2019	B	229	M	7	2.3	39.31	0.614	-0.2118	0.1223	-0.9124	0.3227	-0.4912	0.2004	-0.6981
05/08/2019	B	229	M	8	2.4	41.14	0.643	-0.1918	0.1345	-0.8714	0.3685	-0.4335	0.2341	-0.6307
05/08/2019	D	234	F	1	3.1	34.56	0.392	-0.4067	0.1111	-0.9542	0.2744	-0.5617	0.1632	-0.7872

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
05/08/2019	D	234	F	2	3.2	39.65	0.542	-0.2660	0.1346	-0.8709	0.3385	-0.4705	0.2039	-0.6907
05/08/2019	D	234	F	3	3.3	40.15	0.569	-0.2449	0.1419	-0.8479	0.3402	-0.4683	0.1982	-0.7028
05/08/2019	D	234	F	4	3.4	42.78	0.651	-0.1864	0.1440	-0.8415	0.3999	-0.3980	0.2559	-0.5919
05/08/2019	D	234	F	5	4.1	40.29	0.528	-0.2774	0.1212	-0.9164	0.3368	-0.4726	0.2156	-0.6663
05/08/2019	D	234	F	6	4.2	38.84	0.48	-0.3188	0.1243	-0.9054	0.3907	-0.4081	0.2664	-0.5745
05/08/2019	D	234	F	7	4.3	41.1	0.595	-0.2255	0.1160	-0.9356	0.3370	-0.4723	0.2210	-0.6555
05/08/2019	D	234	F	8	4.4	41.11	0.621	-0.2069	0.1519	-0.8183	0.3562	-0.4482	0.2043	-0.6897
06/08/2019	C	223	M	1	1.1	28.91	0.226	-0.6459	0.0541	-1.2665	0.0878	-1.0563	0.0337	-1.4723
06/08/2019	C	223	M	2	1.2	35.62	0.372	-0.4295	0.0686	-1.1634	0.2080	-0.6818	0.1394	-0.8557
06/08/2019	C	223	M	3	1.3	38.59	0.501	-0.3002	0.0956	-1.0197	0.3248	-0.4884	0.2293	-0.6397
06/08/2019	C	223	M	4	1.4	43.23	0.705	-0.1518	0.1310	-0.8828	0.3610	-0.4424	0.2301	-0.6381
06/08/2019	C	223	M	5	2.1	30.94	0.221	-0.6556	0.0546	-1.2631	0.1113	-0.9534	0.0568	-1.2460
06/08/2019	C	223	M	6	2.2	35.57	0.402	-0.3958	0.0762	-1.1181	0.2443	-0.6120	0.1681	-0.7743
06/08/2019	C	223	M	7	2.3	45.09	0.809	-0.0921	0.1180	-0.9282	0.4250	-0.3716	0.3070	-0.5129
06/08/2019	C	223	M	8	2.4	40.58	0.592	-0.2277	0.1195	-0.9227	0.3672	-0.4351	0.2477	-0.6061

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
06/08/2019	B	233	F	1	3.1	33.37	0.314	-0.5031	0.0805	-1.0943	0.2327	-0.6333	0.1522	-0.8177
06/08/2019	B	233	F	2	3.2	36.56	0.424	-0.3726	0.1034	-0.9855	0.2646	-0.5774	0.1612	-0.7926
06/08/2019	B	233	F	3	3.3	42.36	0.666	-0.1765	0.1465	-0.8340	0.4925	-0.3076	0.3459	-0.4610
06/08/2019	B	233	F	4	3.4	51.35	1.23	0.0899	0.2716	-0.5661	0.6129	-0.2126	0.3413	-0.4668
06/08/2019	B	233	F	5	4.1	39.47	0.468	-0.3298	0.1059	-0.9750	0.3052	-0.5154	0.1993	-0.7005
06/08/2019	B	233	F	6	4.2	42.17	0.585	-0.2328	0.1246	-0.9045	0.3646	-0.4381	0.2400	-0.6197
06/08/2019	B	233	F	7	4.3	42.2	0.684	-0.1649	0.1432	-0.8439	0.4038	-0.3938	0.2606	-0.5841
06/08/2019	B	233	F	8	4.4	43.75	0.766	-0.1158	0.1571	-0.8038	0.4220	-0.3747	0.2649	-0.5769
07/08/2019	A	247	F	1	1.1	32.64	0.277	-0.5575	0.0553	-1.2571	0.1432	-0.8441	0.0879	-1.0562
07/08/2019	A	247	F	2	1.2	36.57	0.431	-0.3655	0.0910	-1.0411	0.2217	-0.6543	0.1307	-0.8837
07/08/2019	A	247	F	3	1.3	36.86	0.426	-0.3706	0.0836	-1.0777	0.2730	-0.5638	0.1894	-0.7226
07/08/2019	A	247	F	4	1.4	42.92	0.732	-0.1355	0.1409	-0.8509	0.4412	-0.3554	0.3002	-0.5226
07/08/2019	A	247	F	5	2.1	37.6	0.44	-0.3565	0.0645	-1.1907	0.1729	-0.7622	0.1084	-0.9648
07/08/2019	A	247	F	6	2.2	37.78	0.492	-0.3080	0.0838	-1.0765	0.2439	-0.6129	0.1600	-0.7959
07/08/2019	A	247	F	7	2.3	38.78	0.524	-0.2807	0.1011	-0.9952	0.3400	-0.4686	0.2388	-0.6219

