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Interactions Between Migrating Salmonids and Low-Head Hydropower Schemes

BY

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Abstract

The redevelopment of existing riverine barriers with modern hydropower turbines is becoming increasingly prevalent on main stem rivers with valuable stocks of migratory salmonids. This is a concern because these fish rely on longitudinal connectivity to complete their lifecycles, and modifications for hydropower could jeopardize that connectivity by obstructing or injuring migrating fish. In order to exploit very low head hydropower potential, novel turbine types are emerging. The Archimedean screw hydropower turbine is one such technology which is becoming increasingly popular for low-head applications. However the impact of these turbines on fish movements remains largely untested. This thesis aims to provide much needed evidence on the effects that these turbines and schemes may have on migrating salmonids.

Fixed radio and passive integrated transponder receivers were used to track the downstream movements of wild migrating juvenile salmonids through a low-head Archimedean screw hydropower scheme. Atlantic salmon smolts were found to pass through the alternative routes of the turbine and main river channel in proportion to flow through these channels. Passage times were generally fast through both routes (median = 17.6, range = 5.1-905.6 minutes over the 350m scheme extent, for radio tagged fish), and longer passage times were associated with daytime presence in both routes. The majority of PIT tagged Atlantic salmon and sea trout smolts that passed through the 100 m long turbine channel, did so in under 27 minutes (median = 6.8 minutes), whilst a few fish had much longer passage times, associated with daytime presence. There were no differences in onward survival (measured as distance survived downstream) between turbine passed and non-turbine passed migrants.

Atlantic salmon smolts were passed through an Archimedean screw turbine to test for harmful effects from the turbine, with comparison to equivalently handled non-turbine passed smolts. There was no evidence of visible damage aside from low to moderate scale loss, which was not significantly associated with turbine passage. Blood chemistry parameters were used to test for subtle turbine-induced damage. This novel application of these techniques did not yield conclusive results, but serves as a useful precedent for future studies.

Radio and PIT telemetry equipment were used to investigate the movements of upstream migrating adult salmonids at three separate low-head hydropower schemes which may act as obstacles to migration. These schemes each had distinct configurations and flow management regimes. Movements within, and progression beyond these schemes varied substantially between sites, and in some cases were related to flow management parameters. Whilst not conclusive, the results suggest that scheme configuration and the management of flows influence the time that fish spend at such schemes, and the proportion of fish that ascend beyond them.

With the global shift towards renewable energy generation, the exploitation of running water for hydropower is likely to become increasingly pervasive. The results of these studies provide valuable information for the informed and ecologically sustainable development of low-head hydropower schemes.

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Author's Declaration

I declare that the work presented in the thesis is entirely my own except where specifically acknowledged or cited. No part of this thesis has been submitted for any other degree or qualification.

Robert Brackley

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Chapter 1

General Introduction

Many riverine fish species make extensive movements within rivers, and between freshwater and marine environments, as part of their lifecycles (Lucas & Baras, 2001). The modification of these migration pathways with hydropower dams, diversions, and turbines can halt, obstruct, kill or injure migrating fish, resulting in the decline or extermination of affected populations (e.g. Aarestrup & Koed 2003; Gauld *et al.*, 2013; Lucas *et al.*, 2009; Ugedal *et al.*, 2008). The impacts of large hydro-electric power (hydropower) schemes on migrating fish are now well understood (Kumar *et al.*, 2011), through experience in the developed world from the large-scale exploitation of this resource.

The global increase in energy demand, and the drive towards sustainable energy generation by renewable means, has led to renewed interest in hydropower (Abbasi & Abbasi, 2011). In regions with a history of large-scale hydropower development such as Western Europe and North America, the opposing force of environmental legislation, and the limited remaining undeveloped large-scale hydropower potential, has resulted in the recent trend of small hydropower development (Abbasi & Abbasi, 2011; Anderson et al., 2015). New installations tend to be either on small, steep streams, or use existing barriers on lowland rivers (Anderson *et al.*, 2015; Robson *et al.*, 2011). In order to exploit these more marginal opportunities efficiently, novel turbine technologies are emerging. One such technology is the Archimedean screw hydropower turbine (ASHT), which is particularly popular for the redevelopment of existing small barriers.

There is a perception that the environmental impacts of small-scale hydropower are likely to be lesser than those of large scale hydropower (Abbasi & Abbasi, 2011; BHA, 2005; Paish, 2002). For migratory fish, however, the factors that impede or facilitate their movements are complex, dynamic and interacting, and include species' biology, scheme design and environmental factors. Hence the scale of a development is just one consideration amongst many. Moreover, there may be cumulative effects of multiple small schemes on these broad-ranging fish species (Fraser *et al.*, 2015). This thesis aims to assess whether small, low-head hydropower schemes may cause reduced survival and stock replenishment of salmonids, via the mechanisms of delay to migration and physical damage to migrating life stages.

In the following sections, the ecology of important migratory species in Scotland is introduced, followed by a background to hydropower; its historic development, technology, recent drivers for growth, and the regulatory framework for its development and operation. The general impacts of hydropower, and specifically large hydropower upon fish movements are then introduced, after which the potential effects of small, low head hydropower on migrating fish are considered and discussed. The chapter concludes with the aims for the thesis chapters.

1.1 The ecology and status of the key migratory fish species in Scotland

There are several key migratory species in Scotland which spend some or all of their lifecycles in rivers and thus may be impacted by hydropower installations. The major threat to these species from hydropower is the disruption of migration pathways: the introduction of obstacles and hazards associated with hydropower can reduce the potential for migrating individuals to complete their lifecycles (Lucas & Frear, 1997; Aarestrup & Koed, 2003; Lucas et al., 2009; Gauld et al., 2013), and hence affect recruitment and continuity of the population.

Atlantic salmon (*Salmo salar* L.) is an iconic species of high conservation significance and is of considerable socio-economic importance within Scotland. Brown trout (*Salmo trutta* L.) can also migrate extensively, and those that move between fresh water and the sea (sea trout, the same species but morphologically distinct) are highly valued. European eel (*Anguilla anguilla,* L.) and sea, river and brook lampreys (*Petromyzon marinus* L., *Lampetra fluviatilis* L. and *L. planeri* Bloch), also have life histories characterised by migrations. Historically river lamprey have supported important fisheries within the UK and low level exploitation still occurs. European eel is also exploited in parts of the UK outwith Scotland. Each of the species above is of high conservation interest and a range of legislative measures are in place to ensure their protection, as summarized in Table 1.1. The general lifecycle and migrations of Atlantic salmon and sea trout, the sea-going form of brown trout, are described below, followed by brief descriptions for European eel and lampreys.

1.1.1 Atlantic Salmon

1.1.1.1 Lifecycle and migrations

Atlantic salmon are anadromous, and this means that they reproduce in freshwater but spend the majority of their adult lives in the sea before returning to their natal rivers to spawn. An illustration of their lifecycle is shown in Figure 1.1. Adult fish bury their eggs in gravel beds with moderate current and depth (Moir *et al.,* 2002) beginning in late Autumn (Fleming, 1996). The hatched fish emerge the following spring, and spend up to four years living in the river, as fry in the first year, and then as ‘parr’. At this stage they feed territorially, but are also known to make broader movements in response to fluctuating river levels (Saunders & Gee, 1964; McCormick *et al.*, 1998; Thorstad *et al.*, 2010), and seasonally, to make use of differing feeding habitats. Some male parr mature sexually and move from rearing to spawning sites (Thorstad *et al.*, *op. cit.*). Surviving parr undergo a physiological transformation called smolting, which is a pre-adaptation for migration to sea (Johnsson & Johnsson 2011). The major migration of smolts to sea occurs in the spring, although there have been numerous observations of autumnal seaward movements by parr likely to smolt the following spring (e.g. Youngson *et al.,* 1983). Smolting normally takes place between April and June in Scotland and other parts of Great Britain (Maitland & Campbell, 1992). The smolt migration is a critical phase, with high mortality due to predation, exacerbated by physiological and osmoregulatory stress as they enter the marine environment (Thorstad *et al.*, 2012). The vulnerability of this life stage and the implications of hydropower passage are discussed fully in Chapters 2 and 3.

At sea, Scottish ‘post-smolts’ migrate vast distances to feeding grounds in the Norwegian sea and west of Greenland (Malcolm *et al.,* 2010). After one to four years at sea, adult salmon return to freshwater to spawn.

Table 1.1. Conservation legislation currently in place to protect native freshwater fish in Scotland. For Atlantic salmon, inclusion on the UKBAP list is for the spring stock component only. For *Salmo trutta*, both ancestral brown trout forms, ferox and sea trout are included.

Scientific name	**W&C Act**	**UK BAP**	**HD Annex**	**Cons Regs Schedule**	**Bern Conv Appendix**	**Bonn Conv Appendix**	**CITES**	**IUCN 2015**
Lampetra fluviatilis (L.)		Y	II, V	3	III			LC
Lampetra planeri (Bloch)			II		III			LC
Petromyzon marinus L.		Y	II		III			LC
Acipenser sturio L.	Sch. 5	Y	II, IV	2	III	I, II	I	CR
Anguilla anguilla (L.)		Y				II	II	CR
Alosa alosa (L.)	Sch. 5	Y	II, V	3	III			LC
Alosa fallax (Lacepede)	Sch. 5	Y	II, V	3	III			LC
Osmerus eperlanus (L.)		Y						LC
Coregonus albula (L.)	Sch. 5	Y	V	3	III			LC
Coregonus lavaretus (L.)	Sch. 5	Y	V	3	III			VU
Salmo salar L.		Y	II, V	3	III			LC
Salmo trutta L.		Y						LC
Salvelinus alpinus (L.)		Y						LC

Notes on Table 1.1

Legislation	**Relevant schedules and appendices as listed in Table 2**
Wildlife & Countryside Act 1981 (as amended)	*Schedule 5* – animals (other than birds) that are protected
UK BAP	*UK BAP priority fish species list*
EC Habitats Directive	*Annex II* - designation as qualifying feature within SACs for the species listed. *Annex IV* – special protection for the species listed. *Annex V* – exploitation may be subject to management.
The Conservation (Natural Habitats etc.) Regulations 1994	*Schedule 2* – European Protected Species in GB *Schedule 3* – animals that may not be taken in certain ways
Bern Convention (Convention on the Conservation of European Wildlife and Natural Habitats)	*Appendix III* – regulation of the exploitation of species listed.
Bonn Convention (The Convention on Migratory Species)	*Appendix I* - migratory species that are endangered. *Appendix II* - migratory species that require international agreements for their conservation and management.
CITES (Convention on International Trade in Endangered Species)	*Appendix I* – trade only in exceptional circumstances. *Appendix II* – trade subject to licensing.
IUCN Red Lists	CR – Critically endangered, VU – Vulnerable, LC - Least Concern, EX – Extinct.

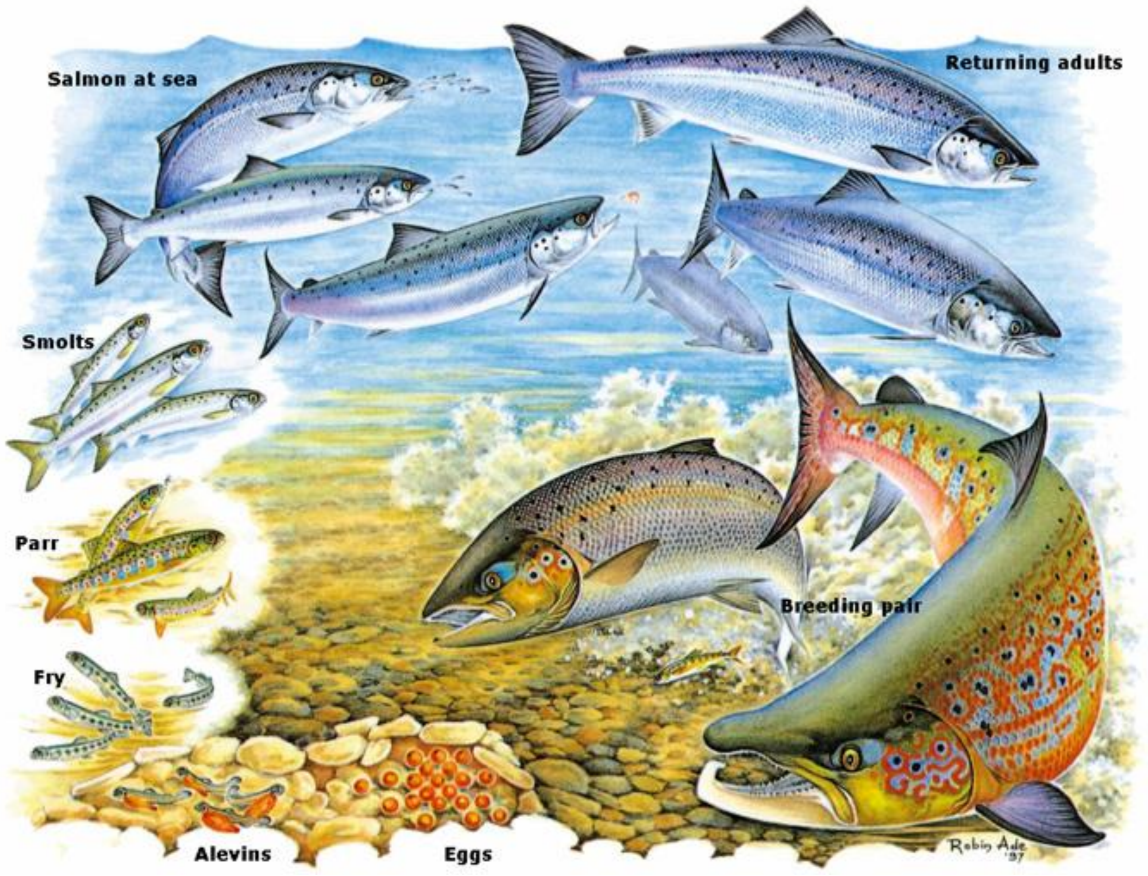


Figure 1.1. The lifecycle of the Atlantic salmon. Image from Robin Ade and the Atlantic Salmon Trust.

Atlantic salmon in general tend to return to their natal spawning sites, and display a strong homing ability (Thorstad *et al.*, 2010). River entry can occur at any time of year in Scotland (Webb *et al.*, 2007), where adult fish are classed into spring, summer and autumn components. In general, salmon arriving early in the year are larger, multi sea winter fish. Migration movements upstream are constrained by discharge, especially at steep sections of river, falls and man-made obstacles. It is thought that early entry is a strategy to maximise opportunities to pass upstream throughout the year, for fish destined to spawn high up in the catchment. These larger, stronger swimmers are more likely to be able to make use of high flows. Moreover, since adult salmon do not feed in the river, only larger fish with greater energy reserves can utilize this strategy. A fuller consideration of upstream adult migration movements and the effects of hydropower schemes on these is given in Chapter 4.

Spawning sites are selected that have clean, silt free gravel through which well oxygenated water can flow. The female lays between 2,000 and 15,000 eggs which she buries under river gravel. After spawning, the majority of adult salmon die, but some (mostly females) survive as kelts to return to sea, and may repeat the spawning migration in years to come (repeat spawners). Repeat spawners are more fecund than single spawners due to their greater size and egg-producing ability, and this has formed the basis for arguments to protect these fish, despite a low rate of return of spawned fish.

1.1.1.2 International status

Atlantic salmon are found in the temperate and arctic regions of the Northern Hemisphere, with freshwater life stages in the rivers flowing into both sides of the North Atlantic Ocean, and the Baltic Sea. Stocks are considered to have declined throughout their range, and within the North East Area Commission (which includes Scotland), declines have been evident since the late 1970s (Figure 1.2, ICES, 2015). These reductions are attributed to mortality at sea, the causes of which have been disputed for many years. There is a growing body of evidence that suggest that mortality from by-catch and the increasing influence of climate change are key contributors to marine losses (Chaput, 2012).

1.1.1.3 National status

Atlantic salmon are known to be present in at least 389 Scottish river systems (Gardiner & Egglishaw 1986; MSS, 2007). Chaput (2012) suggests that most studies of Atlantic salmon abundance have been carried out at geographic or stock levels which cannot identify those populations which are in decline. At its coarsest level Figure 1.3 shows the trend in rod catch at a Scottish (national) level for grilse and MSW fish. This appears to show that stock levels are generally stable or, for the grilse stock component, increasing. This may be a result of changing exploitation patterns, in particular the reduction in the coastal netting industry (Scottish Government 2015a). As of 2016, the killing of wild salmon outside of estuary limits is prohibited. Also evident, however, is the wide levels of inter-annual variability that exists in rod catch, particularly towards the end of the 53-year data series. This period (2000 onwards) includes both the highest and lowest rod catch records.

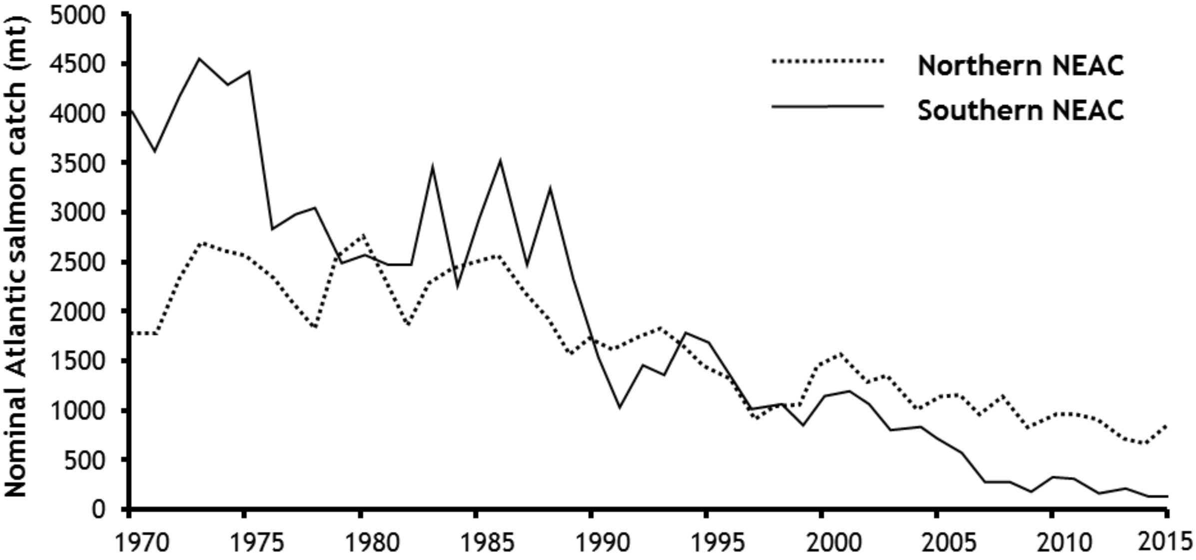


Figure 1.2. Atlantic salmon catch (in biomass) for the Northern and Southern components of the NE Atlantic Commission from 1970-2015. Northern component comprises Norway, Russia, Finland, Sweden, Denmark and the northeastern regions of Iceland. Southern component comprises UK (Scotland, England & Wales and Northern Ireland), Ireland, Spain, and the southwestern regions of Iceland. Figure adapted from ICES (2015).

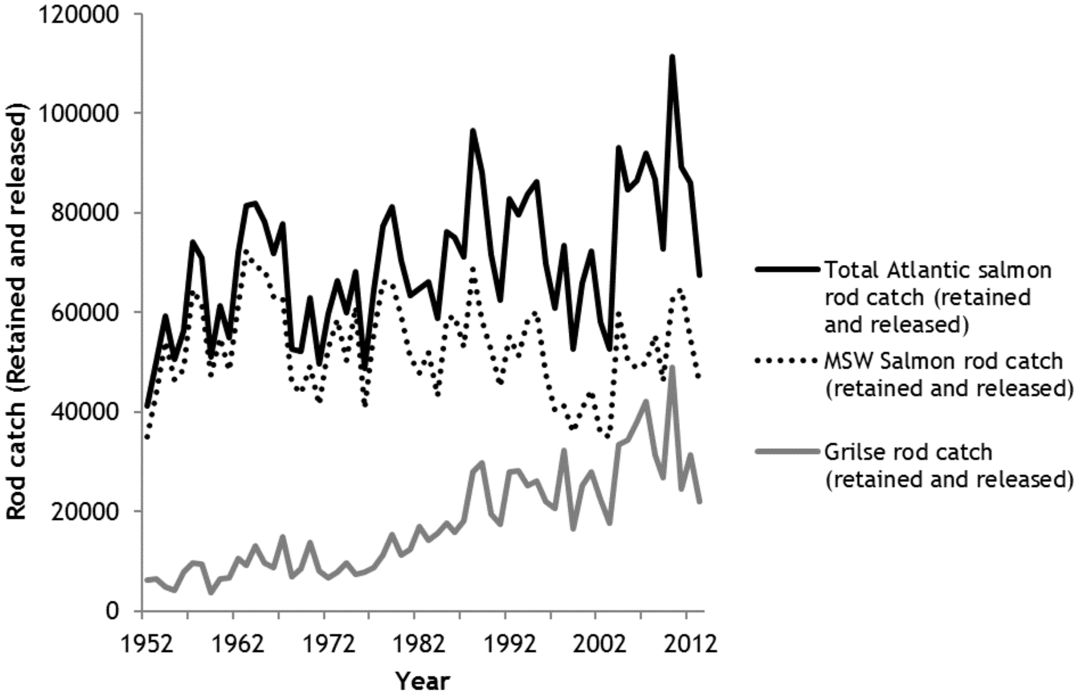


Figure 1.3. Rod catch (retained and released combined) data for Atlantic salmon in Scotland. Data provided by Marine Scotland Science.