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
07/08/2019	A	247	F	8	2.4	41.85	0.613	-0.2125	0.2010	-0.6969	0.3678	-0.4343	0.1669	-0.7776
07/08/2019	D	256	F	1	3.1	31.77	0.274	-0.5622	0.0644	-1.1911	0.1735	-0.7606	0.1091	-0.9620
07/08/2019	D	256	F	2	3.2	37.67	0.439	-0.3575	0.0775	-1.1110	0.2538	-0.5955	0.1763	-0.7536
07/08/2019	D	256	F	3	3.3	41.57	0.59	-0.2291	0.1029	-0.9874	0.3643	-0.4385	0.2614	-0.5827
07/08/2019	D	256	F	4	3.4	48.02	0.926	-0.0334	0.1928	-0.7148	0.6643	-0.1776	0.4715	-0.3265
07/08/2019	D	256	F	5	4.1	34.03	0.314	-0.5031	0.0744	-1.1287	0.2546	-0.5941	0.1803	-0.7441
07/08/2019	D	256	F	6	4.2	40.07	0.526	-0.2790	0.1209	-0.9174	0.3216	-0.4927	0.2006	-0.6976
07/08/2019	D	256	F	7	4.3	41.86	0.641	-0.1931	0.1432	-0.8442	0.5229	-0.2815	0.3798	-0.4205
07/08/2019	D	256	F	8	4.4	43.9	0.708	-0.1500	0.1412	-0.8501	0.3595	-0.4443	0.2183	-0.6610
08/08/2019	C	202	F	1	1.1	33.83	0.362	-0.4413	0.0811	-1.0910	0.1580	-0.8015	0.0769	-1.1143
08/08/2019	C	202	F	2	1.2	34.52	0.384	-0.4157	0.0903	-1.0442	0.1789	-0.7474	0.0886	-1.0528
08/08/2019	C	202	F	3	1.3	40.4	0.57	-0.2441	0.1464	-0.8345	0.3662	-0.4363	0.2198	-0.6579
08/08/2019	C	202	F	4	1.4	42.54	0.673	-0.1720	0.1324	-0.8781	0.4167	-0.3802	0.2843	-0.5462
08/08/2019	C	202	F	5	2.1	36.81	0.469	-0.3288	0.0796	-1.0992	0.2425	-0.6153	0.1629	-0.7881
08/08/2019	C	202	F	6	2.2	36.95	0.476	-0.3224	0.0861	-1.0650	0.2934	-0.5326	0.2073	-0.6834

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
08/08/2019	C	202	F	7	2.3	38.47	0.493	-0.3072	0.1103	-0.9573	0.3090	-0.5101	0.1986	-0.7019
08/08/2019	C	202	F	8	2.4	40.99	0.638	-0.1952	0.1413	-0.8499	0.4269	-0.3697	0.2856	-0.5442
08/08/2019	B	214	M	1	3.1	34.23	0.364	-0.4389	0.0694	-1.1584	0.2794	-0.5538	0.2099	-0.6779
08/08/2019	B	214	M	2	3.2	39.63	0.602	-0.2204	0.1145	-0.9411	0.3930	-0.4057	0.2784	-0.5553
08/08/2019	B	214	M	3	3.3	41.83	0.665	-0.1772	0.1262	-0.8991	0.4049	-0.3926	0.2788	-0.5547
08/08/2019	B	214	M	4	3.4	53	1.37	0.1367	0.2318	-0.6350	0.5439	-0.2645	0.3121	-0.5057
08/08/2019	B	214	M	5	4.1	36.9	0.414	-0.3830	0.0740	-1.1306	0.2522	-0.5982	0.1782	-0.7491
08/08/2019	B	214	M	6	4.2	41.17	0.663	-0.1785	0.1283	-0.8919	0.3473	-0.4593	0.2190	-0.6596
08/08/2019	B	214	M	7	4.3	45.79	0.832	-0.0799	0.1481	-0.8293	0.5280	-0.2774	0.3798	-0.4204
08/08/2019	B	214	M	8	4.4	48.03	0.959	-0.0182	0.1800	-0.7448	0.4825	-0.3165	0.3025	-0.5193
09/08/2019	B	227	M	1	1.1	33.18	0.318	-0.4976	0.0676	-1.1698	0.1160	-0.9355	0.0484	-1.3155
09/08/2019	B	227	M	2	1.2	39	0.568	-0.2457	0.1187	-0.9257	0.2713	-0.5666	0.1526	-0.8164
09/08/2019	B	227	M	3	1.3	44.05	0.79	-0.1024	0.1568	-0.8048	0.3799	-0.4203	0.2231	-0.6514
09/08/2019	B	227	M	4	1.4	49.23	1.071	0.0298	0.2012	-0.6963	0.6051	-0.2182	0.4039	-0.3938
09/08/2019	B	227	M	5	2.1	35.97	0.441	-0.3556	0.0634	-1.1980	0.2302	-0.6379	0.1668	-0.7778

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
09/08/2019	B	227	M	6	2.2	41.56	0.565	-0.2480	0.1103	-0.9572	0.3261	-0.4866	0.2158	-0.6660
09/08/2019	B	227	M	7	2.3	42.41	0.612	-0.2132	0.1199	-0.9210	0.3664	-0.4360	0.2465	-0.6083
09/08/2019	B	227	M	8	2.4	47.53	1.013	0.0056	0.1845	-0.7341	0.6034	-0.2194	0.4189	-0.3778
09/08/2019	A	239	F	1	3.1	39.17	0.528	-0.2774	0.1133	-0.9457	0.3168	-0.4992	0.2035	-0.6914
09/08/2019	A	239	F	2	3.2	38.23	0.453	-0.3439	0.1034	-0.9855	0.3596	-0.4442	0.2562	-0.5914
09/08/2019	A	239	F	3	3.3	41.8	0.649	-0.1878	0.1280	-0.8928	0.4174	-0.3795	0.2894	-0.5385
09/08/2019	A	239	F	4	3.4	45.84	0.819	-0.0867	0.1771	-0.7519	0.6089	-0.2155	0.4318	-0.3647
09/08/2019	A	239	F	5	4.1	36.08	0.356	-0.4486	0.0722	-1.1414	0.2552	-0.5932	0.1829	-0.7377
09/08/2019	A	239	F	6	4.2	38.73	0.493	-0.3072	0.0997	-1.0014	0.3466	-0.4602	0.2469	-0.6075
09/08/2019	A	239	F	7	4.3	42.08	0.676	-0.1701	0.1374	-0.8622	0.4346	-0.3620	0.2972	-0.5270
09/08/2019	A	239	F	8	4.4	46.16	0.832	-0.0799	0.1679	-0.7749	0.4959	-0.3046	0.3280	-0.4842
12/08/2019	C	209	M	5	2.1	33.44	0.35	-0.4559	0.0451	-1.3462	0.1357	-0.8673	0.0907	-1.0425
12/08/2019	C	209	M	6	2.2	41.27	0.688	-0.1624	0.1017	-0.9926	0.4201	-0.3767	0.3184	-0.4971
12/08/2019	C	209	M	7	2.3	43.97	0.775	-0.1107	0.1150	-0.9392	0.4543	-0.3427	0.3393	-0.4695
12/08/2019	C	209	M	8	2.4	45.76	0.921	-0.0357	0.1553	-0.8089	0.5095	-0.2928	0.3542	-0.4507