When broken down into individual stock components, Figure 1.4 shows a long-term decline in the spring salmon rod catch, and a coincident increase in the number of fish captured during the autumn in particular. Underlying these figures, however, are local variations in abundance and capture effort.

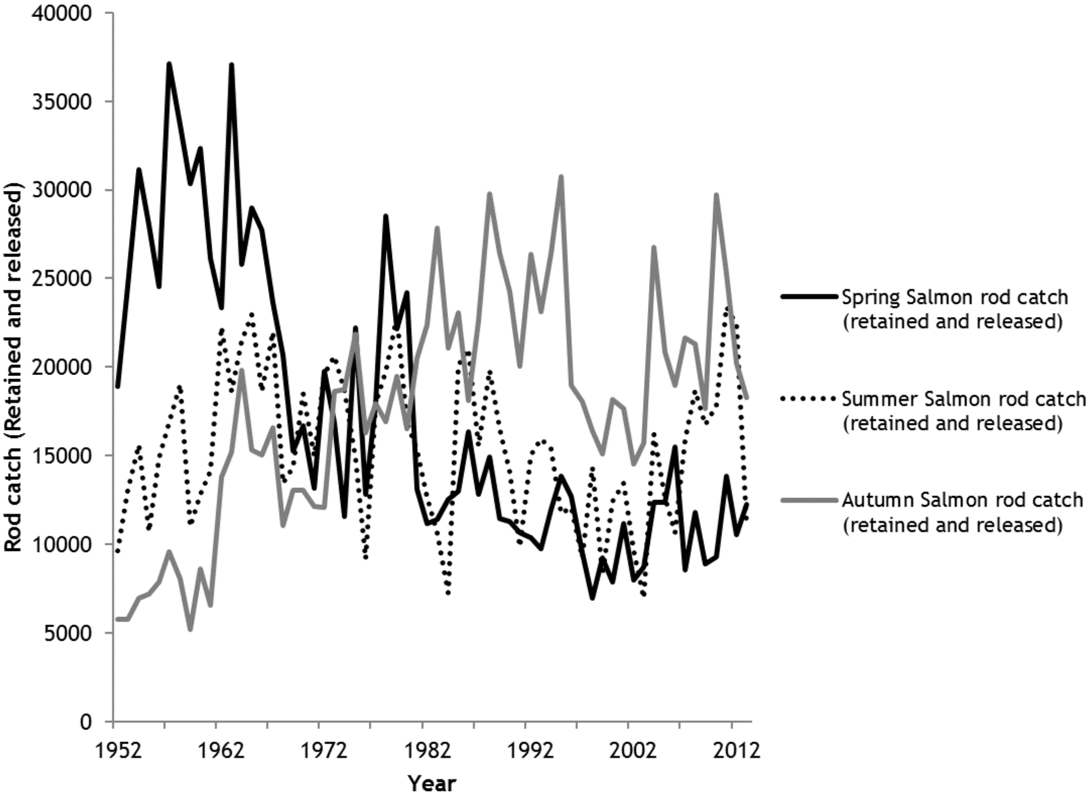


Figure 1.4. Rod catch (retained and released combined) data for the spring, summer and autumn Atlantic salmon stock components in Scotland. Data provided by Marine Scotland Science.

1.1.1.4 Conservation status

Atlantic salmon are listed on Appendix III of the Bern Convention and Annex II and V of the EC Habitats & Species Directive. The multi-sea-winter component of the Atlantic salmon population is included in the UK Biodiversity Action Plan Priority Species List. Atlantic salmon are classified as 'Least Concern' for extinction by the International Union for Conservation of Nature (IUCN, 2015). Continuing concern for Atlantic salmon as a whole in Scotland has led to introduction of The Conservation of Salmon (Scotland) Regulations 2016. This categorises salmon fishery districts according to population status, and requires management action for those under threat.

1.1.2 Sea trout

Sea trout are the anadromous form of brown trout, which are ecologically very diverse. Trout have variable life histories, and individuals from the same populations have differing extents of migratory movements, ranging from entirely within freshwater through estuarine excursions to complete anadromy.

Life History

In terms of their life history, sea trout are like Atlantic salmon in that they migrate to the sea as smolts to feed and grow before returning to fresh water to spawn. Like salmon, migration downstream to the sea usually takes place between the months of April-early June (Elliott, 1994, ICES, 2013).

Sea trout do not, unlike Atlantic salmon, migrate to far off feeding grounds, but instead utilise coastal areas. The time spent at sea can be quite short, with some fish returning to the river after just a few weeks or months between July and September. These small fish are often referred to as 'finnock'. Many adults return as larger 'maiden' fish after 12 or more months at sea and these fish can be seen in the river between May-October. Spawning takes place in their natal river and normally begins in mid-October and continues through to early January.

Neither forms of trout, freshwater resident or sea trout, receive extensive protection within conservation legislation. Some exploitation controls exist within fisheries legislation and sea trout are further protected within fisheries acts relating to the protection of 'salmon'. In 2007 ancestral brown trout and sea trout were added to the UK Biodiversity Action Plan Priority Species List.

International status

The status of sea trout within its European range is variable both between and within countries (ICES, 2013). Noticeable declines have been recorded in many countries (e.g. Finland, Germany, Netherlands, Norway, Poland, Russia, Spain and Sweden), although others report either stable (e.g. France, Latvia) or increasing (e.g. Estonia, Lithuania) trends.

National status

There are concerns over the long-term decline in sea trout numbers across the whole of Scotland (Figure 1.5). The mechanisms behind local and national declines in sea trout numbers are likely to be complex, and one which may involve factors such as climate change, in-stream productivity and their interaction with the genetic quantitative traits which determine whether a trout becomes a ‘sea trout’ or not. Significant knowledge gaps relating to the interaction between sea trout and the environment exist however.

Value of Atlantic salmon and sea trout

Both Atlantic salmon and sea trout contribute to the Scottish and local economies. Estimates in 2002 suggested that Atlantic salmon and sea trout angling in Scotland contributed around £80 million of economic output, leading to around £39 million of income to Scottish households and supporting an estimated 2200 jobs (Radford *et al.*, 2004).

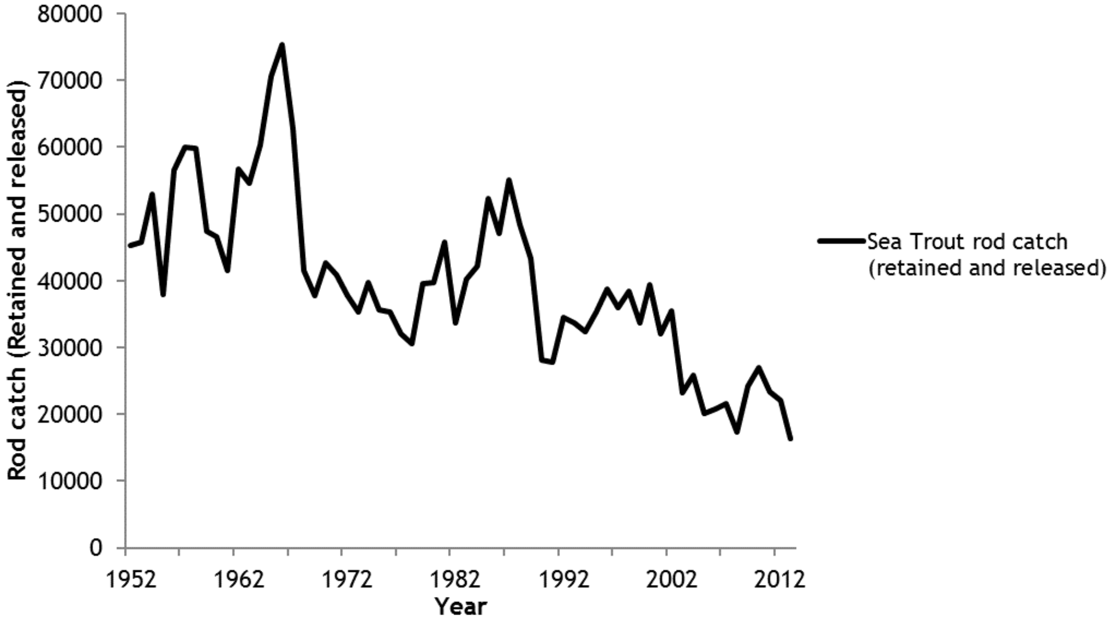


Figure 1.5. Sea tout rod catch data for the whole of Scotland from 1952-2015. Data for fish retained and released are presented. (Data supplied by Marine Scotland Science).

1.1.3 European eel

European eel are a catadromous species that enter freshwater as juveniles, and exit three to thirty years later on their spawning migration to the Sargasso sea (Poole & Reynolds, 1996; Tesch, 2003; Belpaire *et al.*, 2009). There is much concern over the decline in European eel populations, which are classified as being critically endangered (IUCN, 2015).

1.1.4 Lampreys

Three species of lamprey are present in the UK: river lamprey , brook lamprey and sea lamprey, all of which have migratory life stages. After hatching from their gravel redd or nest, the larval stage (ammocoete) exits, and moves downstream to settle and bury in areas of slow flow with soft sediment in late winter and spring. After several years the ammocoete undergoes metamorphosis preparing it for the adult, free swimming, phase. Juvenile anadromous lamprey (macropthalmia or ‘transformers’) make distinct migrations from these larval rearing habitats, to sea (Moser *et al.*, 2014), whilst non-anadromous forms make smaller migrations within freshwater. Adult lampreys migrate upstream to spawn to varying degrees depending on species and life history (Maitland, 2003). For the non-parasitic brook lamprey, this migration may be up to a few kilometres following metamorphosis, whilst anadromous parasitic lamprey swim from the ocean or estuaries many kilometres up river to complete their life cycle.

1.2 Hydropower background

1.2.1 A brief history of hydropower

Man has been harnessing the kinetic energy of water moving under the force of gravity to do work (hydropower) for millennia. Until the technology for hydro-electricity generation emerged in the late 19th century, all hydropower was mechanical, and ‘small scale’ in today’s terms. In some regions it was developed extensively: before the Industrial Revolution in Europe, water was a major power source for milling lumber and grain, and powering small machinery, mainly using water wheels (Moreira & Poole, 1993). For example, in England alone there were some 20000 working mills by the end of the 17th century (Boyle, 2004). The first of the modern water turbines appeared in the early nineteenth century, but it was not until near the turn of the century that these inventions were combined with Faraday’s contemporary discoveries with electricity in the first hydro-electricity generating installations. A spattering of small hydro-electric plants sprung up around the industrialising world and its colonies, for example, in Australia in 1881 and New Zealand in 1885 (Petchey & Bauchop, 2012) and India in 1897 (Abbasi & Abbasi, 2011).

The period from 1930 to 1960 saw hydropower schemes increase in scale, using large dams to store water. Flagship schemes such as Tennessee Valley in the early 1930s in the US demonstrated the concept of integrated use of water for power and other applications over whole catchments. In Scotland the major period for hydropower construction occurred from 1943 to 1965 (Wood, 2002), when twenty-eight hydro-schemes were built comprising 66 dams, 51 power stations, 171 miles of tunnels and 103 miles of aqueducts (Johnson, 1994). These developments pre-dated today's legal requirement for environmental impact assessment, and it was not until later that the sometimes devastating ecological impacts were realised. Particularly concerning was the disruption of migrations of socio-economically important fish populations. Today, much of the large hydropower potential in the developed world has been realised, and the environmental consequences of developing any which remains is usually not acceptable. Yet in the last decade there has been a resurgence of interest in hydropower, particularly at smaller scales. In the following sections this trend is explored, after which the regulation and impacts of hydropower are considered. First it is useful to introduce and define some common terms used to describe hydropower schemes.

1.2.2 Hydro-electric power schemes: definitions and types

Hydro-electric power (referred to hereafter under the term hydropower) schemes can take a variety of forms, with no two exactly alike. They all have the common aim of converting the kinetic energy of water moving under gravity into electrical energy using mechanical means to drive a generator. The kinetic energy of moving water is a function of its mass and velocity, and for water to move under gravity, a drop in height (head), is required. Hence the two physical components needed for hydropower generation are water head and water mass (or equivalently, volume). The generic layout and terms used to describe parts of a hydropower scheme later in this thesis are depicted in Figure 1.6. Two typical small run-of-river hydropower scenarios are illustrated: those which divert water away from the river course for some distance (Figure 1.6A) and those that direct water through a turbine with minimal diversion by placing the turbine on the barrier itself – hereafter referred to as on-weir schemes.

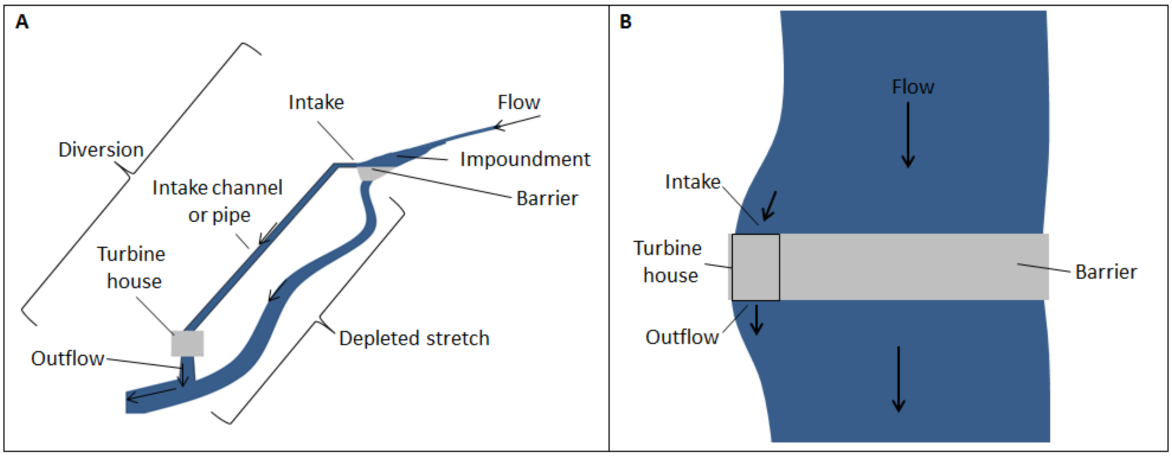


Figure 1.6. The anatomy of typical small diversion (A) and on-weir (B) run of river hydropower schemes.

The design of a particular scheme attempts to match the available water energy resource with the demand for electricity. The variety of designs which have arisen to meet this challenge can be broadly classified into the categories described below. Whilst some of these descriptors are mutually exclusive (e.g. impoundment *vs* run-of-river schemes, high-head *vs* low-head and large scale *vs* small scale), there are no universally applied thresholds separating them, rather regionally accepted norms, dependent on the distribution of hydropower developments along these scales, and so working definitions are given for terms used throughout this thesis.

1.2.2.1 Impoundment schemes

Where continuous generation (or reserve capacity) of power is needed from an intermittent supply of water, dams are built to impound water – storing or smoothing out its supply to the turbines and generators. This has the added effect of increasing the head, or height, of water above the turbines. The higher the head the greater is the energy available to drive the turbines. Impoundment schemes also have the advantage that they can respond to instantaneous electricity demand, since they have water head which can be rapidly converted to electrical energy. By contrast, thermal plants (such as coal-fired plants) operate optimally when producing a constant supply, and have no such capacity for fast response. Hence impoundment schemes can act in compliment with thermal plants to respond to peak demand.

1.2.2.2 Run-of-river schemes

Run-of-river schemes divert water from rivers through turbines without significant impoundment. The power-output depends on the flow available, so intermittent flows generate intermittent power. There is no universally accepted definition of run-of river, but in general it means that only the available supply of water is used, and any barrier is really designed to divert, rather than store, water. In Scotland, schemes that have less than 24 hours' worth of storage are considered to be run-of-river (SEPA, 2015), and this is the convention adopted for this thesis.

1.2.2.3 High-head schemes

High-head schemes use large heads (generally greater than 50 m) to generate power. The height difference between the intake at the top of the scheme, and the turbine at the bottom, may be the result of a large impoundment, the natural river profile over the course of a diversion, or both. Since the two components required for hydropower generation are water head and water volume, a large value of one component can compensate for a small value of the other to provide an economically feasible output. Hence the placement of small high-head schemes in the upper reaches of rivers in Scotland where flow volume is low but high head is available.

1.2.2.4 Low-head schemes

Low-head schemes generate power without much height difference. In general they use the larger flow volumes available in the lower reaches of rivers to compensate for the lack of head. In this thesis, low-head refers to a height difference of less than six metres.

1.2.2.5 Small-scale hydropower

In the UK hydropower generation is generally classed as small-scale below 10 MW installed capacity. The hydropower industry further separates these small-scale schemes into mini (<1 MW), micro (<100 kW) and pico (<5 kW) (Robson *et al.*, 2011). In Scotland, schemes below 100 kW installed capacity have a simplified licensing process. This thesis will focus on schemes with up to 220 kW capacity.

However, in this introduction, when referring to the global context, ‘small’ and ‘small-scale’ hydropower will encompass capacities up to 10 MW.

1.2.2.6 Conventional *vs* emerging turbine technologies

‘Conventional’ turbines

Since the establishment of large hydropower in the 1930s, the turbines favoured have been designed to be used with water flowing downhill through an enclosed pipe or tunnel. Pelton wheels and crossflow turbines are suitable for high-head applications and are impulse turbines, using the kinetic energy of fast-moving water, and discharging to atmospheric pressure. Bulb, Straflo, tube, Kaplan and Francis turbines are reaction turbines, which use a combination of water pressure and momentum, and are suitable for sites with lower head and higher flows.

Emerging turbine technologies

With the increasing exploitation of small, low head and free-flowing hydropower potential, has come the emergence of novel turbine technologies more suited to these opportunities. Water wheels are once again coming into use at low head barriers. The Archimedean screw hydropower turbine (ASHT) is being increasingly favoured for low head applications, because it can generate efficiently at very low head (ASHTs are dealt with in detail in Chapter 3). Hydrokinetic turbines use the natural kinetic energy in a watercourse to generate power, with minimal infrastructure in place to direct water towards them. Various designs of hydrokinetic turbines are available, from undershot waterwheels, to propeller types and vertical axis turbines.

1.3 The drive for hydropower development

1.3.1 International drivers

Much of the renewed interest in hydropower since the turn of the century stems from international targets to reduce greenhouse gas emissions and dependence on fossil fuels. In December 2015 the United Nations Framework Convention on Climate Change (UNFCCC) agreed to reduce anthropogenic global warming to *“well below 2 ^{o}C”*. The UN also set its Sustainable Development Goals, including *“access to affordable, reliable, sustainable and modern energy for all”* (International Hydropower Association 2015). These advances towards reducing global greenhouse gas emissions began in 2001 with the implementation of the Kyoto Protocol.

Hydropower provides a stable alternative to electricity generation from fossil fuels. It is widely recognized (for example Bodis *et al.*, 2014) that hydropower schemes utilizing substantial water storage offer a flexible, stabilizing complement to other more variable or intermittent renewable generation technologies (wind power, for example). Run-of-river schemes, whilst they do not offer the storage or rapid response capabilities of impoundment schemes, do offer a viable replacement for baseload generation by fossil fuels or nuclear power (EURELECTRIC 2011; Glachant *et al.*, 2014).