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
12/08/2019	A	225	M	1	3.1	34.74	0.346	-0.4609	0.0772	-1.1126	0.2601	-0.5848	0.1830	-0.7376
12/08/2019	A	225	M	2	3.2	37.91	0.608	-0.2161	0.0247	-1.6069	0.3096	-0.5092	0.2848	-0.5454
12/08/2019	A	225	M	3	3.3	42.31	0.704	-0.1524	0.1413	-0.8500	0.5204	-0.2836	0.3792	-0.4212
12/08/2019	A	225	M	4	3.4	49.25	1.064	0.0269	0.2001	-0.6988	0.6568	-0.1826	0.4567	-0.3404
12/08/2019	A	225	M	5	4.1	36.93	0.463	-0.3344	0.0957	-1.0190	0.2866	-0.5427	0.1909	-0.7192
12/08/2019	A	225	M	6	4.2	40.9	0.643	-0.1918	0.1193	-0.9232	0.4190	-0.3777	0.2997	-0.5233
12/08/2019	A	225	M	7	4.3	42.65	0.663	-0.1785	0.1207	-0.9181	0.4470	-0.3497	0.3262	-0.4865
12/08/2019	A	225	M	8	4.4	44.6	0.833	-0.0794	0.1557	-0.8076	0.5087	-0.2935	0.3530	-0.4523
13/08/2019	A	242	F	1	1.1	31.77	0.258	-0.5884	0.0537	-1.2701	0.1112	-0.9538	0.0575	-1.2401
13/08/2019	A	242	F	2	1.2	34.61	0.34	-0.4685	0.0726	-1.1389	0.2078	-0.6823	0.1352	-0.8690
13/08/2019	A	242	F	3	1.3	42.27	0.658	-0.1818	0.1289	-0.8899	0.3889	-0.4102	0.2600	-0.5849
13/08/2019	A	242	F	4	1.4	42.48	0.659	-0.1811	0.1151	-0.9388	0.3721	-0.4293	0.2570	-0.5901
13/08/2019	A	242	F	5	2.1	30.73	0.265	-0.5768	0.0611	-1.2141	0.0895	-1.0483	0.0284	-1.5466
13/08/2019	A	242	F	7	2.3	38.89	0.503	-0.2984	0.1032	-0.9863	0.3098	-0.5089	0.2066	-0.6849
13/08/2019	A	242	F	8	2.4	43.2	0.634	-0.1979	0.1314	-0.8814	0.4717	-0.3263	0.3403	-0.4681

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
13/08/2019	A	257	F	1	3.1	35.09	0.347	-0.4597	0.0793	-1.1006	0.2628	-0.5804	0.1834	-0.7365
13/08/2019	A	257	F	2	3.2	38.45	0.463	-0.3344	0.1160	-0.9356	0.3605	-0.4431	0.2445	-0.6117
13/08/2019	A	257	F	3	3.3	42.8	0.649	-0.1878	0.1184	-0.9266	0.4281	-0.3685	0.3097	-0.5091
13/08/2019	A	257	F	4	3.4	47.96	0.987	-0.0057	0.1760	-0.7544	0.6384	-0.1949	0.4623	-0.3350
13/08/2019	A	257	F	5	4.1	36.5	0.432	-0.3645	0.0931	-1.0310	0.3003	-0.5225	0.2072	-0.6837
13/08/2019	A	257	F	6	4.2	41.66	0.547	-0.2620	0.1187	-0.9255	0.3315	-0.4795	0.2128	-0.6720
13/08/2019	A	257	F	7	4.3	42.34	0.574	-0.2411	0.1099	-0.9591	0.3694	-0.4325	0.2596	-0.5857
13/08/2019	A	257	F	8	4.4	43.35	0.664	-0.1778	0.1368	-0.8638	0.4143	-0.3826	0.2775	-0.5567
14/08/2019	D	213	M	2	1.2	38.52	0.5	-0.3010	0.1194	-0.9232	0.2508	-0.6006	0.1315	-0.8812
14/08/2019	D	213	M	3	1.3	41.16	0.612	-0.2132	0.1411	-0.8505	0.4110	-0.3861	0.2699	-0.5687
14/08/2019	D	213	M	4	1.4	48.3	0.993	-0.0031	0.1912	-0.7185	0.5736	-0.2414	0.3824	-0.4175
14/08/2019	D	213	M	6	2.2	42.47	0.64	-0.1938	0.1113	-0.9536	0.2460	-0.6090	0.1348	-0.8704
14/08/2019	D	213	M	7	2.3	42.74	0.68	-0.1675	0.1273	-0.8951	0.3781	-0.4224	0.2507	-0.6008
14/08/2019	D	213	M	8	2.4	47.31	0.937	-0.0283	0.1790	-0.7472	0.5601	-0.2517	0.3811	-0.4190
14/08/2019	C	216	M	1	3.1	34.17	0.327	-0.4855	0.0838	-1.0769	0.2785	-0.5552	0.1947	-0.7106

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
14/08/2019	C	216	M	2	3.2	40.13	0.518	-0.2857	0.1080	-0.9668	0.4001	-0.3978	0.2922	-0.5344
14/08/2019	C	216	M	3	3.3	45.17	0.777	-0.1096	0.1536	-0.8136	0.4212	-0.3755	0.2676	-0.5725
14/08/2019	C	216	M	4	3.4	49.7	1.085	0.0354	0.1851	-0.7326	0.6036	-0.2193	0.4185	-0.3783
14/08/2019	C	216	M	5	4.1	36.94	0.383	-0.4168	0.0581	-1.2360	0.2634	-0.5794	0.2053	-0.6876
14/08/2019	C	216	M	6	4.2	42.54	0.582	-0.2351	0.1156	-0.9369	0.3450	-0.4622	0.2293	-0.6395
14/08/2019	C	216	M	7	4.3	43.02	0.689	-0.1618	0.1376	-0.8615	0.3763	-0.4244	0.2388	-0.6220
14/08/2019	C	216	M	8	4.4	52.61	1.165	0.0663	0.1796	-0.7456	0.7058	-0.1513	0.5261	-0.2789
15/08/2019	D	205	M	1	1.1	38.51	0.489	-0.3107	0.0850	-1.0704	0.2039	-0.6905	0.1189	-0.9248
15/08/2019	D	205	M	2	1.2	38.72	0.527	-0.2782	0.1148	-0.9401	0.2473	-0.6068	0.1325	-0.8779
15/08/2019	D	205	M	3	1.3	41.91	0.621	-0.2069	0.1291	-0.8891	0.4023	-0.3955	0.2732	-0.5635
15/08/2019	D	205	M	4	1.4	48.35	0.939	-0.0273	0.2008	-0.6972	0.5590	-0.2526	0.3581	-0.4459
15/08/2019	D	205	M	5	2.1	37.36	0.469	-0.3288	0.0822	-1.0850	0.2334	-0.6320	0.1512	-0.8206
15/08/2019	D	205	M	6	2.2	38.44	0.508	-0.2941	0.0767	-1.1155	0.2676	-0.5725	0.1910	-0.7190
15/08/2019	D	205	M	7	2.3	44.67	0.771	-0.1129	0.1310	-0.8829	0.4557	-0.3413	0.3248	-0.4884
15/08/2019	D	205	M	8	2.4	49.57	1.129	0.0527	0.1853	-0.7322	0.6102	-0.2145	0.4249	-0.3717