1.3.2 European and UK drivers

In Europe, the EU Renewables directive (2009/28/EC) set a target to increase gross energy consumption of renewables to 20% by 2020. The 2030 Climate and Energy Framework seeks to raise this to 27% by 2030 (EC, 2014). These targets are implemented within a National Renewable Energy Action Plan for each nation. In the UK 15% of energy must come from a mix of renewables by 2020.

At a national level, renewables development is encouraged by financial incentives and statutory quotas. Almost all EU member states, and many more globally have enacted support schemes to promote renewable electricity generation. (Ortega-Izquierdo, 2016). Feed in tariffs (FIT),where electricity generated by renewable means is purchased at above the market price using government subsidies, are by far the more prevalent method in Europe (Glachant *et al.*, 2014). In the UK, FITs were introduced in 2010 and have been the major driver for increased small hydropower development from 2010 to 2014 (Figure 1.7). From 2014, the incentives were reduced, and applications for new developments declined (R. Gosling, SEPA, March 2015, pers. comm.). In Scotland, a renewables obligation was also set by the Scottish Executive (now Scottish Government) for electricity suppliers to supply at least 10% and 15% of electricity from renewable sources in 2010 and 2020 respectively (Bean & Thin, 2008).

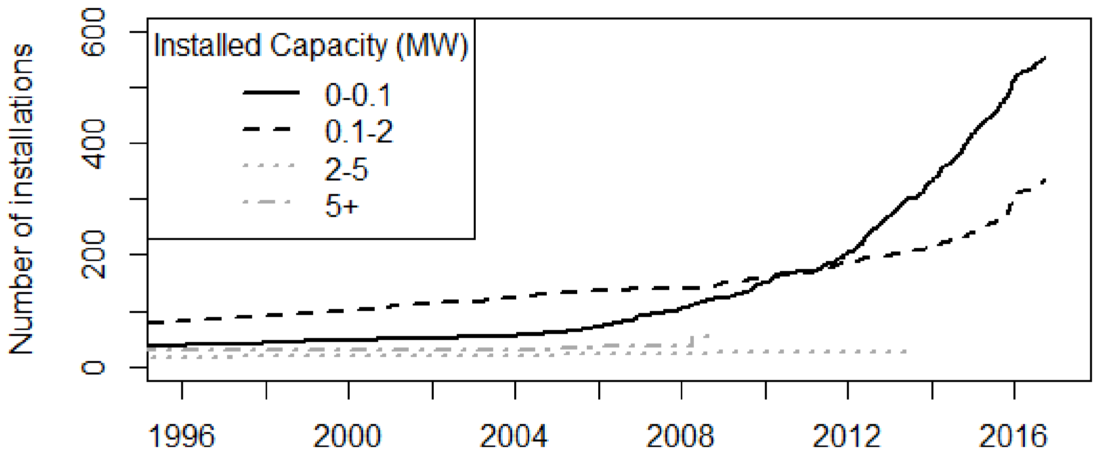


Figure 1.7. Cumulative numbers of hydropower schemes installed in the UK. Data sourced from Ofgem (2016).

1.4 The regulatory framework and guidelines for hydropower development

The management of water resources is multifaceted, with interactions between differing human and ecological demands, and is subject to variable hydrology and changing climate. It follows that the institutional frameworks governing hydropower are complex and geographically varied (Glachant *et al.*, 2014). The following is an overview of the regulatory framework of relevance to small hydropower development in Scotland, beginning with Europe-wide directives through national requirements.

1.4.1 European legislation

The two main pieces of European conservation legislation which impact upon the development and operation of hydropower are the Water Framework Directive (WFD) (European Directive 2000/60/EC) and the Habitats Directive (HD) (92/43/EEC). The WFD requires the protection and restoration of the water environment and the HD requires the protection of certain water-dependant freshwater species and habitat features.

Of particular relevance to hydropower, Member States must: maintain or restore European protected habitats and species listed in the Annexes at a favourable conservation status; encourage the management of features of the landscape which are essential for the migration, dispersal and genetic exchange of wild species; and, ensure strict protection of species listed on Annex IV of the Directive (also known as European Protected Species).

The Habitats Directive annexes include a range of anadromous fish species which could be impacted by hydropower developments, either through abstraction, or the installation of structures which reduce habitat connectivity within river catchments. These include Atlantic salmon, river lamprey, sea lamprey, Allis shad (*Alosa alosa* (L.)), twaite shad (*A. fallax* (Lacepède)) and Atlantic sturgeon (*Acipenser sturio* L.). A wider range of non-fish species which depend on the presence of these fish, or the maintenance of appropriate river flows, such as freshwater pearl mussel (*Margaritifera margaritifera* L.) and otter (*Lutra lutra* (L.)) may also be impacted. The presence of in-stream structures which delay the migration of fish downstream may, conversely, increase predation opportunities for species such as otters, or piscivorous birds which are also protected under the EC Birds Directive (Directive 2009/147/EC).

Also of specific interest is the Eels Regulations (Council Regulation 1100/2007) which calls for *"appropriate measures as soon as possible to reduce eel mortality caused by factors… including hydroelectric turbines, pumps or predators"*. Key to planning policy is the Environmental Impact Assessment Directive (European Directive 2011/92/EU) which sets out the requirements for environmental impact assessment. These Directives have been variously translated into regional law to incorporate and supersede existing legislation with integrated Europe-wide policies.

1.4.1.1 Planning and Licencing for Hydropower in UK Regions

In the UK, hydropower developments are generally subject to three areas of legislation: 1) planning permissions – regulated by local authorities or national government, depending on scale; 2) licensing for the abstraction or impoundment of water bodies – regulated by the environment protection agencies; and 3) compliance with environmental and natural heritage conservation law.

These three aspects are integrated to varying degrees in the application process for hydropower developments. The process as a whole is administered by the national authority responsible for environmental protection: the Scottish Environment Protection Agency (SEPA), the Environment Agency (EA) in England, Natural Resource Wales, and the Northern Ireland Environment Agency (NIEA).

Hydropower regulation in Scotland

The potential conflict between the European Renewables Directive and legislation protecting the water environment prompted Scottish Ministers to make a policy statement in 2010 to clarify the government's standpoint on balancing these needs. In response, SEPA released a guidance document aimed at facilitating the process of run-of-river hydropower licence applications (latest revision: SEPA, 2014).

In Scotland, water abstractions, impoundments and engineering works in or near inland water or wetlands require a Controlled Activities Licence (commonly referred to as a CAR licence), under the Water Environment (Controlled Activities) (Scotland) Regulations 2011. SEPA is responsible for the administration of CAR licences.

Proposals of less than 100 kW capacity will normally only accepted where they cause no degradation of the water environment (SEPA, 2014). That is, if they will be in degraded waters (with no restoration planned), or in small, steep streams, or those which will make improvement to the ecological quality of the water environment, and will operate within river flow standards. These flow standards are designed to protect minimum (or hands off) flows, flow variability, peak flows, and flows for the migration of fish. Development consent must be obtained from the Local Authority, subject to an environmental impact assessment if the site is in a sensitive area. Scottish Natural Heritage play a consultee role for developments that affect sites or species of conservation importance, and District Salmon Fishery Boards are consulted for developments that are planned in river systems where Atlantic salmon are present.

The protection of valued freshwater fish stocks is well established in Scottish law. Specifically with regard to Atlantic salmon and hydropower, the Salmon and

Freshwater Fisheries (Consolidation) (Scotland) Act 2003 allows Scottish Ministers to make further regulations with regard to *"the construction and alteration of dams, lades or water wheels so as to afford a reasonable means for the passage of salmon"*. The governance of freshwater fisheries in Scotland is in the process of review and it is likely that District Salmon Fishery Boards will be replaced by a new National Freshwater Fisheries Unit, supported by a network of local Fishery Management Organisations These may play a statutory consultee role for hydropower issues in future years. The centralised management of fisheries offers the opportunity to facilitate holistic and strategic consideration of pressures such as hydropower, and to develop more standardized policy with regards to planning. This new fisheries management structure will have an 'all species' remit and will not be restricted to providing comments on 'salmon'.

1.5 Small hydropower status, trends and potential

Whilst there is still potential for development of large-scale hydropower in the developing world, in more developed countries small hydro is an important component for the switch to renewables for two reasons: 1) much of the large hydro potential has been developed already, and 2) small hydro is perceived as having less environmental impact (Abbasi & Abbasi, 2011; Robson *et al.*, 2011).

In the UK, there remain opportunities for small-scale hydropower development in upland regions, where the topography and climate favour high-head developments. However, there are also opportunities for low-head generation via the redevelopment of a wealth of existing historic weirs. For example, the Environment Agency identified 25935 such existing barriers as candidates for development in England and Wales (Environment Agency, 2010). Many such low-head barriers were built for mechanical hydropower (Section 1.1) until they were replaced by steam engines and grid power from large scale generation in the last century. These barriers require relatively little modification other than the installation of a modern turbine. This is an especially attractive opportunity since environmental legislation prevents the degradation of waterways by installation of new barriers. The Scottish Hydropower Resource Study (Forest *et al.*, 2008) suggested that up to 657 MW of new small scale hydropower may be viable in Scotland. An update on the 2008 study now estimates there could be up to 1.2 GW of potential new hydro capacity in 7,043 schemes (The Scottish Government, 2011).

1.6 General hydropower impacts

Hydropower at any scale is not without ecological consequences (Abbasi & Abbasi, 2014; Anderson *et al.*, 2015; Robson *et al.*, 2011). All biota present in rivers are adapted to, and selected by, an interplay of hydrological and associated chemico-physical conditions (Vannote *et al.,* 1980). Any modification of this habitat will result to some degree in alterations to the communities present in a particular river reach (Ward & Stanford, 1983). The fundamental effects of hydropower on the physical state of a river can be viewed as:

1) changes to river morphology and hydraulics, by introducing barriers, impoundments and diversions;
2) changes to hydrology, by displacing or isolating flow; and
3) the extraction of energy.

The extent of these alterations varies between schemes, and is related to their characteristics, which are broadly compared for low-head run-of-river, high-head run-of-river and large impoundment schemes in Table 1.2. These fundamental changes then influence other physical, chemical and biological processes, ultimately resulting in some degree of habitat change, or change to habitat connectivity, with consequences for biological communities. A brief and holistic view of these effects is now is given, before focussing specifically on the problem of loss of connectivity faced by highly mobile species (Section 1.8).

Table 1.2. Comparison of features of low head run-of-river, high head run-of-river and large impoundment hydropower schemes. Adapted from Robson *et al*., (2011).

Characteristics	Low head run-of-river schemes	High head run-of-river schemes	Large Impoundment schemes
Location in catchment	Generally in lowland reaches, often constructed around existing weirs.	Upland reaches associated with waterfalls or steep gradient terrain.	Mostly upland catchments impounding steep valleys.
Impoundment types	Generally use existing weir structures <5m high.	Small weirs <3m. May be constructed on existing waterfall.	Large dam.
Reservoirs	No	No	Yes
Hydrological features	Flow diversion through turbine results in a depleted reach between impoundment and outflow except when no abstraction, or isolation of flow through turbine constructed on impounding structure. Operation except at high or low flows.	Diversion of flow through turbine leading to depleted reaches between impoundment and outfall except when no abstraction. Operation except at high or low flows.	Water storage and release linked to electricity demand. Can lead to hydropeaking of regulated flow. Hydrology upstream of impoundment converted from lotic to lentic system. May incorporate diversion resulting in a depleted stretch. Some inter-catchment diversions result in dramatic change to downstream hydrology.

1.6.1 Effects on physical character and processes

Abstraction or impoundment for large hydropower modifies the hydrological regime downstream. Impacts associated with schemes utilizing large impoundments or long diversions include elevated and depressed low flows, diurnal fluctuations, reduced flood frequency and magnitude, unnaturally rapid rates of stage change and elimination of flow and floods all together (Gilvear *et al.,* 2002). Because of the intimate linkage between hydrology and channel morphology, regulated rivers undergo changes in geomorphology. Reduced channel widths, sediment aggradation, large tributary confluence bars and siltation of channel substrates (Gilvear *et al., op. cit.*) are all characteristics of regulated rivers where there has been a net reduction in flow or in peak flows.

The hydraulics in impoundments differ greatly from flowing streams. One physical consequence is that impoundments (of any scale) act as sediment traps. The step-change in gradient at a dam may cause a river to erode its banks downstream and thus recapture the lost sediment load. The extraction of energy from river flow also means that there is less net energy available for the transport of sediment, resulting in siltation. Even in low-head hydropower scenarios, accumulation of fine sediment can occur in the weir pool (Csiki & Rhoads, 2010; Mueller et al., 2011), and depleted stretch, (Jesus et al., 2004). Large impoundments also affect water quality parameters. When a large body of water is impounded in a temperate climate, its temperature is conserved to a greater extent between cold and warm seasons, resulting in thermal stratification in the reservoir. Water released from stratified reservoirs can cause river temperatures below large impoundments to be atypical for the time of year (Gilvear *et al.,* 2002).

1.6.2 Chemical Impacts

Most of the reported changes in water quality due to flow regulation are associated with the effects of large impoundments (Petts, 1984; Gilvear *et al.,* 2002). Temperature stratification in reservoirs results in a warm, well-oxygenated upper layer, and a cooler, anoxic bottom layer. The reduction of oxides from sediment in the lower layer can result in elevated concentrations of iron and manganese (Gilvear *et al.,* 2002). Hence, as for temperature, the effect on the quality of downstream released water depends on the level at which water is drawn off. Dissolved gas concentrations downstream may be elevated to supersaturated levels where high spill over dams occurs, which can cause gas embolism in fishes (Weitkamp & Katz, 1980).

1.6.3 Effects on biological communities

Riverine ecosystems have been described as 'intricate webs of interdependence' (Johnson and Law, 1995), with biotic and abiotic factors interacting at varying spatial and temporal scales to influence the structure and dynamics of biological communities. The fundamental changes associated with hydropower, and the physical and chemical alterations and processes outlined above, are changes (both spatial and temporal) to habitat type, extent and variability. The ecological niches of aquatic organisms are to a large extent determined by water velocity, depth and quality, and these are all influenced by hydropower installations, as described above. The impoundment of large bodies of water for large scale hydropower generation creates a lentic habitat which is suitable for different species to those present in flowing water (Petts, 1984). Reduction of flow in river reaches depleted by diversions reduces available habitat area for sessile and sedentary rheophilic species (Bean & Thin, 2008, Robson *et al.*, 2011). The alteration of temporal dynamics in physical parameters also has profound consequences for biological communities. For example hydrological disturbance, in the form of flood events, is a key determinant in aquatic and riparian community dynamics, and so the artificial modulation of flows can lead to differently structured communities (Robson *et al.*, op. cit). Longitudinal fragmentation can also affect species dispersal, (e.g. for plants, Andersson *et al.,* (2000), and fish (see Section 1.8)). The effects of flow regulation may go far beyond the locality of the impacting scheme, even affecting estuarine ecosystems and communities (Drinkwater & Frank, 1994).

1.6.4 Effects on riverine fish

1.6.4.1 Habitat change

Impounding reservoirs alter the river habitat from lotic to lentic, changing local fish communities according to habitat preference. Flow regulation may change water availability and quality in the depleted stretch, reducing the extent of spawning, rearing, and nursery areas (Robson *et al.,* 2011). The effects on geomorphology of siltation or gravel depletion are particular risks to spawning habitat (Robson *et al., op. cit*) for salmonids, which depend on suitable gravels for spawning (Armstrong *et al.*, 2003). The effects of temperature stratification in reservoirs can also result in altered temperatures and dissolved oxygen concentration downstream.

1.6.4.2 Loss of connectivity

Loss of longitudinal connectivity across hydropower barriers is a major problem for highly mobile fish species (Jungwirth 1998; Lucas & Baras, 2001), but for the most part, the current knowledge base is on large scale hydropower effects and for socio-economically important fish species (Schilt, 2007), particularly on salmonids, and on adult fish (Roscoe & Hinch, 2010). There is increasing awareness of the effects of lesser obstacles or partial barriers, and impacts on migratory species of conservation concern. There is also an increasing recognition that these impacts extend to the movements of other fish species previously regarded as ‘resident’, but which also require or benefit from unimpeded longitudinal connectivity of watercourses to complete their lifecycles (Harris & Mallen-Cooper, 1994; Lucas & Baras., 2001). In Scotland, man-made barriers to fish migration were ranked as the most widespread pressure on the water environment in 2015 (Scottish Government, 2015b).

1.7 The impacts of hydropower on fish movements

It is now recognized that hydropower schemes can have substantial effects on ecosystems and species. Particularly concerning is the loss of river connectivity for mobile fish species. Jiang *et al.,* (2016) found the major academic hydropower research concerns to be related to post-construction environmental, ecologic or sustainability issues, with fish impacts the dominant theme. In their quantitative review, the topic of salmon passage ranked as the second highest proportional content (4.2%) of all hydropower article abstracts.

In general, much more is known about the effects of large-scale hydropower than small-scale hydropower (Anderson *et al.*, 2015; Bilotta *et al.*, 2016). For example, a Web of Science literature search (23 May 2016) revealed a wealth of articles related to fish passage impacts, but this was substantially diminished by the inclusion of the terms “small” or “micro”, indicating that little focus has been given to identifying impacts specifically from small scale schemes (Figure 1.8).

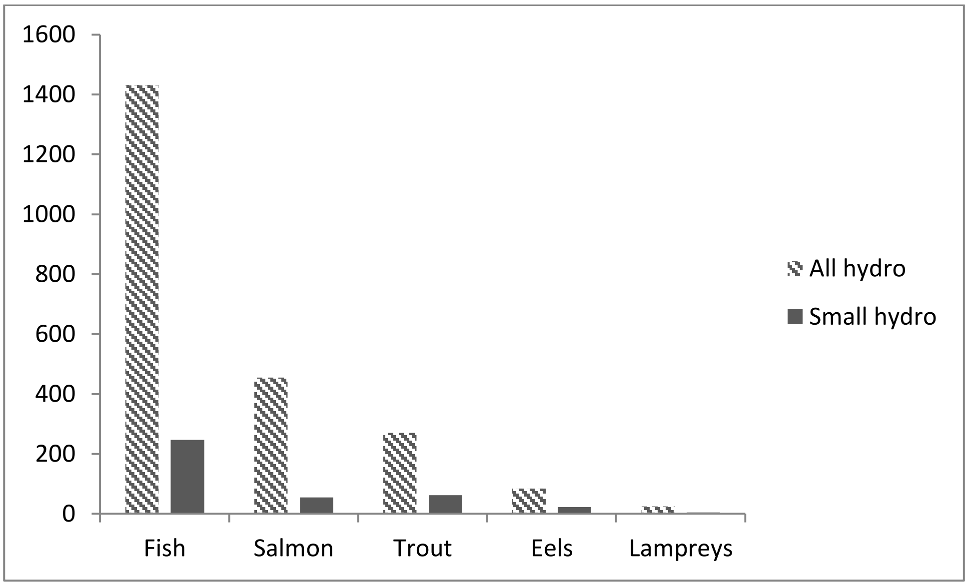


Figure 1.8. Comparison of numbers of articles on fish and hydropower, and fish and small hydropower from a Web of Science topic search. The following search terms and Boolean operators were used ([search terms] “BOOLEAN OPERATORS”]), where the asterisk (*) represents any group of characters, including no character: [hydro*power “OR” hydro*electric*], for all hydro with “AND” [small “OR” micro] for small hydro; [fish “OR” salmon “OR” trout “OR” eel* or lamprey*] (Fish); [salmon] (Salmon); [trout] (Trout); [eel*] (Eels) and [lamprey*] (Lampreys). The search was restricted to articles in the Web of Science Core Collection, from 1900 to 2016.

1.7.1 The effects of large hydropower upon fish passage

1.7.1.1 Downstream passage

Injury

Downstream migrating fish are at risk from injury during passage through hydropower infrastructure. Injury can be direct and acute, resulting from exposure to rapid and extreme change in pressure or velocity, high sheer stresses arising from greatly differing relative velocities between water bodies, or between structures and water bodies, or mechanical damage (Monten, 1985; Coutant & Whitney, 2000).

Rapid or extreme pressure change

Many teleost fish actively maintain neutral buoyancy with a swim bladder (Bone et al. 1995), which is vulnerable to rupture during rapid decompression (Brown et al. 2012).Fish which regulate the volume of the swim bladder slowly by gaseous exchange through the blood vessels in the wall of the swim bladder (physoclists) are more susceptible to mortality from pressure change than those fish which can quickly alter swim bladder volume through the air canal and mouth (physostomes - for example salmonids) (Larinier & Travade 2002; Brown et al. 2012). In experiments with juvenile Chinook salmon, Brown et al. (2012) found the ratio of acclimation pressure to lowest exposure pressure to be the important factor associated with mortal injury during simulated turbine passage. Turnpenny (2000) also identified haemorrhages to the eyes and other areas of the body, and internal haemorrhages resulting from pressure changes under experimental conditions.

If air is entrained in water which is subsequently pressurised, the dissolved concentration of gases will be above those found at atmospheric pressure. This is termed super-saturation, and can occur where air is entrained into high-head turbine intakes or where water overspills high dams. If the dissolved nitrogen in a fish’s blood equilibriates with nitrogen supersaturated water, this will vaporise when the fish is exposed to lower pressures, a condition equivalent to ‘the bends’ experienced in divers, and which can be fatal. There is a large body of literature evidencing mortality of fish downstream of hydropower schemes from this cause, as reviewed in Cheslak & Carpenter (1990).

Shear stress

Fluid movement parallel to a surface such as a fish’s body results in a stress on that surface which is termed shear stress (Cada et al. 2006). This can occur in hydropower systems where two masses of water moving in different directions intersect or where moving water slows near a fixed structure. Shear stress experiments by Turnpenny (2000) identified resulting injuries as: removal or rupture of the cornea, torn gill-covers, red-eye and pop-eye. No significant scale loss was found, although mucous stripping was observed. Some delayed mortality was recorded due to these injuries, including those resulting from fungal infections because of loss of surface mucous.

Turbine strike

Turbine strike is recognized as a dominant cause of injury and mortality from passage through conventional turbines. Larinier & Travade (2002) summarize mortality rates for conventional turbine designs as follows: Pelton turbines, 100%; Francis turbines, 5-90%; Kaplan turbines, 5-20%. It is clear that mortality rates are highly variable. For any system they depend both on scheme and operating characteristics (turbine properties, head, mode of operation) and on the characteristics of the fish passing (species, length, mass). More detail on methods for predicting and assessing injury or mortality from mechanical damage is given in Chapter 3.

These injury mechanisms may also cause subtle or latent effects which can affect the fitness of a fish and hence, in the longer term, the population (Budy, 2002). Subtle injury or stress may lead to increased likelihood of disease or predation (Mesa, 1994) after passage, and could act in combination with other process to lead to delayed mortality. For example in a telemetry study on Chinook salmon (*Oncorhynchus tshawytscha* (Walbaum)) smolts, Ferguson *et al.*, (2006) estimated that delayed mortality contributed 46% to 70% to the total mortality resulting from passage through a hydropower scheme.

Delay

Hydropower impoundments and infrastructure can also halt or delay the downstream movements of fish (e.g. Venditti *et al.*, 2000). As well as the physical barriers introduced by dams, there can also be behavioural obstacles, such as lack directional cues from flow, or hydraulic effects that discourage onward movement. A full consideration of these is given in Chapter 2, especially with reference to Atlantic salmon smolts). Delay may also occur after passage, potentially due to disorientation, stress, or because of water circulation patterns that hold migrants (Schilt, 2007). Migrations are often seasonal and their timing can be crucial to onward survival (Thorstad, 2012), hence any delay could result ultimately in population decline. Aggregations of migrants due to delay at barriers may also act to increase predation rates.

1.7.1.2 Upstream passage

Large dams can halt upstream fish movement. Historically this has led to the development of fish passage technologies to restore connectivity for upstream migrating fish. Anthropocentric concerns about socio-economically important fisheries have been the main motivation for improving fish passage and protection, and so this effort has been mostly biased towards salmonids for cultural and economic reasons (Clay, 1995). This is changing worldwide (Meyer, 2007) due to conservation concerns manifested in legal requirements (for example the Water Framework Directive and Habitats Directive in Europe, see section 1.4).

Basic research has quantified the movement abilities of some species, with a view to optimising fishway design. Field assessments and experimental evaluations have been performed to assess and optimise the utility of fishway technologies. With these studies has come the recognition of exogenous and endogenous influences on a fish's ability to traverse an obstacle. Temperature, flow, water quality, species, lifestage, size, physiology and life history are known to affect swimming performance (Wardle, 1975; Beamish, 1978; Blake, 1983; Beach, 1984; Videler, 1993; Pon, 2009). Exogenous and endogenous influences are considered in more detail, particularly for Atlantic salmon, in Chapter 4. Despite substantial research into, and implementation of, technologies to aid fish passage, understanding of the effectiveness of these measures remains poor, and even well targeted species do not always pass as well as expected (Castro-Santos *et al.*, 2009).

1.7.2 Potential effects of small, low-head run-of-river hydropower upon fish passage

1.7.2.1 Downstream passage

Injury

As for large hydropower, small, low-head hydropower infrastructure can cause injury to downstream moving fish. However, there is a perception that certain hazards associated with high head schemes are less likely to cause issues at low-head schemes. The smaller magnitude of height differential means that changes in water pressure or velocity are less extreme, as are the associated sheer stresses. Additionally, because the impacts of turbine passage are now well understood, fish protection is usually required in the form of intake screens and bypasses. However, there are several emerging hydropower turbine technologies designed for use on low-head barriers, or in natural flow without the requirement for screens and bypasses. These turbines tend to have slow rotational speeds, which are perceived to reduce the risk of injury from turbine strike, pressure or sheer.

Archimedean screw turbines are one such technology that are becoming increasingly popular for low-head applications. At present there is no requirement to prevent fish from entering these turbines. The regulating agencies in the UK have developed guidelines for the design and operation of these turbines with regard to fish protection. These recommendations are based on the outcomes of a series of commercial consultancy reports (Kibel, 2007; Kibel & Coe, 2008; 2009; 2011). Although these studies are valuable evidence, they have not resolved uncertainties about the potential for damage to the whole range of species and life stages they may impact upon. There is a clear need for a greater body of evidence.

Delay

Obstacles such as small weirs associated with low-head hydropower infrastructure have the potential to halt or delay the downstream movements of fish (Aarestrup & Koed, 2003; O'Connor *et al.*, 2006; Gauld *et al.*, 2014). Physical obstructions such as hydropower intake screens can halt fish which cannot find a suitable route downstream. Behavioural obstructions, that fish are unwilling to pass, or lack of directional cues can lead to delay or halting of migration (Haro *et. al.*, 1998; Enders *et al.,* 2009) even where a safe passage downstream exists.

1.7.2.2 Upstream passage

Low-head obstacles can halt or delay upstream fish movements (Beasley & Hightower 2000; Ovidio & Philiphart, 2002; Cooke & Leach 2004; Zigler *et al.*, 2004, Weigel *et al.*, 2013). The degree of impediment depends on the characteristics of a particular obstacle, and with fish species, size, and other factors, but can also be dynamic with environmental influences such as flow and temperature. There is now an appreciation of the concept of 'partial' barriers (hereafter referred to as obstacles), which impede fish movements differently depending on conditions. Run-of-river hydropower schemes reduce the flow available over a section of river or obstacle, and this modifies the potential for fish passage. From a fisheries management perspective, an obstacle should allow sufficient passage to achieve management targets for a population. From a conservation perspective, it is desirable to minimize the fitness consequences to individual fish of obstacle passage (Roscoe & Hinch 2008; Castros-Santos, 2009; Noonan, 2012). In the worst case, of complete obstruction to movement, fish may be prevented from completing their lifecycle. Where fish are impeded but not obstructed, the delay and energy expenditure resulting from such an inefficient passage experience could decrease fitness. For example, stress in fish is known to affect not only spawning behaviour and timing, but the survival of offspring (Schreck, 2001).

1.7.2.3 Cumulative impacts

Where multiple schemes are installed in series along a river, the accumulation of the above effects on individuals and populations passing through them may become important, even where the effects of each scheme are acceptable (Fraser *et al.*, 2015). Cumulative impacts have been demonstrated for upstream migrating Atlantic salmon (Gowans, 2003), Pacific lamprey (*Entosphenus tridentatus* Richardson) (Moser, 2002) at large hydropower schemes, and river lamprey (Lucas *et al.,* 2009), at a tidal barrage and series of low-head weirs.

Similarly, cumulative effects have been shown on downstream migrants for European eel (Winter *et al.*, 2006; 2007), and Pacific salmon smolts (Williams *et al.*, 2001) at large hydropower schemes.

1.8 Thesis aims

The effects on downstream migrating fish of turbine passage and delay at large hydropower schemes that use conventional turbines is relatively well understood. However, small, low-head, run-of-river hydropower schemes are proliferating in Scotland, and little is known about the potential for delay or effects of turbine passage, especially at ASHTs, where there is no requirement to prevent fish from entering turbines. This evidence is lacking even for the most socio-economically valuable of species, the Atlantic salmon and sea trout, which migrate to sea as juveniles (smolts). In order to assess the population effect of turbine passage, it is first necessary to quantify the proportion of the migrating population that is exposed. This line of questioning relates to the behaviour of smolts approaching and passing such schemes, and is linked to the question of potential delay.

> **Chapter Two** aims to determine the proportion of smolts,if any,exposed to turbine passage at an ASHT, and assess whether this depends on flow management or other environmental or endogenous factors. Also addressed is the question of whether delay to smolt migration occurs. Finally, the onward consequences of ASHT passage on migration success are investigated.
>
> **Chapter Three** then aims to assess the prevalence and severity of damage to Atlantic Salmon smolts from ASHT passage: both the occurrence of external injury, and also possible subtle effects which are not readily apparent.

Low-head barriers, such as those associated with low-head hydropower schemes are known to constitute obstacles to upstream fish passage. The degree to which movement is hampered is dependent on physical characteristics of the barrier, hydrology, fish characteristics, and other environmental influences such as light levels and temperature. Diversions through turbines reduce the flow available over these obstacles, and depending on the scheme layout, also deplete flow through a section of river. Although all hydropower schemes are required to maintain flows over these reaches for fish passage, the effectiveness of this strategy for ensuring efficient movements is uncertain. In addition, fish may be attracted to the introduced outflow below hydropower turbines. These obstacles: a low-head barrier, depleted stretch, and competing flow, all modulated by the degree of abstraction by the turbine, may combine to reduce upstream passage success, and induce delays at the scheme.

> **Chapter Four** aims to establish whether low-head ASHT schemes can pose an obstacle to upstream migrating adult salmonids, and whether this effect is related to flow management, scheme layout, or other environmental and endogenous factors. It is recognized that such effects may be highly site specific, and so this chapter incorporates the study of adult fish movements at three ASHT schemes in order to gain insights into the influence of scheme design and operational regime on these movements. A subsidiary aim is to characterise the downstream return movements of post-spawned salmonids at these schemes.

Chapter 2

Smolt passage behaviour at an Archimedean screw hydropower scheme

Possibly the most divisive issue associated with hydropower development is its potential effect on fish species of socio-economic and conservation value. Of particular concern are the risks posed to fish species which rely on longitudinal connectivity for migration between habitats to complete their lifecycles. In Scotland, Atlantic salmon and sea trout (the anadromous form of *Salmo trutta* L.) are economically valued species of conservation concern. They spend portions of their lifecycle in both freshwater and marine habitats, and thus are potentially at risk from hydropower installations when moving between these environments.

Prior to migrating to sea, juvenile anadromous salmonids (smolts) undergo a series of morphological, physiological and behavioural changes (McCormick *et al.*, 1998) preparing them for marine life. This is a critical life stage, with high levels of mortality (Thorstad *et al.,* 2012). Migration usually occurs in spring, between April and June in Scotland, and arrival at sea appears to be synchronized within catchments, regardless of the origin of individuals. At broader geographic scales, the timing of sea entry is associated with a narrow range in sea temperatures (Thorstad *et al.*, op.cit). This is believed to be an adaptation to meet favourable physiological and feeding conditions (McCormick *et al.,* 1998).

During migration, passage through hydropower turbines can result in direct or delayed mortality to smolts, and these effects are dealt with in Chapter 3. The risks can be mitigated by the use of fishways and guidance systems, screens, ‘fish-friendly’ turbine designs and the preservation of environmental flows to allow passage of fish through the depleted section of river. Such mitigation measures aim to provide safe passage, without undue delay, to migrating fish, but their efficacy at achieving these goals depends on sound understanding of the passage preferences of downstream migrants.

The recent trend of redeveloping existing low-head barriers for hydropower generation is concerning, because whilst these small schemes are assumed to have little or no impact on emigrating juvenile salmonids, this has not been tested. Archimedean screw turbines are a popular choice for such redevelopments, in part because they are purported to be benign for fish passage. For this reason these turbines are exempted from the requirement to prevent fish from passing through them. In this chapter, the effects of such a scheme upon smolt migration behaviour is characterised on the River Don, as an initial step towards assessing their potential impacts.

2.1.1 The migratory behaviour of salmonid smolts in relation to hydropower schemes

There is a generally accepted view that salmonid smolts of all species migrate near passively in the fastest moving part of the water column, close to the surface, and at night (Johnsson & Johnsson, 2011; Thorstad *et al.* 2012). However, migration can involve active swimming (Davidsen *et al.*, 2005; Dempson *et al.*, 2011), may also occur near the bottom of the water column (Davidsen *et al.,* 2005), and the reliance on nocturnal migration periods lessens as temperature increases (Ibbotson *et al.*, 2006). These variations in behaviour are also likely dependent on flow conditions and temperature, and are believed in part to be adaptations to avoid predation under differing conditions (Thorstad *et al.,* 2012). Certain behaviours appear to act to preserve smolts from damage during semi-passive drifting downstream. For example smolts will turn to face upstream to escape accelerating water velocities (Haro *et al.* 2000; Kemp *et al.* 2008; Enders *et al.*, 2009; Vowles *et al.,* 2014a), and avoid passing under overhead cover (Kemp *et al.*, 2008).

The controlled drifting behaviour of smolts suggests that they are likely to pass through hydropower facilities with the bulk flow (Coutant and Whitney, 2000, Williams *et al.,* 2012). Most research into smolt behaviour in relation to hydropower schemes has been at large scale installations, typically with large impoundments, and alternative fish passage routes consisting of the turbine, a designated fish bypass system, and a variable spill over the impounding structure. A primary concern has been in evaluating the proportion of migrating populations which are exposed to hazardous passage routes. This has been found to vary with the proportion of flow through these various routes in a curvilinear way (Coutant & Whitney, 2000).

Aside from considerations of passage route, hydropower infrastructure can delay migration by presenting behavioural obstacles such as accelerating flow and overhead cover described above, even when a safe passage route exists. Smolts have been observed to halt their migration in dam forebays before resuming downstream migration (Venditti *et al.*, 2000; Beeman & Maule, 2001). This delay to migration has also been observed at low-head barriers in Scotland (Gauld *et al.* 2014) for trout smolts. These delays may increase exposure to predation (Gauld *et. al.*, op. cit; Aarestrup, 2003), and reduce post-smolt survival, by causing a mismatch between marine arrival and optimal conditions for survival and growth (McCormick *et al.* 1998; Otero *et al.* 2013). Additionally, smolts have a limited period of time during which the fish are physiologically prepared for seawater entry, and migratory delay, along with increasing temperatures, could result in 'desmolting' and a return to freshwater residency (McCormick *et al.*, 1999). McCormick *et al.* (2009) noted that a range of perturbations acting in the freshwater environment, including impoundments, can affect smolt physiological development, post-smolt behaviour, early seawater survival, growth and homing. Recent evidence (Stich *et al.* 2015), has also highlighted the importance of physiological preparedness for early seawater survival, and the delayed, negative impacts of dam passage.

2.1.2 Aims

This study aims to assess the behaviour of downstream migrating salmonid smolts at a small, low-head hydropower scheme on the River Don in Aberdeenshire, Scotland, in order to inform scheme design and management which is sympathetic to the needs of this migratory life-phase, both at Craigpot, and for low-head hydropower schemes in general. The key aims are to:

> establish whether naturally migrating wild Atlantic salmon and trout smolts pass through the turbine channel and Archimedean screw turbine;

estimate the proportion of the migrating population that take the turbine channel route, and relate this to flow apportionment through the turbine, and environmental conditions;

identify any undue delay to smolts passing through the scheme; and

establish whether the onward survival of migrating smolts is affected differentially by passage through the turbine or alternative route.

2.2 Methods

2.2.1 Study Site

2.2.1.1 River Don catchment

The study was carried out between 30 March 2013 and 30 May 2014 at Craigpot hydropower scheme (57.26°N 2.63°W) in the middle reaches of the River Don, Aberdeenshire, Scotland. The main stem of the River Don (Figure 2.1) is 131 km long, and is the sixth longest river in Scotland. The catchment area is 1312 km^2, and can be viewed as two distinct topographical areas. The western portion of the catchment is a high gradient system which rises into the Cairngorm Mountains. Towards the east, the catchment encompasses a relatively flat floodplain and farmland between Kildrummy and the coast. The upper Don exhibits a rapid hydrologic response to rainfall, with a snow melt component in the spring. The lower river has more consistent flows. The 46-year mean daily flow at Parkhill, the SEPA gauging station furthest downstream (57.22°N, 2.19°W) and approximately 10.5 km from the tidal limit, is 21.15 m^3s^{-1} and the 95% exceedance flow (Q_{95}) is 5.55 m^3s^{-1} (National River Flow Archive, 2015). Land use in the upper catchment is predominantly moorland grazing for deer and sheep, along with areas of coniferous forestry. The lower catchment is agricultural land.

2.2.1.2 River Don salmonid stocks

As the river nears the urbanized coast at Aberdeen the gradient increases for the final 8 km. Historically this section of the River Don was used as a power source for numerous mills. There are currently four main points of abstraction on the River Don: two paper mills at Inverurie and at Stoneywood, the ASHT scheme at Craigpot, and a similar scheme in the upper catchment at Strathdon. Another ASHT scheme has been licenced in the lower catchment but has not as yet been developed.

Alantic salmon are present in all parts of the catchment which are accessible to this species (River Don Trust, 2008, Gardiner & Egglishaw, 1986). There are no fish counters present on the Don and so no information on adult Atlantic salmon and sea trout abundance, other than rod catch numbers, is available. Annual rod catches of salmon and grilse for 2005-2015 are shown in Figure 2.2, which suggests a declining trend, although in the longer term, fluctuations of comparable magnitude are frequent (DDSFB, 2015).

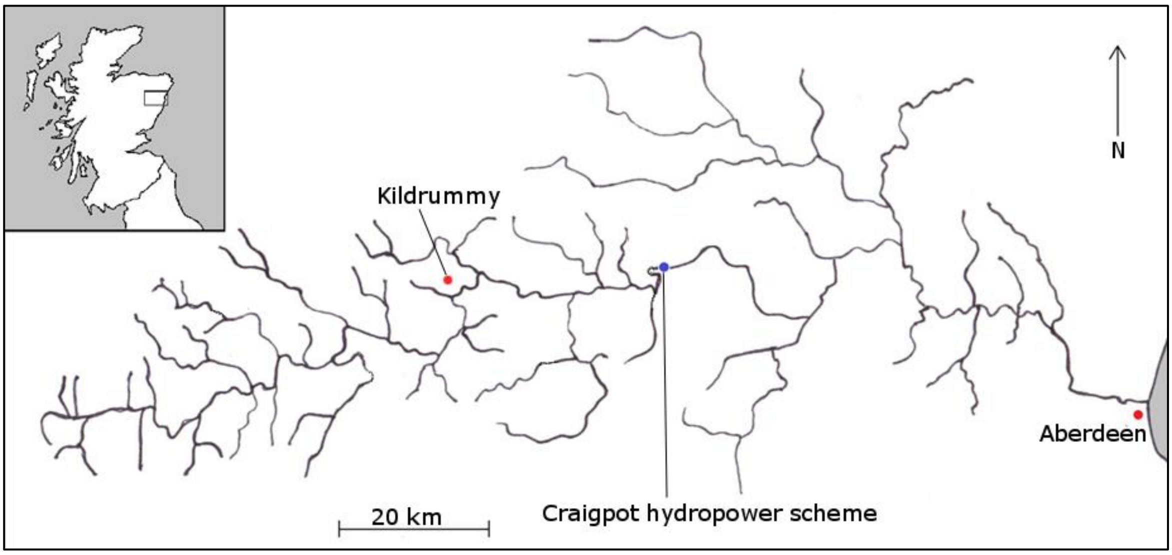


Figure 2.1. The River Don Catchment, and its location in Scotland (inset).