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
15/08/2019	B	236	F	1	3.1	37.85	0.483	-0.3161	0.1120	-0.9508	0.4219	-0.3748	0.3099	-0.5088
15/08/2019	B	236	F	2	3.2	41.92	0.616	-0.2104	0.1261	-0.8992	0.3093	-0.5096	0.1832	-0.7371
15/08/2019	B	236	F	3	3.3	46.93	0.956	-0.0195	0.1800	-0.7447	0.5183	-0.2854	0.3383	-0.4707
15/08/2019	B	236	F	4	3.4	56.65	1.659	0.2198	0.3114	-0.5067	0.8163	-0.0882	0.5049	-0.2968
15/08/2019	B	236	F	5	4.1	36.55	0.44	-0.3565	0.0978	-1.0095	0.3466	-0.4602	0.2487	-0.6043
15/08/2019	B	236	F	6	4.2	39.93	0.511	-0.2916	0.0991	-1.0040	0.2794	-0.5537	0.1804	-0.7439
15/08/2019	B	236	F	7	4.3	43.92	0.736	-0.1331	0.1608	-0.7936	0.4841	-0.3150	0.3233	-0.4904
15/08/2019	B	236	F	8	4.4	48.83	1.102	0.0422	0.2372	-0.6248	0.5826	-0.2347	0.3453	-0.4618
16/08/2019	D	212	M	1	1.1		0.288	-0.5406	0.0676	-1.1703	0.1750	-0.7569	0.1074	-0.9688
16/08/2019	D	212	M	2	1.2	34.68	0.36	-0.4437	0.0801	-1.0963	0.2416	-0.6168	0.1615	-0.7917
16/08/2019	D	212	M	3	1.3	44.73	0.801	-0.0964	0.1779	-0.7498	0.5118	-0.2909	0.3339	-0.4763
16/08/2019	D	212	M	4	1.4	45.51	0.853	-0.0691	0.2026	-0.6933	0.6622	-0.1790	0.4595	-0.3377
16/08/2019	D	212	M	5	2.1	33.9	0.363	-0.4401	0.0734	-1.1341	0.1878	-0.7262	0.1144	-0.9415
16/08/2019	D	212	M	6	2.2	38.43	0.55	-0.2596	0.1050	-0.9787	0.2466	-0.6080	0.1416	-0.8490
16/08/2019	D	212	M	7	2.3	40.94	0.469	-0.3288	0.1054	-0.9771	0.3766	-0.4242	0.2712	-0.5668

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
16/08/2019	D	212	M	8	2.4	47.58	0.89	-0.0506	0.2232	-0.6513	0.5670	-0.2464	0.3438	-0.4637
16/08/2019	C	241	F	1	3.1	32.91	0.323	-0.4908	0.0848	-1.0716	0.3036	-0.5177	0.2188	-0.6599
16/08/2019	C	241	F	2	3.2	35.75	0.397	-0.4012	0.1076	-0.9684	0.3309	-0.4802	0.2234	-0.6509
16/08/2019	C	241	F	3	3.3	40.42	0.596	-0.2248	0.1597	-0.7968	0.4474	-0.3493	0.2877	-0.5410
16/08/2019	C	241	F	4	3.4	51.81	1.219	0.0860	0.2116	-0.6746	0.9640	-0.0159	0.7524	-0.1235
16/08/2019	C	241	F	5	4.1	31.88	0.256	-0.5918	0.0762	-1.1178	0.2760	-0.5591	0.1998	-0.6995
16/08/2019	C	241	F	6	4.2	41.79	0.569	-0.2449	0.1238	-0.9072	0.4980	-0.3028	0.3742	-0.4269
16/08/2019	C	241	F	7	4.3	43.03	0.737	-0.1325	0.1301	-0.8856	0.4664	-0.3312	0.3363	-0.4733
16/08/2019	C	241	F	8	4.4	47.89	0.874	-0.0585	0.1396	-0.8552	0.5252	-0.2797	0.3856	-0.4139
17/08/2019	C	220	M	1	1.1	36.32	0.447	-0.3497	0.0716	-1.1452	0.1582	-0.8009	0.0866	-1.0626
17/08/2019	C	220	M	2	1.2	36.95	0.488	-0.3116	0.1006	-0.9973	0.3654	-0.4372	0.2648	-0.5771
17/08/2019	C	220	M	3	1.3	40.88	0.632	-0.1993	0.1269	-0.8964	0.3871	-0.4122	0.2601	-0.5848
17/08/2019	C	220	M	4	1.4	43.32	0.732	-0.1355	0.1666	-0.7782	0.5114	-0.2912	0.3448	-0.4624
17/08/2019	C	220	M	5	2.1	38.65	0.473	-0.3251	0.0751	-1.1244	0.2187	-0.6601	0.1436	-0.8428
17/08/2019	C	220	M	6	2.2	38.16	0.47	-0.3279	0.0638	-1.1953	0.2419	-0.6164	0.1781	-0.7494