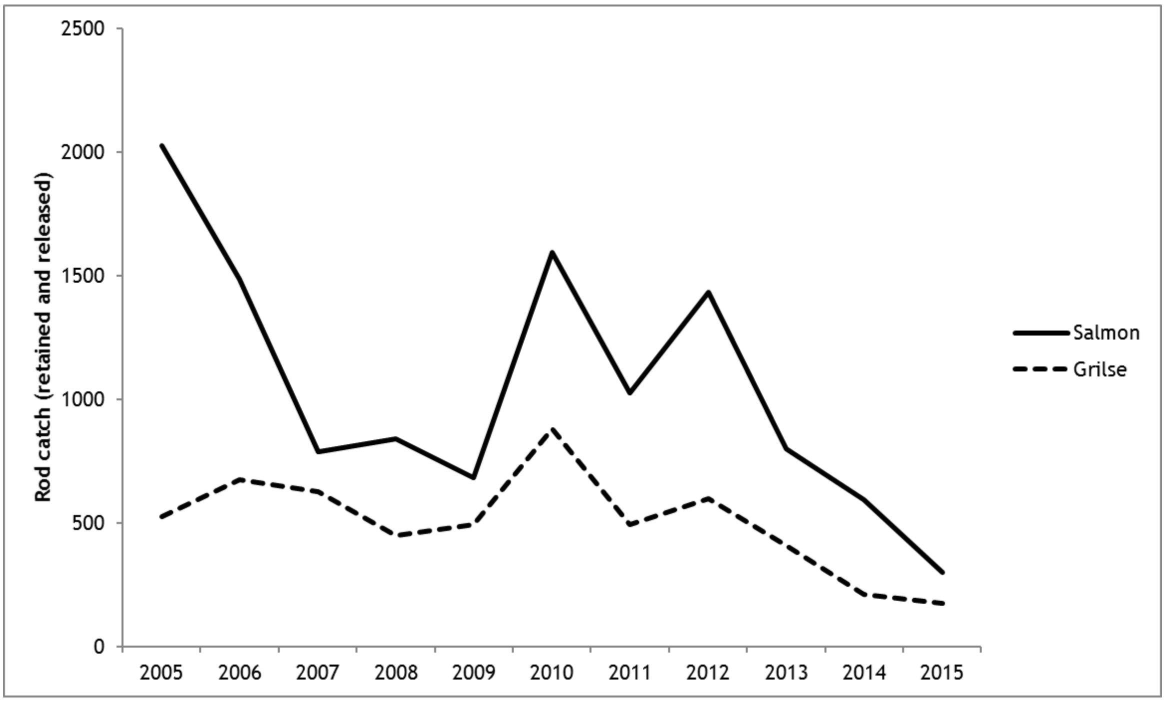


Figure 2.2. Rod catch data for salmon and grilse from the River Don during the period 2005-2015. Data supplied by Marine Scotland Science.

Under the Aquaculture and Fisheries (Scotland) Act 2013, the River Don is classed as category 3, which means that conservation limits are met for 60-80% of the time.

Salmo trutta is present as both the freshwater-resident brown trout and anadromous sea trout forms. Within the angling community the River Don is renowned as a wild brown trout river, and the distribution of trout extends to all parts of the catchment accessible to adult fish (River Don Trust, 2008). Trout fry and parr have been found throughout the catchment, with the greatest densities in the tributary sites (Shields, 1996). Annual rod catch for the period 2005-2010 appears to show a decline (Figure 2.3)

Stocking of salmonids was carried out by the Don and District Salmon Fishery Board from the late 1950s until 2012 (James Kerr, River Don Superintendent, 2014, pers. comm.). From 2006 the stocking activity was reduced from 750,000 to 300,000 salmon eggs, and approximately 100,000 trout eggs (River Don Trust, 2008). All were derived from broodstock migrating to the upper catchment in late October or November and collected at Strathdon. Stocking with juvenile trout of hatchery origin also occurred.

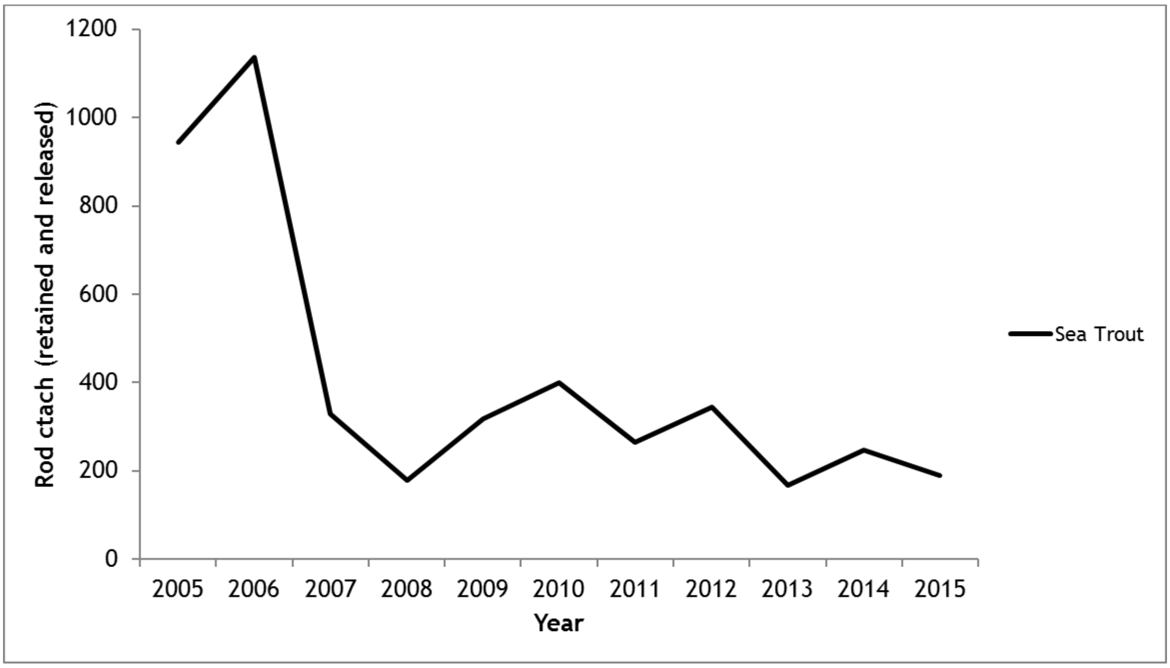


Figure 2.3. Sea tout rod catch data for the River Don from 2005-2015. Data for fish retained and released are presented. (Data supplied by Marine Scotland Science).

2.2.1.3 Craigpot hydropower scheme

Craigpot hydropower scheme (the study site: Figure 2.4) is a redevelopment of an historical mill site, originally built for a saw mill and subsequently used for hydropower for Castle Forbes Estate, where it is located, until the 1950s. A small water wheel was installed in the late 1990s which abstracted 0.4 m^3s^{-1}. This was replaced by the new Archimedean screw turbine, which has a maximum abstraction limit of 4 m^3s^{-1}. The site layout is shown in Figure 2.5. The impounding structure is an 89 m length sloped profile weir which is oriented 15-25 degrees to the main flow. The construction is of boulder and cobble, with some concrete augmentation. The hydraulic head is 0.9 m. Mean effective weir face length is 4.3 m ranging from 3.9 to 5.1 m. The weir is set oblique to the flow on a left hand bend, and leads to a 100 m long intake channel which feeds the hydropower turbine. For the purpose of facilitating fish passage a 1.8 m wide breach is present 8 m from the true right side of the weir. There is no fish screening on water intake to the channel, but there a 100 mm spaced vertical bar trash rack is in place 5 m upstream of the turbine.

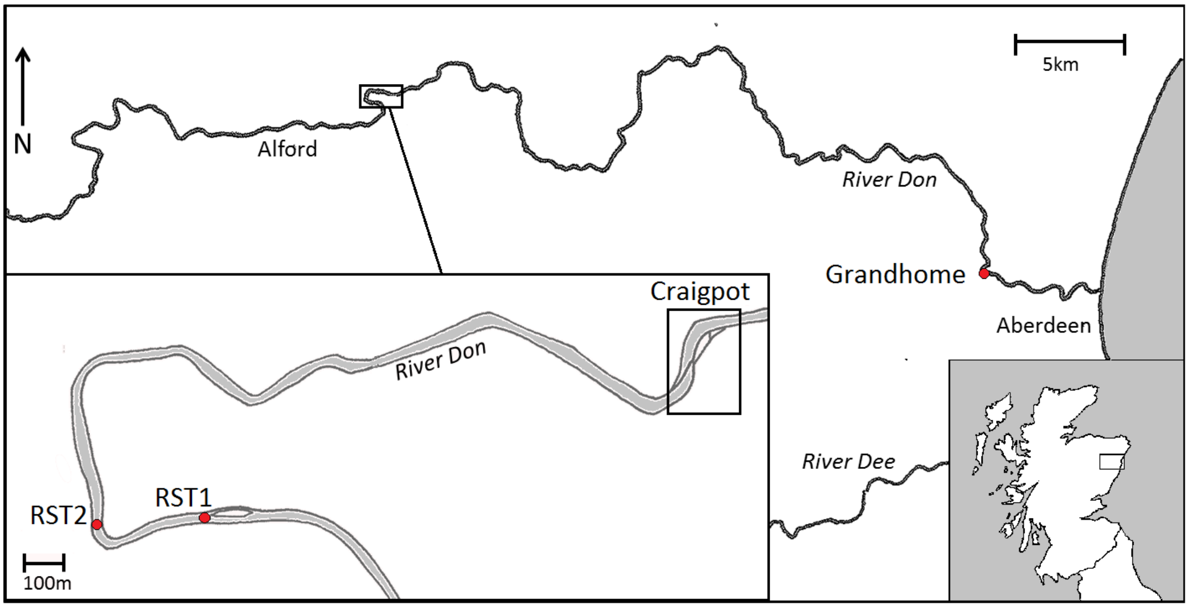


Figure 2.4. Map showing the location of the river Don in Scotland (right inset), and the trapping and monitoring locations on the river. The rotary screw trap was positioned at point RST1 (left inset) in spring 2013 and at RST2 in autumn 2013 and spring 2014. The hydropower scheme is labelled as “Craigpot” and was instrumented with fixed radio and PIT logging receivers. A further fixed radio receiver was installed for monitoring at the point labelled “Grandhome” in 2014.

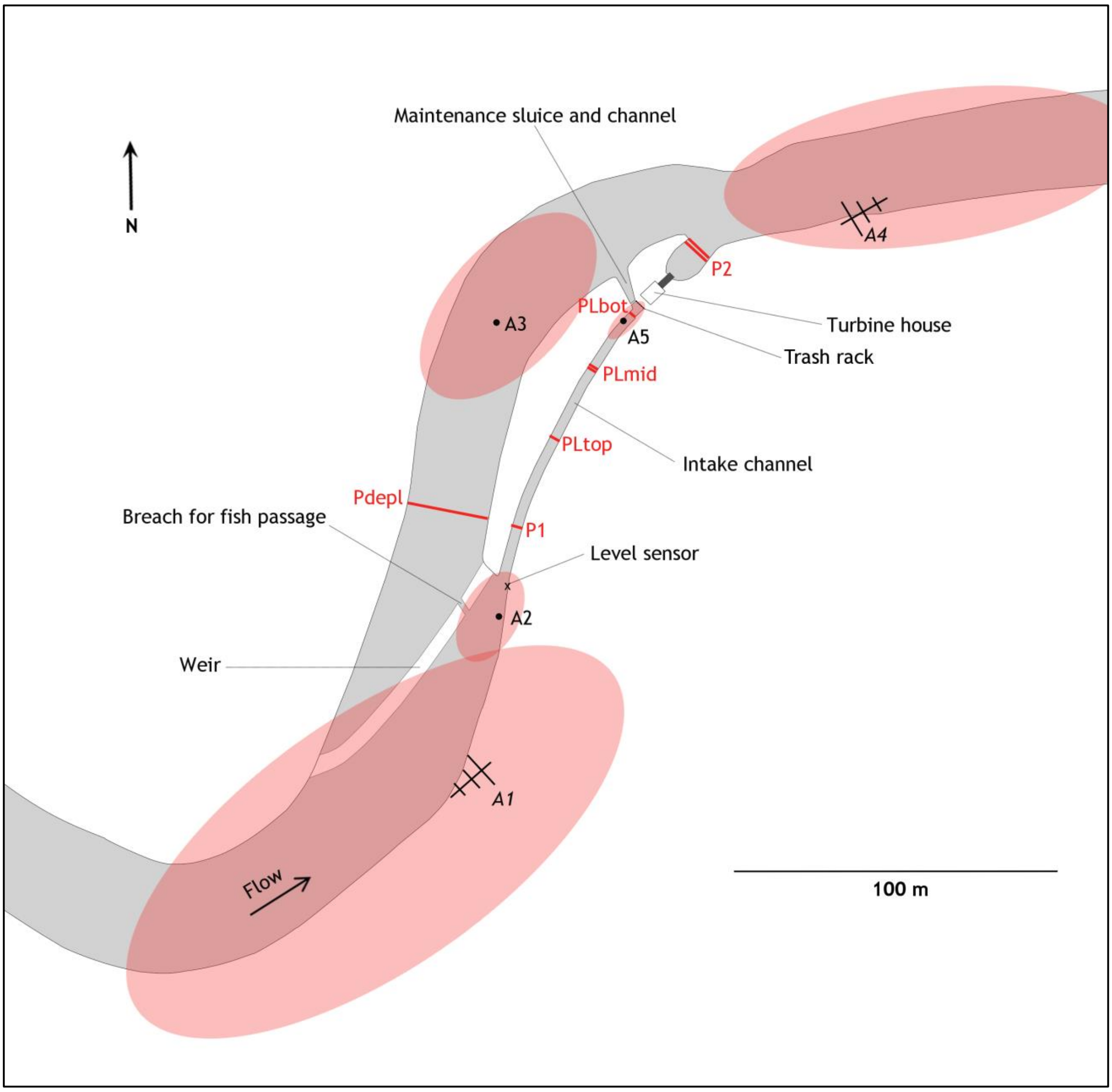


Figure 2.5. Site layout, PIT and radio monitoring. PIT antennas are shown as red lines and labelled with the prefix P, radio antenna locations are represented as the hatched symbols (Yagi antenna) and black dots (dipole antenna), and labelled with the prefix A. Radio detection zones determined from range testing are shaded red.

The scheme uses a 4-bladed Archimedean screw turbine (Landustrie, Sneek, the Netherlands) connected to a generator, to convert the movement of water over the available head of 2.2 m to electricity, up to a maximum of 60 kW at its full capacity of 4 m^3s^{-1}. The length of bladed screw is 5.4 m, and the diameter is 2.9 m. The screw is mounted in a steel trough set at 22 degrees to the horizontal, through which the water flows, driving the screw. The upstream leading edges of the turbine blades are fitted with rubber bumpers with 35 mm of compression to mitigate blade strike to fish. The maximum gap between the screw blades and trough is 5 mm.

The turbine operates on a variable abstraction regime specified by the operational licence (SEPA CAR licence CAR/L/1096350), and is shown in Table 2.1. No abstraction is allowed when river discharge falls below 4.43 m^3s^{-1}, equivalent to Q_{90} based on historical records. The maximum permissible abstraction under any other flow condition is set at 4 m^3s^{-1}. Flow through the screw is controlled by limiting the rotational speed using electrical resistance applied to the generator according to a control algorithm. The target abstraction rate between the upper and lower limits is calculated using Equation 2.1.

$$q_{target} = -0.0289Q^2 + 0.9216Q + 9.2694$$

Equation 2.1

where:

q_{target} is the target abstraction flow, in m^3s^{-1}, and

Q is the total river discharge, calculated as the sum of the turbine and weir flows (given by Equations 2.2 and 2.3, which follow).

Table 2.1. Abstraction regime at Craigpot hydropower scheme as defined by the operating licence (SEPA CAR licence CAR/L/1096350) Exceedance discharge is the river flow which is exceeded for the percentage of time given in the "% Exceedance" column based on historical daily mean flow data. At flows above 25% exceedance the turbine is operating at its maximum capacity of 4 m^3s^{-1}.

% Exceedance	Exceedance discharge (m^3s^{-1})	Maximum proportional take (m^3s^{-1})
25	14.876	-
60	8.016	0.3
70	6.74	0.25
80	5.543	0.2
85	4.985	0.11
90	4.43	0

The control system uses, as its inputs, real-time data from a water level gauge in the impoundment at the entrance to the turbine intake channel (see Figure 2.5), and the rotational speed of the turbine (which is considered proportional to flow through the turbine), and thus adjusts the abstraction according to the river discharge calculated from these two variables. Discharge over the weir is calculated as:

$$q_{weir} = l\, c_w h^{1.5}$$

Equation 2.2

where:

q_{weir} is the flow over the weir, in m^3s^{-1},
c_w is the weir coefficient, 2.5,
h is the crest depth of water, in m taken from the level gauge, and
l is the length of the weir crest, 89 m.

The actual turbine flow under normal operating conditions is calculated using Equation 2.3, with flow directly proportional to turbine rotational speed, and limited to 4 m^3s^{-1} at the maximum of 26 revolutions per minute (RPM), and 0 m^3s^{-1} at 0 RPM.

$$q_{turbine} = \frac{r}{r_{max}}\, q_{max}$$

Equation 2.3

Where
$q_{turbine}$ = turbine flow, in m^3s^{-1},
q_{max} = maximum flow through turbine, 4 m^3s^{-1}
r = turbine rotational speed, in revolutions per minute (RPM) , and
r_{max} = maximum turbine rotational speed (RPM) at full capacity.

2.2.2 Scheme layout and fish passage monitoring

Before fish tagging commenced the hydropower scheme was instrumented with telemetry equipment to enable the movements of Atlantic salmon and sea trout smolts in the vicinity of the scheme to be recorded. Passive integrated transponder (PIT) technology was used to determine passage route, and precisely record the time of passage at monitored locations, with the particular aim of determining the time spent in the turbine channel. PIT tags are small, unpowered transponders which emit a unique digital signal when in close proximity (usually less than 1 m) to a powered PIT antenna. This signal is then recorded by a logging receiver, along with the time of detection. The antenna comprises a loop of stranded wire with electrical current passing through it. This produces an electromagnetic field, which powers transponders within range by induction. Antennas can be a loop of cable around a channel (pass-through antenna) or a flat loop on a surface (flat-bed antenna). Generally, antenna loops work best when the cable is separated by no more than 1 metre from the other side of the loop, although this can depend on the operating environment, construction materials and loop configuration. As well as the high temporal resolution of PIT technology, the low cost of these transmitters afforded a larger sample size than was feasible for other, more costly transmitter options. A disadvantage of this technology is that tags may fail to be detected for several reasons: available systems do not allow the concurrent detection of multiple tags; larger span antennas are less efficient at detection; and antennas are susceptible to damage from high flows.

In order to circumvent these issues it was decided to also tag a subsample of PIT-tagged fish with radio tags. Radio telemetry was chosen in preference to acoustic technology because of the shallow depths and acoustic interference, both from natural turbulence and the hydropower turbine at the site. The low conductivity of the river water (81 to 127 μS cm^{-1} during monitoring setup) made VHF radio telemetry viable. Under the conditions at the site, radio tags could be detected by fixed receiving loggers with a range up to several hundreds of metres, and also be tracked using mobile receivers. This enabled more definitive allocation of passage route for the radio-tagged sample, and also the determination of the movements and fate of these fish outwith the areas monitored by fixed receivers, in particular the onward survival of smolts after passage through the scheme.

2.2.2.1 PIT monitoring

Five half-duplex (HDX) PIT detection antennas (based on the design of Castro Santos *et al.*, 1996) were installed in-stream in the turbine channel above and below the turbine, in the locations shown in Figure 2.5. PIT antenna P1 was positioned 20 m downstream from the turbine channel offtake and 80 m from the leading end of the turbine. PIT antenna P2 spanned the outflow channel 11.5 m below the turbine at the opening to the river, PIT antennas PLtop, PLmid and PLbot were 50, 25 and 9 m from the turbine mouth respectively. The antennas comprised of a loop of insulated, finely braided single-core speaker cable (model S12102R, Shark, Oud-Beijerland, The Netherlands), of 413 strands of 0.1 mm diameter oxygen free copper, and nominal conductor area 3.24 mm^2. P1 and P2 encircled the channel perpendicular to the flow, were tensioned above the flow by static line and were weighted to the bed by rocks and sand bags so that the cable followed the shallow U-shape of the bed. The cable was crossed mid flow on a supporting upright to form a figure of eight loop. Maximum separation between the top and bottom of the loop was 1.2 m, at the midpoint. PLtop, PLmid and PLbot were 5.1 m rectangular figure of eight loops mounted on timber frames secured within the channel, with loop heights of 1.4 m, 1.35 m and 1.2 m respectively.

These antennas were each monitored with a logging reader (single or multiplexing readers, Oregon RFID, Portland, Oregon based on hardware from Texas Instruments). P2 comprised two antennas (P2A1 and P2A2) spanning the flow in series and separated by a metre, and was driven by one multiplexing reader, whilst the other antennas were individually operated by single readers, or by multiplexing readers set to operate a single antenna. An energise-read-write rate of 14 cycles per second was used, which was halved in the multiplexing reader driving P2 to 7 cycles per second, per antenna. Each detection was logged individually. A reader-integrated logger stored the data, which were the unique code of the transponder and date and time of the read, as well as reader operational information (reader start and stop times, and changes to reader configuration by the user, for example) and this was downloaded as a text file. Date and time were reset periodically due to observed reader clock drift.

Pdepl was installed ready for smolt monitoring in 2014. This was a 26 m span flat-bed antenna comprised of one American wire gauge (AWG) welding cable, housed in PVC pipe threaded through 30 concrete building blocks wedged amongst the boulders of the river bed. The range of this antenna was augmented by increasing the normal 12 V battery power supply used for the other readers to 18 V.

Range tests were carried out along the entire length of all antenna loops. P1 and P2 gave continual detection of 12 and 23 mm PIT tags oriented perpendicular to the loop, within a range of 25 and 50 cm from the plane of the loop for the two respective tag sizes. The range for the flat-bed antenna Pdepl was between 40 and 50 cm for the 23 mm tag, and was 20 cm for the 12 mm tag. PLtop, PLmid and PLbot gave continual detection of 23 mm PIT tags oriented held perpendicular in the centre of the loop, but for the 12 mm PIT tag, there was a vertical gap in coverage which varied between tests during the study, but ranged from approximately 40 cm to 100 cm. In general, the larger the aperture of the loops, the lesser was the detection range, resulting in less efficient tag detection. In the case of Pdepl, the limited vertical range of this antenna likely allowed tagged fish to pass undetected when channel depth exceeded this range.

Antennas were tested using a PIT tag on a pole, and antenna tuning capacitors (Texas instruments model RI-ACC-008B-30) were checked and tuned with a tuning indicator (Texas instruments model RI-ACC-AT12) at least at every battery change. Battery changes, interim tests and data downloads were carried out within the expected running intervals of the batteries (between one and four 110 Ah 12 V, or 200 Ah 6 V deep cycle leisure batteries per reader) and there were no gaps in the operation of PIT readers during the smolt monitoring periods.

Efficiencies were calculated for each of the antennas as the proportion of PIT-tagged fish detected at downstream antennas which were also detected at the antenna of interest. Detections of fish tagged with both radio and PIT tags by fixed radio antennas downstream were included in these estimates, and for PDepl, radio detections comprised the only downstream antennas from which to derive estimates. The efficiency of P2A1 was calculated using detections at P2A1 and at P2A2. Then for P2A2 it was assumed that all fish passing P2A2 had passed P2A1, there being only a metre separating these sequential antennas. The efficiency of P2A2 was taken as the proportion of PIT-tagged fish detected at the upstream antenna (P2A1) which were also detected at P2A2. The composite efficiency of P2, and of the combined turbine channel antennas was calculated as the complementary probability of no detection at all sequential antennas:

$$\hat{p}_{composite} = 1 - \prod_{i=1}^{n} (1 - \hat{p}_i)$$

Equation 2.4

where

$\hat{p}_{composite}$= composite efficiency estimate for n sequential antennas, and

$\hat{p}_i$ = individual efficiency estimate for antenna *i*.

2.2.2.2 Radio monitoring

Radio tagged smolts (2014 only) approaching the scheme were logged using five antennas monitoring separate detection zones as shown in Figure 2.5. Antennas were connected to receivers in the turbine house with 50Ω, RG58 co-axial cable with a copper clad aluminium inner conductor of 2.74 mm diameter, screened by tinned copper wire braid (Antennax ANT-400).

Antennas A1 and A4 were 3-element Yagi antennas oriented with their elements in the vertical plane. A2 and A5 were low-range underwater antennas made by stripping the coaxial cable. The length of exposed cable was 9.4 cm, which is half the wavelength of the radio tag frequency in water, as reccommended by Beeman *et al.* (2004), and calculated as:

Wavelength (in cm) = 0.32649 x frequency (in MHz)$^{-0.9998}$ = 18.8 cm

The coaxial sheath was securely sealed from water ingress by covering with hot-melt glue, then wrapping first with self amalgamating tape and finally with electrical tape. The antenna was protected by housing in PVC pipe, and oriented axial to the flow (and therefore parallel to the expected orientation of the transmitter antenna on a swimming smolt). Antenna A3 was constructed in the same way, but attached above water on a rock in the middle of the river. Antennas A1 through A4 were monitored with a single data-logging receiver (Lotek SRX-4000, Ontario, Canada) equipped with a switcher which enabled sequential recurrent monitoring of each antenna. Scan duration at each antenna was set at 6 s, 1 s longer than the longest pulse interval of the transmitters used. Antenna A5 was continuously monitored using a dedicated logging receiver

(Sigma-eight Orion, Ontario, Canada). Range tests were carried to ensure that transmitters were detected in all zones at full depth, whilst travelling dowstream through each zone at approximately the surface water velocity. These tests were carried out with a weighted test tag dragged along the substrate from a kayak. Range tests showed an effective range distribution for these antennas as approximated in Figure 2.5. The efficacy of the antenna and receiver configuration for detecting five co-located tags was tested by floating the tags in a water-filled bottle through each antenna zone. No tags were missed during tests with the final antenna and receiver configurations. All tests were carried out with the tag antennas trimmed as described in section 2.2.4. A test tag was introduced within range of antenna A4 on 28 April 2014 and was detected at this antenna until the receiver was switched off on 9 June 2014.

An additional data-logging SRX-4000 radio receiver was placed at a weir in Aberdeen (Grandhome, Figure 2.4), 49.7 km downstream from Craigpot and 8.2 km upstream of the tidal limit, in order to assess in-river survival. This receiver monitored two 4-element Yagi antennas pointing upstream and downstream of the weir, with the receiver switching between antennas every 10 s and set to log first and last detections within a 20 minute window in order to reduce stored data. The efficacy of the antenna and receiver configuration was tested during setup with a weighted test tag in the river approximately 100 m upstream and downstream of the antennas, with a result of continual detection during these tests. All radio receivers operated continuously throughout the 2014 smolt monitoring period. The radio telemetry system was also operational in 2013, although no smolts were tagged with radio transmitters.

Radio tagged salmon smolts were manually tracked opportunistically on nine days by vehicle, by kayak and on foot. All manual tracking was carried out using a Lotek SRX4000 receiver. For vehicle based tracking this was connected to a roof-mounted, omni-directional dipole antenna; for tracking on foot a 3-element Yagi antenna was used and for tracking by kayak a small dipole antenna was mounted on the top of the kayak. Table 2.2 shows the dates and extent of manual radio tracking events. Tracking coverage by vehicle between the distances given was only partial because, in some areas, the distance between the river and the road was beyond signal range. The method used to analyse these data (Section 2.2.6.3), do not make the assumption of complete tracking coverage during these events.

Table 2.2. Manual radio tracking events during the smolt migration study. River distances covered from start to end of tracking are given with Craigpot as the datum, hence starting points for tracking sessions beginning upstream are shown as negative distances. Coverage was not complete for sections covered by vehicle.

Date	Method	Reach	Distance covered (km)
29/04/2014	Vehicle	Smolt trap to Craigpot	-2.1 – 0
30/04/2014	Vehicle	Smolt trap to Craigpot	-2.1 – 0
01/05/2014	Vehicle	Smolt trap to Aberdeen	-2.1 – 52.8
04/05/2014	Vehicle	Smolt trap to Aberdeen	-2.1 – 52.8
06/05/2014	Vehicle	Smolt trap to Grandhome	-2.1 – 50.7
08/05/2014	Kayak	Kemnay to Dyce	15 – 46.5
08/05/2014	Foot	Persley bridge to Bridge of Don	52.9 – 57.9
10/05/2014	Vehicle	Craigpot to Kemnay	-2.1 – 15
14/05/2014	Vehicle	Kemnay to Grandhome	15 – 50.7
16/05/2014	Vehicle	Kemnay to Grandhome	15 – 50.7
22/05/2014	Foot	Smolt trap to Craigpot	-2.1 – 0
22/05/2014	Vehicle	Craigpot to Kemnay	-2.1 – 15
22/05/2014	Foot	Persley bridge to Bridge of Don	52.8 – 57.9

2.2.2.3 Underwater video monitoring

Underwater closed circuit television (CCTV) (camera: Sony model IR 37CSHR-IR, and digital video recorder: Avtech model KPD674 4-channel CCTV DVR H.264) was used to observe wild smolts approaching the trash rack. The camera was mounted at mid depth, 0.5 m in front of the trash rack and angled towards it. These cameras were equipped with infra-red (IR) illumination and their image sensors were IR sensitive for night time imaging. However it was found that at night the field of view was blocked by the illumination of air bubbles and other materials entrained in the flow immediately in front of the camera. Tests with separate IR lights illuminating from an alternative aspect to the camera did not produce clear images, as the range of the IR lights was very low due to suspended materials. The extent of view was also reduced in the day time by turbidity during high flows. Fouling by drifting plant material and algal growth on the camera lenses was minimal due the the downstream angle of the cameras.

The digital video recorder was capable of recording only periods where movement was detected, but the prevalence of drifting material resulted in continuous recording. The video recordings were examined at accelerated speed to identify fish presences. No quantitative analysis was performed because of a paucity of observations. Wild migrating smolts were also recorded at the trash rack and passing into the turbine intake basin using a GoPro camera. Video and incidental visual observations are reported collectively in the results as contextual evidence of behaviour in these areas, but have not been analysed formally.

2.2.3 Fish capture

Nineteen Atlantic salmon and six trout smolts were PIT tagged in spring 2013. In 2014, 324 salmon and 42 trout smolts were PIT tagged, of which 28 salmon smolts were also radio tagged. Salmon and trout smolts were captured using a 0.91 m diameter rotary screw trap deployed upstream of Craigpot. In 2013, the trap was installed 2.5 km from Craigpot (RST1 in Figure 2.2), but in 2014 the trap was moved 400 m downstream in an effort to improve trapping efficiency. The dates of operation were 09/04/2013–18/06/2013 and 02/04/2014-10/05/2014, during which time the trap was checked at least every two days, and usually daily. Trap operation was ceased occasionally by lifting the rotating drum to protect the trap in high water, or when the drum was by blocked debris, but this was only a concern in 2013.

Salmon and trout parr were also captured and PIT tagged during autumn 2013 between 9 September and 21 October, in an effort to increase the pool of tagged emigrants for monitoring the following spring. These were captured using the rotary screw trap installed at the 2014 trap location, and by electrofishing in several upstream tributaries (see Appendix A2.1 for numbers of fish tagged at each location). Parr exceeding 110 mm were PIT tagged, based on prior experience of smolt size (2013 capture and J. Urquart, River Don Fishery Trust, 2013, pers. comm.).

2.2.4 PIT and radio tagging

The radio tags used (Lotek Nanotag NTQ-1, 5x3x10 mm, 0.26 g, 173.8 MHz, burst interval 3-4.4 s, predicted longevity 23-30 days) were of the trailing-whip antenna type, and the expected smolt length necessitated shortening of the antennas from 180 mm to 120 mm. A test tag was trimmed in 5 mm iterations with concurrent in-river range testing to ensure that range was not compromised at the relevant scale. Tests were carried out with the tag at maximum channel depth (3 m) at the hydropower scheme up to a horizontal distance of 150 m, using a 3-element Yagi antenna and a Lotek SRX-4000 receiver set at a high receiver gain setting (90 on an available scale of 0-99). The trimmed test tag was found to be logged continuously under these conditions, and was then used to arrive at the antenna range distributions summarized in Figure 2.5. River conductivity during these tests was 84 µS cm^{-1}, and ranged from 81 to 127 µS cm^{-1} during subsequent antenna range tests and checks, which confirmed the ranges shown in Figure 2.5.

All procedures were carried out under UK Home Office Licence (project licence number PPL 40/3425) and complied with the UK Animals (Scientific Procedures) Act 1986. Smolts were removed from the trap and immediately placed in a holding tub filled with highly aerated river water. Fish were anaesthetised by immersion in 50 ppm benzocaine solution with river water. The appropriate level of anaesthesia for tagging was indicated by a total loss of reactivity and a slowed operculum rate, reached after two to five minutes. A conservative approach was taken, using a low concentration for the first fish, with the concentration raised until the appropriate level of anaesthesia was reached. When suitably anaesthetized, the fish was placed upside down on a V-shaped surface. Fork length and mass were recorded and the fish were photographed on both sides before those sufficiently large for tagging were placed in a V-shaped surgical tray. The fish’s gills were aspirated with fresh river water during surgical tagging, using either a gravity-fed hose into the mouth or a squeezable bottle. Fish exceeding 120 mm fork length and 30 g mass were selected for PIT and radio tagging (Larsen *et al.*, 2013). A 6 mm incision was made, left of the centre-line, on the ventral surface of the fish, anterior to the pelvic girdle. The whip antenna of the disinfected radio tag was run through a hole in the abdominal wall punctured with an 18-gauge surgical needle 10 mm posterior to the incision, so that the antenna trailed behind the fish. The radio tag and a 23 mm HDX PIT tag (Texas instruments, USA) were then implanted in to the peritoneal cavity through the incision. The incision was closed with one dissolvable suture (4-0 Vicryl Rapide, Ethicon Ltd, Livingston, UK). The same method was used on fish which were PIT tagged only, without the need to accommodate the whip antenna. 12 mm HDX PIT tags were used for fish under 120 mm fork length (Larsen *et al.*, 2013). For these tags a smaller incision (3 mm) negated the need for a suture. The gills were aspirated with river water during the procedure.

After tagging, the fish were returned to a recovery tub filled with highly aerated water. When recovered the fish were placed in a keep box in the river channel before release into the river in the evening at the capture location. Fish were released in groups, mixing tagged and untagged fish whenever possible (Gauld et al., 2013). All fish recovered and were seen to swim normally, with good balance and no signs of distress. No attempt was made to test the effect of tagging. Studies using surgically implanted transmitters on migrating Atlantic salmon smolts generally accept that tagged fish represent the closest observable approximation to natural behaviour (e.g. Aarestrup et al., 2002; Gauld et al., 2013; Moore et al., 1998; Thorstad et al., 2007).

2.2.5 Environmental and turbine operational data

Turbine operational data were provided by Highland Ecodesign, Perth, and were 15-minute logged turbine rotational speed (revolutions per minute, RPM) and water level (m) above the weir. Turbine flow and flow over the weir was calculated according to equations 2.2 and 2.3. Cross sectional flow profiles were taken in the river and turbine channel using 1 or 2 m sampling intervals and a hand held electromagnetic current meter (Marsh McBirney Flo-Mate 2000, Maryland, USA). Temperature data (in degrees Celsius at 15-minute intervals) were collected using a temperature logger (Fourtek Picolite, USA) installed at mid depth in the turbine intake channel. Sunrise and sunset times were calculated using the sun-methods functions in the package “maptools” (Bivand & Lewin-Koh, 2015) for R statistical software 3.1 (R Core Team, 2014) with the geographical coordinates of the scheme. The influence of daylight on passage metrics was investigated by plotting individual fish passage spans in relation to dawn and dusk times (Figure 2.8, Results). Two diel variables were tested for their influence on responses: (1) whether a fish was first detected in either the day or the night; and (2) whether or not any of an individual's presence at the scheme was during the day time.

2.2.6 PIT data pre-processing

The observed reader clock drift necessitated time corrections for all data. The reader time resets carried out in the field were stored within the reader data files, and these were used to correct all logged tag detection times by interpolation between reset times.

2.2.7 Data analysis

In 2013, only two of 24 PIT tagged smolts (none were radio-tagged) were detected at the hydropower scheme, hence these were excluded from the analysis, which is restricted to telemetry data from 2014. All statistical analyses were carried out using R statistical software 3.1.

2.2.7.1 Passage route

Radio tagged fish which were detected at antenna A5 and then A4 (see Figure 2.5 for antenna locations) were considered to have passed through the turbine, whilst those which were detected at upstream antenna A1 and then A4 without detection at A5 were considered to have passed over the weir. Passage through the turbine was confirmed using the data from the PIT antennas in the turbine channel. A generalised linear model (GLM) with binomial error structure and logit link function was used to test the influence on passage route (turbine or weir) of the covariates turbine flow, weir flow, temperature, diel timing, fish length, condition factor (100 x fish mass (g)/ [fork length $(\text{mm})^3$]) and time of passage. Turbine flow and weir flow were log transformed in order to meet the assumption of proportionality between passage route odds and these flows on the log scale (an explanation of this assumption is given in Appendix A2.3). A maximal model without interactions was fitted and simplified by backwards deletion of terms which did not contribute significantly to model fit, as tested by a likelihood ratio tests (LRT) with a significance threshold of $p \leq 0.1$ for retention of variables. A probability of $p \leq 0.05$ was used as the significance level for evaluating terms within the model. In order to test the hypothesis that the probability of passage through either route was equal to flow apportionment between the two routes, a binomial GLM with the logged turbine:weir flow ratio as the only covariate, was used.

The influence of these covariates on the detection of PIT-tagged fish by PIT readers in the turbine channel or the depleted stretch was also investigated using Binomial GLM with the same model selection procedure.

2.2.6.2 Time until passage

Time until passage for the radio-tagged smolts was defined as the interval between first detection at the approach zone antenna (A1) until the first detection at the exit zone antenna (A4). Linear regression was used to test for the influence on time until passage of the covariates passage route, fish length, condition factor, date and time of arrival and diel variable (separate regressions for each diel variable defined in section 2.2.5).

Residence time in the turbine channel was calculated for PIT-tagged smolts which were detected at both P1 (entrance) and P2 (exit) PIT antennas. Linear regression was used to test for influence of covariates on these residence times. Residence time was $\log_{10}$ transformed to comply with the assumption of normally distributed errors. Model diagnostics were assessed by graphically examining the residuals for heterogeneity.

2.2.6.3 Survival/migration success

Survival distance downstream from the hydropower scheme was considered a measure of migration success. Fisher’s exact test of independence was used to test for association between passage route and proportion surviving beyond thresholds of 10, 20, 30 and 50 km downstream of the scheme. The fixed receivers at 0 and 50 km provided the most reliable data, since these monitored continuously throughout the study. Fish that were recorded passing downstream through the fixed receiver monitored areas, with the last detection on the downstream-most antenna, were considered to have survived past these distances. Manual radio tracking coverage was only partial and unevenly distributed in time, and hence did not provide exact survival distances for all fish. Therefore survival distance inferred from manual tracking was quantised into thresholds at 10, 20 and 30 km. These thresholds were chosen based on the distribution of tracking effort and the aggregation of tracking points, so that thresholds fell within gaps in coverage and known observations were grouped to either side of a threshold. Fish which were located in the same position beyond an interval of two days were considered to have died at that position. Fish which were located but movement not confirmed beyond the threshold being tested (lost to follow-up) were excluded from the calculation of survival proportion for that threshold, since their final position was unknown. Thus, these proportions are the number of smolts surviving beyond each threshold divided by the total number known to have survived or died prior to that threshold. This is the least biased, Kaplan-Meier estimate for censored data of this type (Chapter 2, Kleinbaum & Klein, 2012). A higher increase in mortality was noted at 20 km, and a binomial GLM was used to test for the influence on survival at that threshold of passage route, discharge at passage, time until passage, timing of passage, fish length and condition factor. *Post-hoc* power analysis for Fisher's exact test was carried out by simulation using function "power.fisher.test" in the R package statmod, implemented within the "optimize" function with a target of 80% statistical power.

2.3 Results

2.3.1 Captured and tagged fish

During spring smolt trapping over the two years, 1184 Atlantic salmon smolts and 56 trout smolts were captured. Tables 2.2 and 2.3 give a breakdown of the numbers, fork length, mass and tag/body mass ratio information for captured and tagged Atlantic salmon smolts and trout respectively, and Figure 2.6 summarizes capture success during trapping for the two seasons.