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
17/08/2019	C	220	M	7	2.3	40.16	0.596	-0.2248	0.1079	-0.9669	0.3775	-0.4231	0.2696	-0.5693
17/08/2019	C	220	M	8	2.4	45.01	0.784	-0.1057	0.1492	-0.8263	0.5471	-0.2619	0.3979	-0.4002
17/08/2019	A	248	F	1	3.1	32.08	0.292	-0.5346	0.0721	-1.1423	0.2522	-0.5982	0.1802	-0.7443
17/08/2019	A	248	F	2	3.2	41.9	0.593	-0.2269	0.1271	-0.8957	0.4492	-0.3476	0.3220	-0.4921
17/08/2019	A	248	F	3	3.3	44.62	0.847	-0.0721	0.1551	-0.8095	0.6063	-0.2173	0.4513	-0.3456
17/08/2019	A	248	F	4	3.4	50.66	1.227	0.0888	0.2415	-0.6170	0.9190	-0.0367	0.6775	-0.1691
17/08/2019	A	248	F	6	4.2	39.93	0.528	-0.2774	0.1176	-0.9297	0.3800	-0.4202	0.2625	-0.5809
17/08/2019	A	248	F	7	4.3	44.35	0.763	-0.1175	0.1530	-0.8152	0.6108	-0.2141	0.4578	-0.3393
17/08/2019	A	248	F	8	4.4	45.23	0.807	-0.0931	0.1496	-0.8252	0.5995	-0.2222	0.4500	-0.3468
18/08/2019	A	232	M	1	1.1	30.85	0.28	-0.5528	0.0609	-1.2151	0.1710	-0.7671	0.1100	-0.9585
18/08/2019	A	232	M	2	1.2	33.02	0.323	-0.4908	0.0776	-1.1100	0.1872	-0.7278	0.1095	-0.9604
18/08/2019	A	232	M	3	1.3	40.4	0.583	-0.2343	0.1226	-0.9115	0.3943	-0.4042	0.2717	-0.5659
18/08/2019	A	232	M	4	1.4	49.78	1.114	0.0469	0.2522	-0.5982	0.7159	-0.1452	0.4636	-0.3338
18/08/2019	A	232	M	5	2.1	40.41	0.539	-0.2684	0.0998	-1.0010	0.3306	-0.4808	0.2308	-0.6368
18/08/2019	A	232	M	6	2.2	41.9	0.652	-0.1858	0.1082	-0.9658	0.3251	-0.4880	0.2169	-0.6638

Date	Treatment	Compartment	Parental Sex	Fish ID	Chamber	Fork Length	Mass	log(Mass)	SMR Abs	log(SMR Abs)	MMR Abs	log(MMR Abs)	AS Abs	log(AS Abs)
18/08/2019	A	232	M	7	2.3	41.98	0.622	-0.2062	0.1338	-0.8735	0.4031	-0.3946	0.2693	-0.5698
18/08/2019	A	232	M	8	2.4	49.68	1.074	0.0310	0.2010	-0.6968	0.6090	-0.2154	0.4080	-0.3893
18/08/2019	C	238	F	1	3.1	39.07	0.478	-0.3206	0.0966	-1.0151	0.3751	-0.4258	0.2785	-0.5551
18/08/2019	C	238	F	2	3.2	39.9	0.602	-0.2204	0.1336	-0.8741	0.4499	-0.3469	0.3163	-0.5000
18/08/2019	C	238	F	3	3.3	43.71	0.691	-0.1605	0.1443	-0.8408	0.4812	-0.3177	0.3369	-0.4725
18/08/2019	C	238	F	4	3.4	47.68	0.929	-0.0320	0.1695	-0.7708	0.5942	-0.2261	0.4247	-0.3720
18/08/2019	C	238	F	6	4.2	38.85	0.48	-0.3188	0.0865	-1.0630	0.3398	-0.4688	0.2533	-0.5964
18/08/2019	C	238	F	7	4.3	42.52	0.681	-0.1669	0.1303	-0.8851	0.5065	-0.2954	0.3762	-0.4246
18/08/2019	C	238	F	8	4.4	45.64	0.77	-0.1135	0.1587	-0.7995	0.5345	-0.2721	0.3758	-0.4250

Chapter 6: General Discussion

This thesis aimed to examine how catch and release (C&R) angling of wild adult Atlantic salmon (*Salmo salar*), shortly prior to spawning, affected both the parents and offspring. To achieve this, pre-spawned salmon underwent a simulated C&R protocol with pre-determined periods of exercise and air exposure. There was also a control group present that experienced the whole process of collection, tagging and housing, similar to the disturbed fish, and which was used as a baseline comparison for the additional stressors (exercise and air exposure). The offspring of each family, produced by crossing an experimentally treated parent with a non-experimental parent, were then tracked during the first year of their life (November 2018 – October 2019). This provided a comprehensive overview of how stressors related to C&R angling not only influenced the reproductive success of the parents (Chapter 2), but also the development and phenotype of the offspring (Chapter 3 – 5).

Chapter 1, which was a review of the current published literature, determined that one way to combat the consistent international drop in Atlantic salmon numbers (MacCrimmon and Got, 1979; Parrish et al., 1998; Gibson et al., 1993; Friedland et al., 2009; Chaput et al 2012; Mills et al., 2013; Lenders et al., 2016), is the conservation initiative known as C&R angling (Cooke and Schramm, 2007; Wedemeyer and Wydoski, 2008; Smukall et al., 2019). Recently, this initiative was also adopted by the Scottish government (Marine Scotland, 2018a; Marine Scotland, 2018b; Marine Scotland, 2018c; Scottish Government, 2018). One issue with this approach, however, is that even though a large amount of research has been conducted on the effects of C&R angling on the survival, physiology, behaviour and reproduction of adults fish (Ferguson and Tufts, 1992; Tuft et al., 2000; Cooke et al., 2002; Dempson et al., 2002; Suski et al., 2004; Arlinghaus et al., 2007; Thompson et al., 2008; Donaldson et al., 2011; Olsen et al., 2010; Arlinghaus et al., 2013; Richard et al., 2013; Donaldson et al. 2014; Richard et al., 2014; Cook et al., 2015; Lennox et al., 2015; Raby et al., 2015), there is almost no information on the effects of catch and release angling on offspring (exceptions being Campbell et al., 1992; Booth et al., 1995; Smukall et al., 2019). Yet, this does not mean that the possible

impacts of C&R angling on offspring cannot be anticipated, since a tremendous amount of research has been conducted on disentangling the effects (both positive and negative) that various other parental stressors, or experimental increases in levels of the stress hormone cortisol (release during the stress response from the HPI axis), can have on offspring (McCormick, 1998; Eriksen et al., 2006; Eriksen et al., 2007; Sloman, 2010; Andersson et al., 2011; Eriksen et al., 2013; Sopinka et al., 2014; Eaton et al., 2015; Stringwell, 2015; Ghio et al., 2016; Sopinka et al. 2016a; Sopinka et al., 2016b; Taylor et al. 2016; Bautista and Burggren, 2019; Lehto and Tinghitella, 2019; Warriner et al., 2020). However, most of these investigations have examined the effects of maternal stress on the initial developmental stages of the progeny (Eriksen et al., 2006; Eriksen et al., 2007; Allen et al., 2008; Eriksen et al., 2015; Thayer et al., 2018), and only a handful have studied the influences on offspring performance beyond this point (Eriksen et al., 2007; Andersson et al., 2011). Moreover, there has been little research into the effects of paternal stress on offspring, as until recently it was believed that the effects of parental stress were only transmitted by the mother.