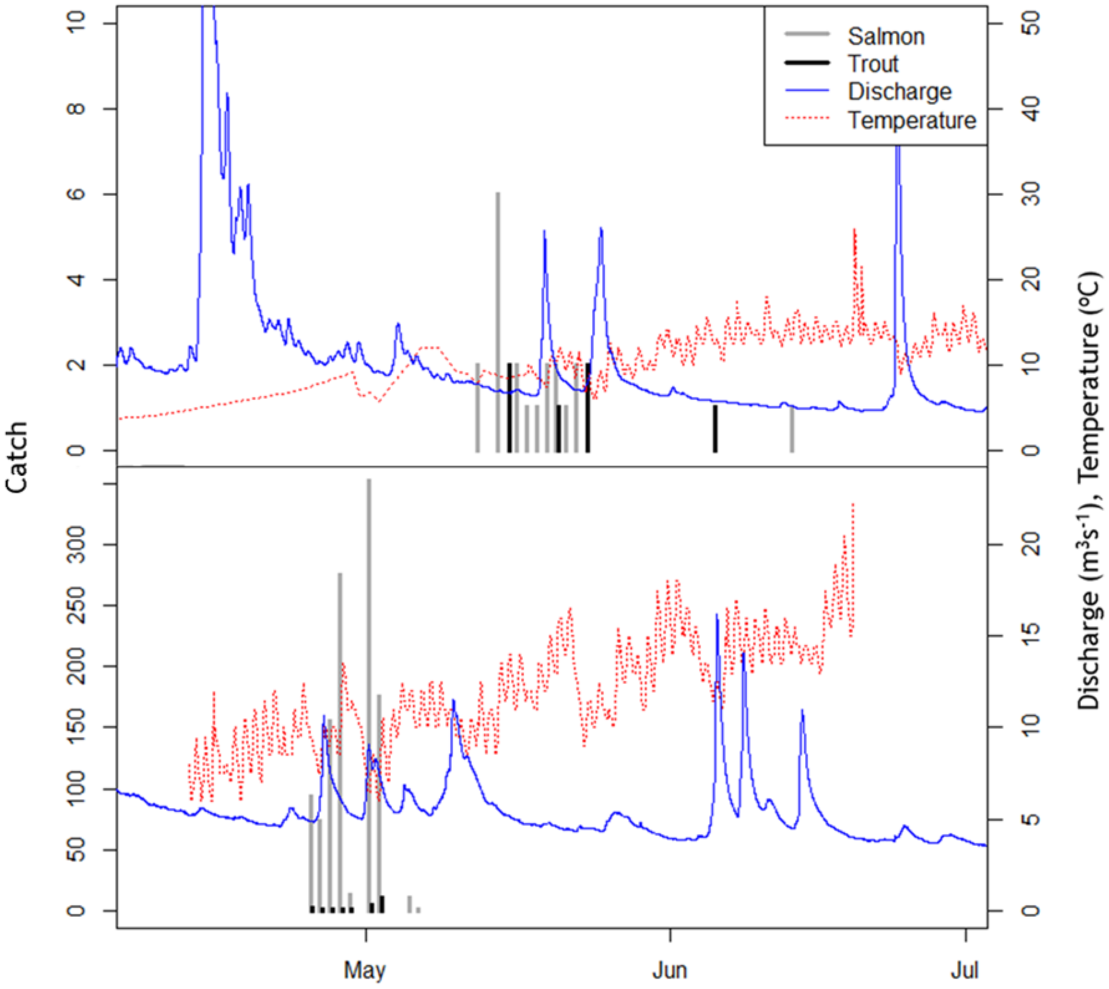
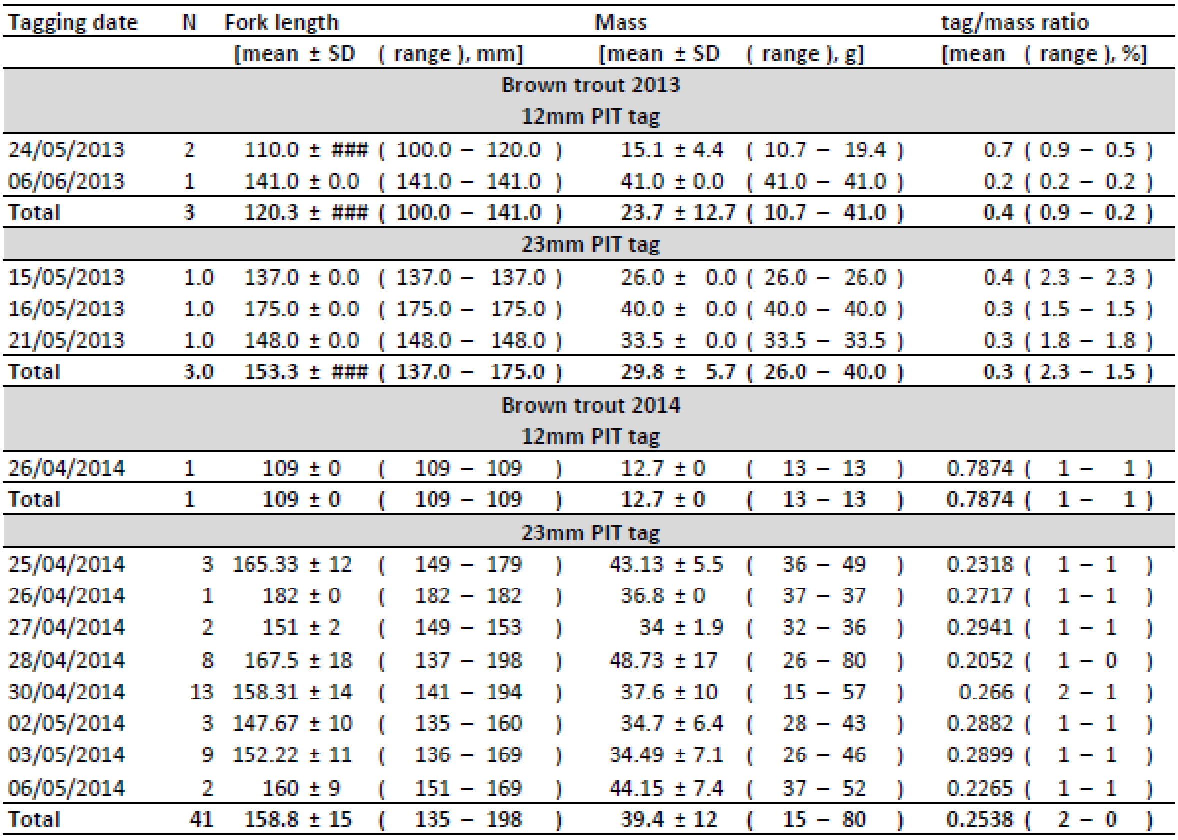


Figure 2.6. Daily catch during smolt trap operation for 2013 and 2014. Temperature data (^{o}C, red dashed line) were measured at least weekly by hand before 17 May 2013, and was logged at 15 minute intervals thereafter. River discharge is 15 minute logged data from the nearest gauging station at Alford.

Table 2.3. Number, fork length (FL, mm), mass and tag:body-mass ratio of salmon smolts captured and tagged in 2013 and 2014, separated into smolts tagged with 12mm PIT tags, 23mm PIT tags and with both 23mm PIT and radio tags.

Tagging date	N	Fork length		Mass		tag/mass ratio	
		[mean ± SD	(range), mm]	[mean ± SD	(range), g]	[mean	(range), %]
Salmon smolts 2013 12mm PIT tag							
15/05/2013	6	121.2 ± 9.5	(103.0 – 134.0)	17.2 ± 3.1	(12.0 – 21.0)	0.6	(0.8 – 0.5)
17/05/2013	2	118.0 ± 3.0	(115.0 – 121.0)	17.0 ± 3.0	(14.0 – 20.0)	0.6	(0.7 – 0.5)
18/05/2013	1	124.0 ± 0.0	(124.0 – 124.0)	19.5 ± 0.0	(19.5 – 19.5)	0.5	(0.5 – 0.5)
19/05/2013	2	126.0 ± 8.0	(118.0 – 134.0)	22.4 ± 4.1	(18.3 – 26.5)	0.4	(0.5 – 0.4)
20/05/2013	2	110.5 ± 5.5	(105.0 – 116.0)	14.6 ± 1.4	(13.2 – 16.0)	0.7	(0.8 – 0.6)
21/05/2013	2	123.0 ± 4.0	(119.0 – 127.0)	17.7 ± 1.6	(16.1 – 19.3)	0.6	(0.6 – 0.5)
22/05/2013	1	124.0 ± 0.0	(124.0 – 124.0)	19.5 ± 0.0	(19.5 – 19.5)	0.5	(0.5 – 0.5)
23/05/2013	2	115.0 ± 7.0	(108.0 – 122.0)	15.5 ± 3.4	(12.1 – 18.9)	0.6	(0.8 – 0.5)
14/06/2013	1	133.0 ± 0.0	(133.0 – 133.0)	24.4 ± 0.0	(24.4 – 24.4)	0.4	(0.4 – 0.4)
Total	**19.0**	**120.7 ± 8.6**	**(102.0 – 133.0)**	**17.9 ± 3.8**	**(12.0 – 26.5)**	**0.6**	**(0.8 – 0.4)**
Salmon smolts 2014 12mm PIT tag							
25/04/2014	17	116.6 ± 6.7	(102.0 – 131.0)	15.9 ± 2.9	(11.0 – 22.2)	0.6	(0.5 – 0.9)
26/04/2014	32	115.8 ± 4.7	(107.0 – 124.0)	15.2 ± 2.1	(10.0 – 19.7)	0.7	(0.5 – 1.0)
27/04/2014	40	113.6 ± 7.4	(93.0 – 130.0)	14.8 ± 2.7	(7.9 – 19.9)	0.7	(0.5 – 1.3)
28/04/2014	4	124.5 ± 2.7	(120.0 – 127.0)	19.2 ± 0.7	(18.0 – 19.8)	0.5	(0.5 – 0.6)
29/04/2014	14	120.9 ± 3.0	(114.0 – 125.0)	17.7 ± 1.2	(15.0 – 19.3)	0.6	(0.5 – 0.7)
30/04/2014	3	125.3 ± 1.2	(124.0 – 127.0)	18.6 ± 0.5	(18.0 – 19.2)	0.5	(0.5 – 0.6)
02/05/2014	47	112.3 ± 8.7	(88.0 – 126.0)	14.0 ± 13.9	(6.6 – 19.2)	0.7	(0.5 – 1.5)
Total	**157**	**115.13 ± 7.6**	**(93.0 – 131.0)**	**15.2 ± 7.9**	**(6.6 – 19.9)**	**0.7**	**(0.5 – 1.5)**
23mm PIT tag							
25/04/2014	4	138.3 ± 5.1	(131.0 – 145.0)	25.6 ± 2.5	(22.1 – 28.0)	2.3	(2.1 – 2.7)
26/04/2014	20	133.5 ± 3.7	(126.0 – 141.0)	22.9 ± 2.0	(19.0 – 27.9)	2.6	(2.2 – 3.2)
27/04/2014	13	133.3 ± 5.4	(126.0 – 142.0)	23.6 ± 3.0	(20.0 – 28.7)	2.5	(2.1 – 3.0)
28/04/2014	30	136.6 ± 5.9	(125.0 – 149.0)	25.5 ± 3.5	(20.5 – 32.8)	2.4	(1.8 – 2.9)
29/04/2014	28	133.8 ± 5.3	(124.0 – 144.0)	24.5 ± 2.4	(20.5 – 30.0)	2.5	(2.0 – 2.9)
30/04/2014	16	135.2 ± 5.7	(127.0 – 149.0)	24.1 ± 2.8	(20.1 – 29.4)	2.5	(2.0 – 3.0)
02/05/2014	21	130.5 ± 3.8	(125.0 – 137.0)	22.4 ± 2.5	(15.3 – 26.5)	2.7	(2.3 – 3.9)
03/05/2014	1	152.0 ± 0.0	(152.0 – 152.0)	35.4 ± 0.0	(35.4 – 35.4)	1.7	(1.7 – 1.7)
06/05/2014	4	125.3 ± 4.3	(120.0 – 132.0)	20.9 ± 1.9	(19.4 – 24.0)	2.9	(2.5 – 3.1)
07/05/2014	2	122.5 ± ###	(108.0 – 137.0)	19.0 ± 3.8	(15.2 – 22.8)	3.2	(2.6 – 3.9)
Total	**139**	**133.81 ± 6.2**	**(108.0 – 152.0)**	**23.96 ± 3.2**	**15.2 – 35.4)**	**2.5**	**(1.7 – 3.9)**
23mm PIT + Radio tag							
26/04/2014	2	145.5 ± 0.5	(145.0 – 146.0)	30.5 ± 0.5	(29.9 – 31.0)	2.8	(2.8 – 2.9)
28/04/2014	7	150.4 ± 6.4	(143.0 – 165.0)	34.4 ± 4.2	(30.5 – 44.0)	2.5	(2.0 – 2.8)
29/04/2014	3	151.7 ± 3.1	(149.0 – 156.0)	36.6 ± 1.8	(34.0 – 38.1)	2.4	(2.3 – 2.5)
30/04/2014	3	150.0 ± 1.6	(148.0 – 152.0)	33.1 ± 1.0	(31.8 – 34.2)	2.6	(2.5 – 2.7)
03/05/2014	13	149.8 ± 3.0	(146.0 – 156.0)	32.7 ± 1.7	(30.6 – 35.8)	2.6	(2.4 – 2.8)
Total	**28**	**149.86 ± 4.2**	**(143.0 – 165.0)**	**33.45 ± 2.9**	**(29.9 – 44.0)**	**2.6**	**2.0 2.9**

Table 2.4. Numbers, fork lengths (FL, mm), mass and tag:body mass ratio of brown trout captured and tagged in 2013 and 2014, separated into fish tagged with 12mm and 23mm PIT tags.



Tagging date	N	Fork length	Mass	tag/mass ratio
		[mean ± SD (range), mm]	[mean ± SD (range), g]	[mean (range), %]
Brown trout 2013 **12mm PIT tag**				
24/05/2013	2	110.0 ± ### (100.0 – 120.0)	15.1 ± 4.4 (10.7 – 19.4)	0.7 (0.9 – 0.5)
06/06/2013	1	141.0 ± 0.0 (141.0 – 141.0)	41.0 ± 0.0 (41.0 – 41.0)	0.2 (0.2 – 0.2)
Total	3	120.3 ± ### (100.0 – 141.0)	23.7 ± 12.7 (10.7 – 41.0)	0.4 (0.9 – 0.2)
23mm PIT tag				
15/05/2013	1.0	137.0 ± 0.0 (137.0 – 137.0)	26.0 ± 0.0 (26.0 – 26.0)	0.4 (2.3 – 2.3)
16/05/2013	1.0	175.0 ± 0.0 (175.0 – 175.0)	40.0 ± 0.0 (40.0 – 40.0)	0.3 (1.5 – 1.5)
21/05/2013	1.0	148.0 ± 0.0 (148.0 – 148.0)	33.5 ± 0.0 (33.5 – 33.5)	0.3 (1.8 – 1.8)
Total	3.0	153.3 ± ### (137.0 – 175.0)	29.8 ± 5.7 (26.0 – 40.0)	0.3 (2.3 – 1.5)
Brown trout 2014 **12mm PIT tag**				
26/04/2014	1	109 ± 0 (109 – 109)	12.7 ± 0 (13 – 13)	0.7874 (1 – 1)
Total	1	109 ± 0 (109 – 109)	12.7 ± 0 (13 – 13)	0.7874 (1 – 1)
23mm PIT tag				
25/04/2014	3	165.33 ± 12 (149 – 179)	43.13 ± 5.5 (36 – 49)	0.2318 (1 – 1)
26/04/2014	1	182 ± 0 (182 – 182)	36.8 ± 0 (37 – 37)	0.2717 (1 – 1)
27/04/2014	2	151 ± 2 (149 – 153)	34 ± 1.9 (32 – 36)	0.2941 (1 – 1)
28/04/2014	8	167.5 ± 18 (137 – 198)	48.73 ± 17 (26 – 80)	0.2052 (1 – 0)
30/04/2014	13	158.31 ± 14 (141 – 194)	37.6 ± 10 (15 – 57)	0.266 (2 – 1)
02/05/2014	3	147.67 ± 10 (135 – 160)	34.7 ± 6.4 (28 – 43)	0.2882 (1 – 1)
03/05/2014	9	152.22 ± 11 (136 – 169)	34.49 ± 7.1 (26 – 46)	0.2899 (1 – 1)
06/05/2014	2	160 ± 9 (151 – 169)	44.15 ± 7.4 (37 – 52)	0.2265 (1 – 1)
Total	41	158.8 ± 15 (135 – 198)	39.4 ± 12 (15 – 80)	0.2538 (2 – 0)

2.3.2 Detections at the hydropower scheme

2.3.2.1 Radio tag detections

Twenty-five radio-tagged Atlantic salmon smolts were detected at the hydropower scheme, and twenty-four passed downstream. One smolt was present within range of the approach zone antenna from 3 May until monitoring was halted on 9 June, and is assumed to have perished above the dam.

2.3.2.2 PIT tag detections

Due to the small sample size of PIT tagged Atlantic salmon in 2013 no further consideration is given to these fish. During the 2014 smolt study period, 130 PIT-tagged Atlantic salmon and trout smolts were detected by PIT-readers at Craigpot hydropower scheme. Four of the 2014 detections had been tagged in upstream tributaries in autumn 2014 (see Appendix A2.1 for details of fish captured and tagged in this way). Two of the 2014 detections that were tagged the previous autumn occurred before completion of the turbine passage trials

(Chapter 3), when passage behaviour would have been influenced by turbine manipulations and the presence of screens in the turbine channel. These are excluded from the analysis. Figures 2.7 and 2.8 give an overview of detections at Craigpot with reference to discharge, turbine discharge and diel timing for the radio-tagged and PIT tagged fish.

2.3.3 General conditions on approach and passage

The mean total river discharge during which radio tagged smolts were present was 7.3 m^3s^{-1} (range 6.3-11), and averaged 7.4 m^3s^{-1} (range 6.2-14) at first PIT detection for all detected PIT tagged fish. Mean turbine flow at which radio tagged smolts were present was 1.79 m^3s^{-1} (range 1.3-3), corresponding to a mean proportional take of total discharge of 0.24 m^3s^{-1} (range 0.21-0.28). At first PIT detection for all fish the mean turbine flow was 1.86 (range 1.2-3.3), corresponding to a mean proportional take of 0.24 (range 0.2-0.3). Mean temperature was 10 ^oC during presence of PIT tagged or radio tagged fish. Detections occurred at night for 91.4% (117/128) of PIT detections, and 84% (21/25) radio tag detections.

2.3.4 Passage route

No fish were recorded (with either PIT or radio monitoring) entering either passage route (the turbine channel or depleted stretch) and subsequently using the alternative route. None of the fish that were recorded passing via the depleted stretch route were subsequently recorded by PIT antenna P2 as entering the turbine outflow. Of the 24 radio-tagged smolts which passed beyond the scheme, 29% (7/24) went through the turbine, with the remaining 71% (17/24) passing over the weir and depleted stretch. Detection efficiencies for antennas A1 and A5 were estimated as 100% based on the numbers of tagged fish detected downstream at antenna A4 which were also detected by these antennas (24/24 for A1 and 7/7 for A4). All 19 tagged smolts located downstream by manual tracking or by the receiver at Grandhome had also been logged at antenna A4, and hence the efficiency of antenna A4 was 100%. These estimates of complete efficiency imply that the above are the known proportions of radio tagged smolts passing by each route.