To put the effects of C&R on the adults into perspective (in relation to what is already known), this study started by investigating the effects that a C&R simulation can have on the survival, reproduction and vulnerability to disease (the universal fungus *Saprolegnia* spp.) of wild adult Atlantic salmon close to spawning (within 5 – 18 days before spawning; Chapter 2). The results revealed that exercise and air exposure had no effect on the immediate mortality of the adults. This corresponds with previous findings on Atlantic salmon (Thorstad et al., 2007; Havn et al., 2015; Lennox et al., 2016; Van Leeuwen et al., 2021), Pacific salmon (*Oncorhynchus* spp.; Raby et al., 2013; Donaldson et al., 2014), and Steelhead (*Oncorhynchus mykiss*; Whitney et al., 2019). As discussed in Chapter 2, salmonids might be physiologically equipped to deal with such acute stressors due to their anadromous nature, as well as their ability to overcome extreme obstacles as they undertake such a demanding spawning migration while relying on stored reserves (Raby et al., 2013; Elmer, 2020; Whitney et al., 2019). In the present study, adults in the disturbed groups 'exercise' and 'exercise + extended air' experienced a substantial increase in the percentage body cover of the fungus *Saprolegnia* spp. infection over time. Under the controlled conditions of this investigation the fungus did not seem to affect adult mortality.

Saprolegnia infection could however have other effects on adults. Firstly, it could cause discomfort, as well as disrupt the ability to swim effectively (i.e. either by restricting locomotion or diminishing aerobic capacity (as could occur if the fungus spreads over the gills), making it difficult to swim against the current or leap over obstacles (natural – waterfall, or man-made – dams). This in turn could slow down their migration or force them to migrate over shorter distances. Arriving late at spawning grounds could reduce the reproductive output of angled individuals, since most individuals would have already spawned. Cutting their migration short could also adversely affect their reproduction since fewer individuals would be available to spawn. Downstream spawning grounds could also be sub-optimal, with poorer quality spawning or rearing substrates (e.g. too silted). Similarly, the fungal infection on adult females might impact their skills to successfully form and cover redds, which could restrict the number of eggs deposited or allow eggs to get dislodged by the current during later months. Males may experience constrained competitive potential when trying to compete for mates against conspecifics. There is also evidence that infection in the wild prior to spawning may leave salmonids more vulnerable to secondary stressors (e.g. higher water temperatures) and opportunistic pathogens (Bordeleau et al., 2018; Elmer, 2020). This could intensify the adverse effects of enhanced fungal infection of the adults already discussed.

The C&R simulation altered several important traits that could influence the reproductive success of salmon. Males that experienced 'exercise + extended air' produced sperm that survived for a longer duration of time once activated. At highly competitive spawning grounds, this change in sperm phenotype could be considered as a disadvantage, as previous investigations have demonstrated a trade-off between sperm velocity and longevity (Levitan, 2000; Lehnert et al., 2018; Taborsky et al., 2018). This might mean that the sperm of undisturbed or less disturbed males would swim faster and so reach and fertilize the ovum first, making sperm longevity irrelevant. However, if males spawn where there is lower conspecific competition, or where mates are rarer (e.g. further downstream from optimal spawning grounds, or near the C&R event), then increased sperm longevity could be an advantage. In females, the stressors (exercise + air exposure) that were presumed to represent the disturbance of a typical catch and release practice caused a reduction in clutch size. This result was also revealed by other studies that examined the effects of air

exposure or increased maternal cortisol (McConnachie et al., 2012; Richard et al., 2013; Cook et al., 2015). Since the eggs had already developed by the time the stressors were applied, this indicates that the eggs were lost rather than never produced or resorbed (re-absorbed). This would result in a smaller quantity of eggs being available for males to fertilize during spawning. If the proportion of female salmon caught through C&R angling is too high, this could reduce the future recruitment of the species as there will not be enough offspring to replace the current population. A contrasting scenario is where the eggs lost from stress may be compensated by a drop in mortality after emergence, mainly due to a reduction in competition for the available resources and through diminished predation risk. Another point to consider are the findings of Burton et al (2013), where depending on the position of the eggs within the female's ovary, the offspring exhibit differences within traits (i.e. social dominance rank and initial size). If the eggs lost from the disturbed females were from specific locations of the ovary, and not lost equally across the egg mass, then this could reduce the variability in traits expressed by the offspring of those females. This reduction in variability was identified in a few of the traits measured within this thesis – i.e. reduced within-family variability in yolk sac volume for offspring from parents exposed to air (Chapter 3), and reduced within and among family variability on emergence time in offspring whose parents were either exercise and/ or air exposed (Chapter 4).

Chapters 3 – 5 followed the life of the offspring, rather than the parents, with chapter 3 focusing on early development. This investigation revealed that exposing the parents to the air for 120s after intense exercise resulted in higher egg mortality. This could suggest that even though the loss of progeny from the C&R angling groups may be low at each developmental stage (see chapter 2 & 3), it could potentially accumulate to a significant loss in population that could affect future recruitment. Furthermore, air exposure can decrease across-family variability, in addition to the within-family variability, as more individuals from non-angled parents will have the capacity to survive to the next developmental stage due to a reduction in competition for available niche. It is important to note, however, that most of the mortality was caused during shocking (the aquaculture practice used to reveal non-viable eggs), with egg mortality prior to this point being insignificant. This agrees with previous findings on salmonids (Atlantic, chum, sockeye salmon and brown

trout) which show that neither direct nor indirect manipulation of egg cortisol nor C&R affect egg viability (Booth et al., 1995; Sloman, 2010; Sopinka et al., 2016a; Sopinka et al., 2016b; Smukall et al., 2019). This indicates that the stressors (exercise and air exposure) themselves might not be intense enough to reduce viability yet can leave the eggs vulnerable to additional disturbances, such as a flash flood. Furthermore, the yolk sac of the eggs was smaller in the offspring whose parents' experienced disturbance of any duration. Yolk sac reserves are important for the developing embryo, especially in salmonids, who cannot produce their own essential developmental hormones (McCormick et al., 1998; Eriksen et al., 2007; Andersson et al., 2011). The reserves also provide the offspring with the energy necessary to transition from endogenous to exogenous feeding, diminishing the likelihood of starvation (McCormick et al., 1998; Eriksen et al., 2006). Even though the yolk sac was reduced in groups with disturbed parents, there was still no detectable differences in the offspring's date of first feeding. Offspring whose parents were air exposed however, for any duration of time, had a shorter fork length at first feeding (restricted structural development). Analogous results were detected in farmed female Atlantic salmon and Pacific salmon that were exposed to elevated cortisol concentration, by endogenous and exogenous techniques respectively (Eriksen et al., 2006; Capelle et al., 2017). In the current study, it was also observed that offspring size at first feeding was smaller when the female was disturbed. This outcome is logical as all the nutrients incorporated into the embryo are from the female (Warriner et al., 2020).

Three months post feeding, there was no longer any difference in fork length across treatment, with this consistency being maintained for the duration of the experiment. One explanation is that the offspring from disturbed groups had a growth spurt, which allowed them to catch up with the control group, especially since no food restriction were applied to the offspring (offspring fed to excess). Another possible justification is that the smaller individuals died off from size-selective mortality, leaving behind the bigger siblings. In nature, where food is not introduced at a consistent rate, and where there may even be periods of limited availability, the mortality of individuals of dissimilar size would be more pronounced (Einum & Fleming, 1999). Larger, more dominant fish can take control of the most profitable habitats, along with all the resources, while smaller individuals will be

pushed to the side to fade away (Reid et al., 2011; Sanchez-Gonzales and Nicieza, 2021). Smaller fish are also more vulnerable to predation, especially if they do not have the reserves to escape. Under laboratory conditions, offspring mortality within the first three months post feeding, was highest in families whose parents were air exposed. When this mortality was investigated further, it was discovered that the shift in mortality for the air exposed groups was triggered by the 12-day *Saprolegnia* spp. fungal outbreak in the system. As with the egg mortality, this could imply that the stressor of C&R might not be intense enough to kill the salmon but could leave the offspring susceptible to secondary disturbances or infections/ disease. Since *Saprolegnia* is a ubiquitous, opportunistic fungus that can affect salmonids in the wild (Casselman, 2005; Wedemeyer and Wydoski, 2008; Arlinghaus et al., 2013; Smukall et al., 2019), this could lead to a more serious problem for the species. Offspring of parents who experience C&R would have a higher probability of being infected with the fungus, both as juveniles and as adults which could: 1) lower their probability of first reaching adulthood; and if they manage that, 2). suffer high infection rates when they return to spawn. For this reason, it is critical to not only recognize the direct, but also the indirect implications of C&R angling of salmon.

Chapter 4 went a step further and examined how stressors related to C&R angling of adults close to the time of spawning affected the behaviour of offspring during early life. This is an area where very little previous research has been conducted and our knowledge has been limited. The investigation revealed that offspring whose parents were either exercised, or exercised and then air-exposed for an extended period, exhibited less locomotor activity and exploration of a novel environment. Reduction in the activity of progeny of stressed parents was previously implied by other investigations on salmonids, where pre-spawned maternal stress was simulated by either direct (cortisol implant in the female) or indirect (cortisol bath for the eggs) manipulation of the cortisol concentration (Eriksen at al., 2006; Burton et al., 2011; Sopinka et al. 2016b). Interestingly, individuals from the 'exercise + extended air' group in the present study explored more of the novel environment as the size of the offspring increased. In a high-risk environment, where conditions can be unpredictable, obtaining a low activity and exploration may be favourable. This makes individuals less visible, and thus drops the probability of being eaten by a predator. For example, female largemouth bass (*Micropterus salmoides*) whose

cortisol levels were raised through intraperitoneal injections, became motionless/inactive for an extended period once a predator was introduced (Redfern et al., 2017). Yet, larger experimental fish explored more of the novel environment, as their risk-benefit analysis indicated a greater advantage in locating the best territory compared to the risk of predation. Bigger fish lower the chances of being eaten through the gape-limitations of the hunter, and because they can escape faster. Offspring from the group's 'exercise' and 'exercise + extended air' also showed a higher level of aggression compared to individuals from the control group. Eaton et al. (2015) showed similar results in offspring aggression when they subjected female guppies (*Poecilia reticulata*) to a mild stressor (routine husbandry procedure) prior to reproduction. Displaying higher levels of aggression might be beneficial to fish, especially when it comes to juvenile salmon that must establish territories to ensure their survival and success in the wild, as it offers them a competitive edge against conspecifics, particularly when they emerge into an unpredictable environment (Royle et al., 2001; Sloman, 2010; Eaton et al., 2015; Ahmed et al., 2016).

Lastly, Chapter 5 focused on how simulated C&R angling of parents affects metabolism and social dominance of offspring. The data revealed that there was no difference in offspring SMR alive at a specific temperature, across the treatment groups. In addition, offspring MMR and AS were lower in treatments where the experimental parents were exercised. AS is associated with other behavioural attributes including activity (Halsey et al., 2018; Hollins et al., 2018). A diminished AS could lead to a decrease in the spontaneous activity of individuals, possibly reducing their predation risk and likelihood of being captured by angling (Killen et al., 2015a; Redfern et al. 2017; Hollins et al., 2018). On the other hand, this could also mean that if spotted by a predator, they may be less reactive or less able to recover from an escape response (Killen et al., 2015b). In contrast, displaying a higher AS can be favourable, as it is linked to other traits such as dominance, boldness, and growth rate (Auer et al., 2015; Eliason and Farrell, 2016). The stressors 'exercise' and 'exercise + extended air-exposure' also affected the dominance of the offspring, where in both treatment groups the individuals were overall subordinate to offspring whose parents were not disturbed. A dominant individual has a higher probability of acquiring the most profitable feeding territory

and the associated benefits (e.g. food, shelter and water flow), however this may also result in a greater cost of living while defending a territory against conspecifics, and maintaining a high activity and growth (Gilmour et al., 2005; Schjolden et al., 2009; Reid et al., 2011; Hoogenboom et al., 2013). The dominance trials in this study produced some very interesting and dynamic results. Although offspring from disturbed parents were more aggressive than those from controls (Chapter 4), this did not translate into higher dominance status. Indeed, offspring from the control group dominated over offspring from disturbed parents on the first 2 days of the trial, but on the third day no dominance was detected across treatments (Chapter 5). In a controlled environment, this shift in dominance was possible, primarily due to the offspring being raised under optimal conditions (i.e. fed to excess, ideal water temperature and no predation). In the wild this shift in dominance would be unlikely, firstly due territorial disputes rarely lasting 3 days, and secondly because once a difference in dominance status is formed between two conspecifics it tends to grow. This is mainly due to the cumulative effect of reduced feeding opportunities or sub-standard nutrition on body condition and growth rates, poorer microhabitat availability leading to exposure to stronger water flows or greater predation risk, so making it difficult for subordinates to reverse their social standing.

The behavioural (activity, exploration, aggression and dominance) and physiological traits (metabolism) expressed by the fish in chapters 4 and 5 are important to record and understand, because they might not only be linked to the life history of the offspring as juveniles, but as future adults as well. This might be an important area of future research, as it can provide information on how the dynamics and traits of fish populations are altered over time by human activities such as C&R. For example, the reduced activity expressed by the 'exercise' and 'exercise + extended air' treatments in the freshwater environment as juveniles, may translate to reduced swimming performance of the fish in the marine environment. This in turn may result in lower overall food consumption, or a narrower, more targeted feeding diet, that may lead to reduced growth and fitness. Lower activity might also make them more vulnerable to natural (once spotted by a predator, they won't have the capacity to escape it) and anthropogenic predation (fish with lower activity will be less able to outswim a trawl net). Another example is the increased aggression expressed by the 'exercise' and 'exercise + air' treatments, where as adults this may assist in the

acquisition of mates, especially during male-to-male competition. This however is an area not yet examined and which requires further investigating.

6.1 Practical Applications

Based on the findings from the simulated C&R angling protocol (see chapter 2 – 5 and summary table 1), it is clear that this form of conservation management could have adverse effects on both adults and offspring if not implemented properly. Most of the effects are a result of the parents being exposed to air (especially for a prolonged period), therefore during an angling event air exposure should be minimized as much as possible and should definitely not exceed 60 s (Ferguson and Tuft, 1992; Killen et al., 2003). It is advised, that if feasible, adult pre-spawned salmon should not be removed from the water while the hook is removed and when photographs or measurements of the fish are taken. Furthermore, handling of the fish should be limited so as to reduce the spread of fungus *Saprolegnia* spp.. This is because both exercise and air exposure leave salmon more vulnerable to the spread of fungus after release. It should be noted that the C&R simulations conducted in this project were carried out very close to the time of spawning (and so after the end of the normal fishing season in Scotland, and greater UK area), and comparable information is lacking on the impacts on both parents and offspring of earlier C&R. It is not known whether adults caught earlier would recover more fully from the experience, so that C&R would have fewer effects on their offspring. However, given the range and extent of adverse effects recorded in this study, it is clear that the season for C&R should not be extended close to the time of spawning.

Table 1. Summary of all the results presented in the thesis in simplified form. For more detailed analysis see corresponding chapter as indicated in the table. Blank cell means no significant difference compared to the control; ↑ and ↓ means significant increase or decrease respectively in the specified group compared to the control; N/A means no information available.

		Exercise	Exercise + Air Exposure	Exercise + Extended Air Exposure
Adults				
Ch. 2	Mortality			
	Fungal Infection	↑		↑
	Sperm Quantity			
	Sperm Survivability			↑
	Females Ripeness			
	Egg Volume			
	Clutch Size	↓	↓	↓
Offspring				
Ch. 3	Total Egg Mortality			↑
	Immediate Egg Mortality Prior to Eyed Stage			
	Egg Mortality - Shocking			↑
	Yolk Sac Volume	↓	↓	↓
	Date of First Feeding			
	Size at First Feeding			↓
	Growth Rate			
	Total Fry Mortality		↑	↑
	Fry Fungal Mortality		↑	↑
	Fry Residual Mortality			
Ch. 4	Risk-Taking Traits			
	Activity	↓		↓
	Exploration			↓
	Exploration: Mass			↑
	Aggression	↑	↑	
Ch. 5	Standard Metabolic Rate			
	Maximum Metabolic Rate	↓		
	Aerobic Scope	↓		
	Overall Dominance	↓	N/A	↓
	Dominance Trial 1 (Day 1)	↓	N/A	↓
	Dominance Trial 2 (Day 2)	↓	N/A	↓
	Dominance Trial 3 (Day 3)			

6.2 Conclusion

In my thesis I have shown that two of the main stressors related to catch and release angling, namely exercise (time take to land a fish) and air exposure, can have multiple significant sublethal effects on both the wild adult pre-spawned Atlantic salmon and their offspring if experienced close to the time of spawning. I have demonstrated that these stressors can influence particular reproductive traits, of both male and female adult salmon, that could affect their future reproductive success. I have also shown that the presence and intensity/duration of the disturbance experienced by the parents during a C&R angling event close to the time of spawning can affect numerous characteristics in the offspring during the first year of their life that can alter both their phenotype and life history. It is important to highlight that virtually all traits examined in the offspring were equally affected, regardless of which parent (male or female) experienced the C&R simulation. Therefore, it is necessary to determine both maternal and paternal influences to establish the whole spectrum of effects caused by a stressful situation on the offspring. This was not anticipated, as the majority of the hormones and nutrients provided to offspring come from the mother, and thus the intensity of influence was expected to be higher in that direction. However, as Immler (2018) summarises, there are numerous non-genetic and epigenetic pathways by which males can alter the phenotype of the offspring, including DNA methylation, proteins, RNAs and histone modifications. In any case, the mechanism by which males who experience C&R angling influence the offspring needs further investigation.

My research has assisted in our understanding of the effects that C&R angling of the parents can have on essential morphological (i.e. structural development), behavioural (i.e. activity, exploration, aggression and dominance) and physiological (i.e. metabolic rate) traits of the offspring. It has also revealed that we shouldn't only investigate the direct effects of C&R angling, but also how it can indirectly influence the progeny (e.g. through vulnerability to disease). Lastly, to reduce most of the effects on the offspring, adult pre-spawned salmon caught by angling should not be removed from the water during hook removal and while taking a photo or

measurements of the fish. If it is necessary to remove the fish from the water, then air exposure should not exceed 60 s.

6.3 References

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