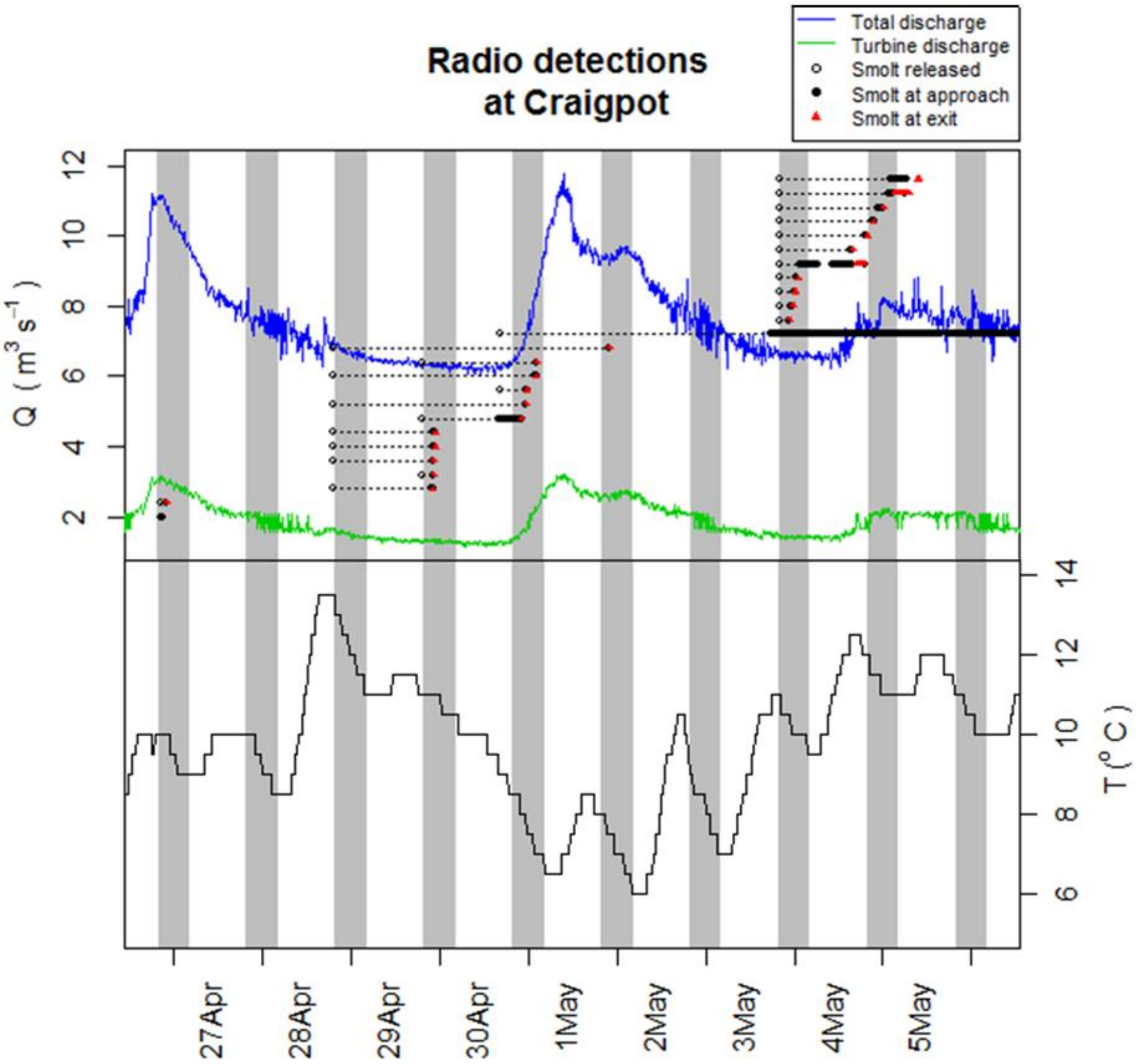


Figure 2.7. Presence of radio-tagged Atlantic salmon smolts (upper panel) at Craigpot in relation to total discharge (upper blue line), turbine discharge (lower green line), diel timing (grey shaded areas represent night time) and river temperature (lower panel). Each horizontal series of points represents an individual fish. Release times at the screw trap (open circles) are shown and connected to detections by the dotted lines. Detections of radio tagged smolts are shown at the approach (black circles) and exit (red triangles) zone antennas (A1 and A4 in Figure 2.5 respectively). The extended presence (thick black horizontal line beginning 3 May) is the smolt assumed to have perished above the dam.

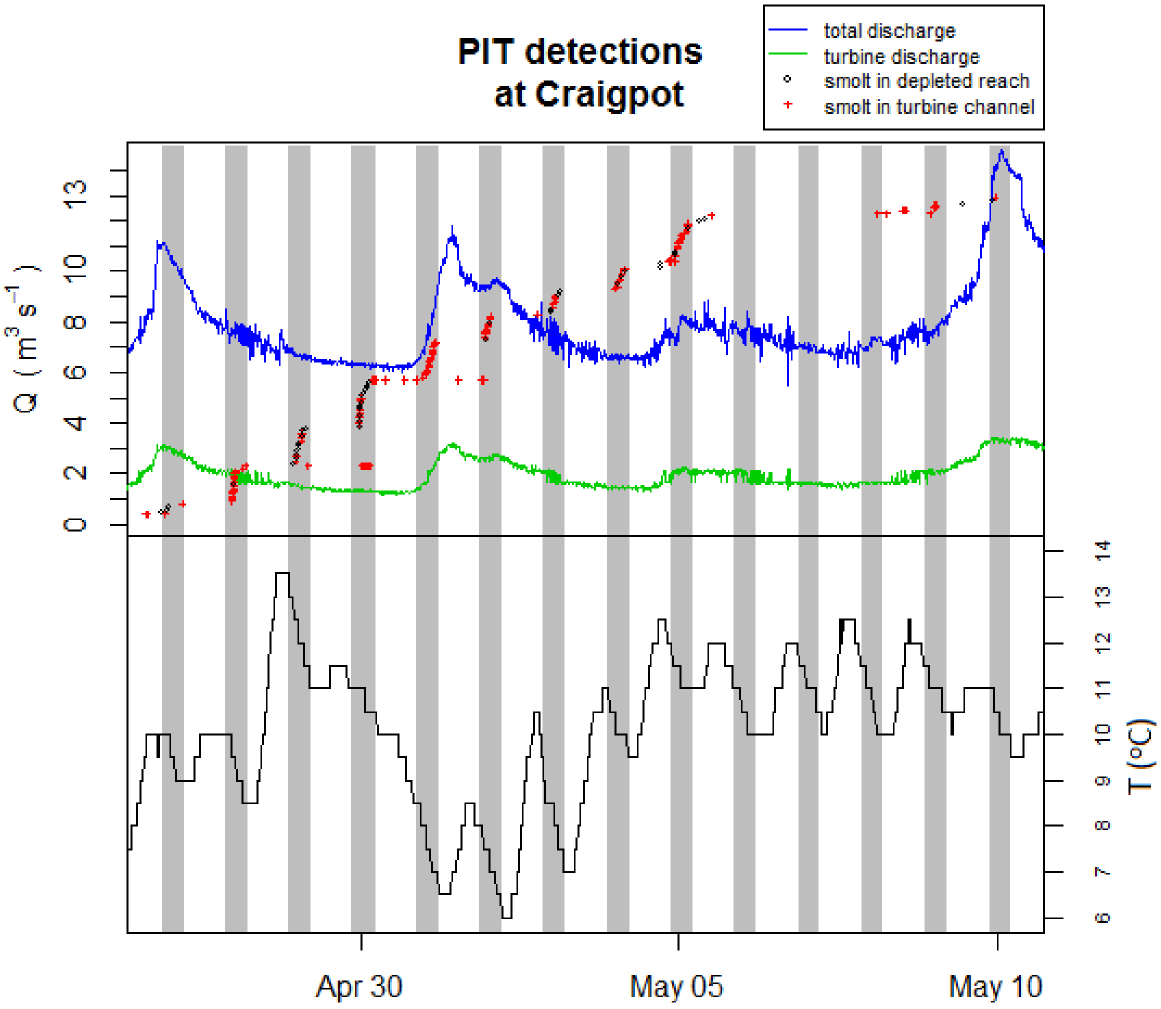


Figure 2.8. Presence of PIT-tagged Atlantic salmon smolts (upper panel) at Craigpot in relation to total discharge (blue line), turbine discharge (green line), diel timing (grey shaded areas represent night time) and river temperature (lower panel). Each horizontal series of points represents an individual fish. Detections of PIT-tagged smolts are shown at the depleted stretch (black circles) or turbine channel (red crosses) PIT antennas. A larger version of this figure, including release times, is included for reference in Appendix A2.3)

Of the 128 PIT-tagged smolts detected at the scheme, 84 were at the turbine channel antennas, and 48 were detected at both the entrance and exit of the turbine channel. Table 2.5 shows the numbers of smolts detected at each route by tag size. Composite efficiency of the combined turbine channel antennas was 0.99 for the 23mm PIT tag, and 0.33 for the 12mm PIT tag. The high efficiency for the 23mm tag is a result of the greater read-range of this larger tag, and the greater overall detection resulting from five antennas installed in series in the turbine channel. The lesser efficiency of detection of the smaller PIT tags is expected because of the shorter read range of these tags leading to gaps in coverage in the large span antennas used. Efficiency of Pdepl was 0.47 for the 23mm PIT tagged fish that were also tagged with radio tags. This lower efficiency is likely because at higher flows the depth of water allowed tagged smolts to pass above the vertical range of this pass-over antenna. It was not possible to estimate efficiency of Pdepl for 12 mm PIT tagged fish from detection data since there was no other means of detecting these fish if they passed down the depleted stretch. Efficiency estimates for all antennas are given in Appendix A2.2. Applying these efficiencies to the numbers of 23 mm transponders detected gives an estimated 22.2% of fish passing into the turbine channel (as shown in Table 2.5), similar to the 29% of the smaller sample of radio tagged fish passing through this channel.

Table 2.5. Efficiency estimates ($\hat{p}$), actual numbers (N), estimated numbers ($\hat{N}$), and estimated proportions ($\hat{P}$) of tagged fish passing each route. Subscripts denote PIT tag size. It was not possible to estimate efficiency of detection for 12 mm PIT tags passing over the depleted stretch PIT antenna, since there was no other means of detecting these fish.

	$\hat{p}_{23mm}$	$\hat{p}_{12mm}$	N_{23mm}	N_{12mm}	$\hat{P}_{23mm}$	$\hat{N}_{12mm}$	$\hat{P}_{23mm}$
Depleted stretch	0.47	-	40	4	85.10638	-	0.78
Turbine channel	0.99	0.33	60	24	24.24242	73.46189	0.22

2.3.4.1 Relation to operational and environmental conditions

For the 24 radio tagged smolt that passed beyond the scheme, passage route was not significantly related to turbine and weir flows at first detection in the approach zone (binomial GLM with log-transformed flows, $p>0.1$). Neither these flows nor temperature, fish length, condition factor, diel timing, and date and time of arrival contributed to model fit (LRT between models with and without these terms deleted, $p>0.1$) and were excluded from the model. Using a binomial GLM to test the relationship between passage route and the logged flow ratio resulted in an intercept term which was not significantly different to 0 (estimate= -3.14, se=3.75, *p=0.4*) and a slope term not significantly different to 1 (estimate=1.92, se=3.14, one-sample T-test: *t(22)=0.29*, *p=0.77*). Hence there is no evidence to reject the hypothesis that route selection probability is equal to flow apportionment.

For the PIT detections, detection in the turbine channel as opposed to the depleted reach was positively significantly associated with the smaller tag size. That is, the proportion of smaller tags detected was higher in the turbine channel than in the depleted stretch. This effect cannot be attributed separately to either a difference in detection efficiency between these routes, or difference in route selection between fish tagged with small and large tags. Since there was no other means of detecting 12 mm tagged fish passing down the depleted stretch, the efficiency of Pdepl at detecting 12 mm tags cannot be estimated, nor can the proportion of 12mm tagged smolts passing each route be estimated. However, if the assumption is made that overall route apportionment of 12mm tagged smolts is the same as for the 23mm tagged smolts, (22:78 turbine-channel:depleted-stretch, as corrected by PIT antenna efficiencies), then, on average, the composite efficiency of the turbine channel antennas was 21.28 higher than for the depleted stretch, for the 12mm tag size (the ratio of efficiencies between the turbine channel and depleted stretch routes is then the ratio of actual numbers of 12mm tags detected down the two routes, each divided by the estimated proportions of 23mm tags down those routes = (24/22)/(4/72) = 21.28). The alternative complement to this assumption is that the ratio of efficiencies of the turbine-channel:depleted-stretch antennas was the same for both tag sizes. Under this assumption it would appear that, on average, 12mm tagged smolts were 2.84 times more likely to pass into the turbine channel than over the depleted stretch (here the ratio of proportions down the turbine and depleted stretch routes = the ratio of number of 12mm tags detected at each route divided by the efficiency of detection of the antennas for the 23mm tags = (24/0.99)/(4/0.47) = 2.84.

Based on the tested vertical range of Pdepl (ratio of efficiencies = 20 cm for 12mm tags and 40-50cm for 23 mm tags) it is felt that reduced efficiency of this antenna at higher flows, when depth would exceed range, is the reason for this apparent effect, not a greater tendency of 12mm tagged smolts to use the turbine channel. Detection in the turbine channel was not related to turbine or weir flow (binomial GLM, Table 2.6), although these terms contributed to model fit (LRT, $p<0.1$). Detection at either route was not related to species, fish length or condition factor: these variables did not contribute to model fit (LRT between models with and without these terms deleted, $p>0.1$), and were eliminated from the final model.

Table 2.6. The probability (odds ratio with 95% confidence interval) of detection of PIT-tagged smolts passing into the turbine channel as opposed to the depleted stretch, in relation to covariates, as estimated by binomial GLM. Probability of detection at either route is a composite of route selection and detection efficiency, which cannot be separated for the 12mm tags (see text).

Predictor	Odds ratio	95% CI	*p*
Intercept	0.086	0.005—1.33	0.079
Weir flow	1.72	0.81—3.66	0.16
Turbine flow	0.23	0.05—1.02	0.054
Tag size 12mm	5.12	1.43—18.38	0.012

2.3.5 Time until passage

Median passage time for the radio-tagged smolts through the monitored area as a whole was 17.6 minutes (range 5.1-905.6). A linear regression on the covariates, including a binary factor for arrival during the day or night, failed to identify any significant effects (all $p>0.05$). However using a binary factor, which indicated whether any portion of the fish's presence was during the day, produced significant results. Figure 2.9 illustrates the classification of individuals into this binary variable. Time until passage was significantly related to this diel factor (linear model, $p<0.05$): smolts which arrived and/or passed the scheme during the day were significantly slower than smolts arriving and passing at night. There was also a significant interaction between this diel factor and route ($p<0.05$, Figure 2.10): slower passage times were associated with the weir route during the day (for estimates, standard errors and p-values, see Table 2.7). The distance from entry to the detection zone at the upstream end of the scheme (A1), to the entrance of the zone at the lower end of the scheme (A4) was approximately 350 m. Using this distance and the modelled estimate for time to pass, the estimated ground speed over the weir route at night (the passage condition for the majority of smolts) was 0.73 kmh^{-1}. Taking the upper 95% confidence limit on time to pass (estimate + [1.96 x *se*]), gives a lower estimate for ground speed of 0.20 kmh^{-1}.

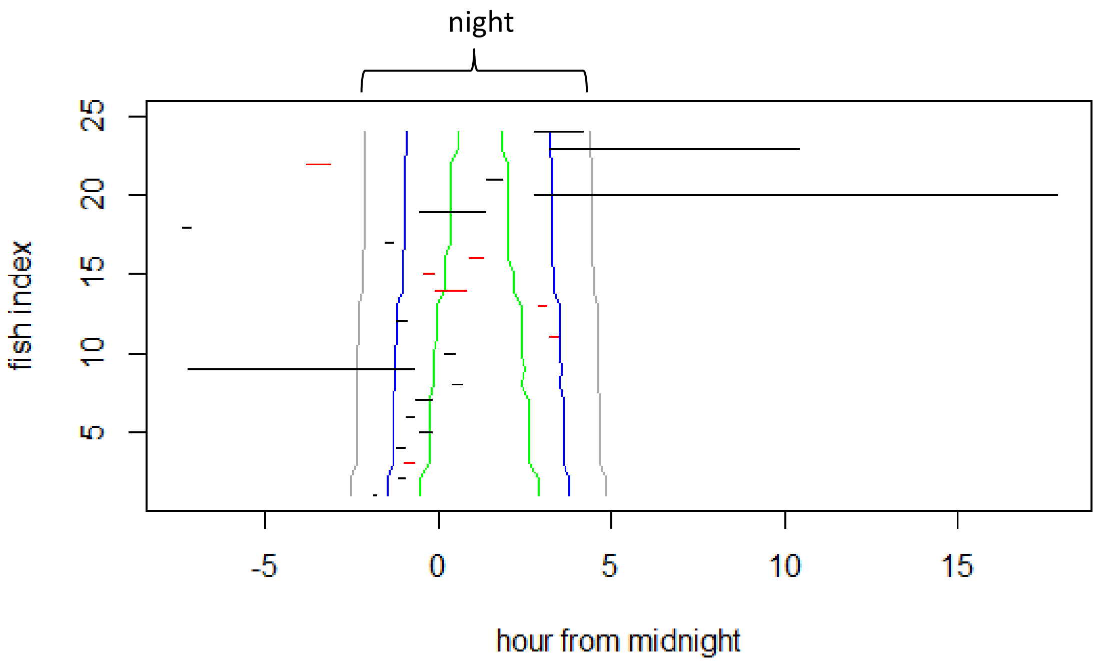


Figure 2.9 Classification of radio tagged individuals at the scheme into a binary diel variable. Each horizontal line represents the interval from arrival until passage of an individual fish, with red lines representing turbine-passed fish. Fish index is order in which individual smolts arrived. Those fish whose presence include time before civil dusk or after civil dawn were classified as being present during the day, as distinct from those that arrived and passed through at night. Solid grey lines represent civil dawn and dusk, nautical and astronomical dawn and dusk are included for comparison as the green and blue lines.

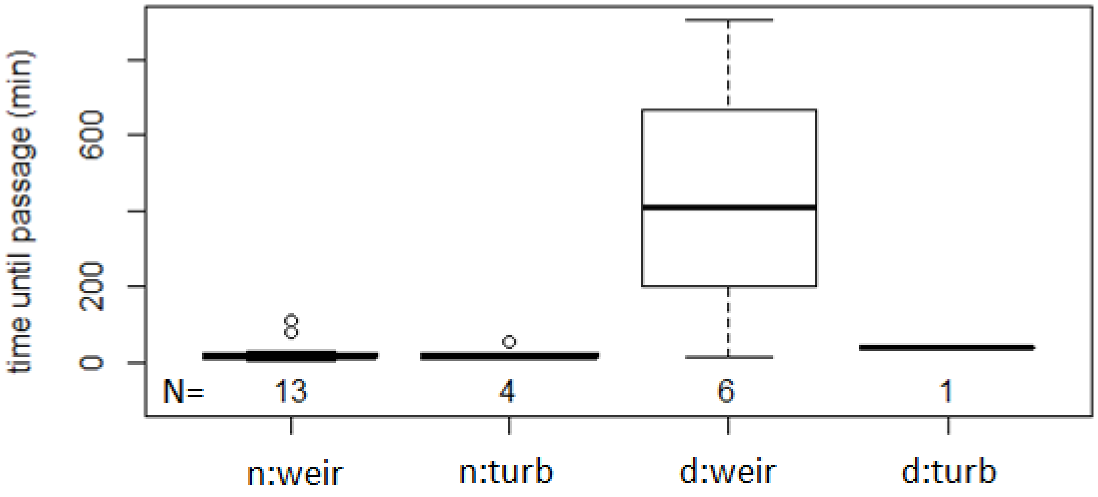


Figure 2.10. Time until passage of radio tagged smolts, partitioned by passage route (turb=turbine-passed, weir= weir-passed) and diel timing (d=present during the day, n=arrival and passage at night). Sample size of each subset is given below the boxplots.

Table 2.7. Passage time in minutes (mean and standard error) of radio-tagged smolts that passed through the turbine and the depleted stretch (weir) with the defined binary diel factor using a linear model ($F_{3,20}=6$, $r^2=0.47$, $p=0.004$).

Predictor	Estimate	SE	*p*
Intercept	28.79	39.85	0.48
Presence during day	406.45	82.15	0.00007
Passage through turbine	-6.16	70.91	0.93
Presence in day : Passage through turbine	390.25	175.59	0.037

Residency in the turbine channel

Of the 84 PIT-tagged Atlantic salmon and trout smolts detected in the turbine channel, 48 (57%) were detected at the first (P1) and last (P2) PIT antenna in the turbine channel. Median residence time in the turbine channel was 6.8 minutes, and ranged from 2.72 minutes to 1.77 days. Residence times were approximately log-normally distributed, with the exception of four fish (two salmon and two trout) with much longer residence times, which represented an obvious discontinuity in the distribution (Figure 2.11). Excluding these fish, residence times for the remaining 91.2% (44 fish) were under 27 minutes. The long residences were retained in the analysis since they represent real data with potential management implications. Three of these fish were present beyond one night. Two remained in the turbine channel until the following night, and the other left on the third night since it arrived. These longer residents were not associated with extreme values of temperature or turbine flow although their arrival times fell either towards the start or end of arrivals of the other detected PIT tagged smolts (Figure 2.12).

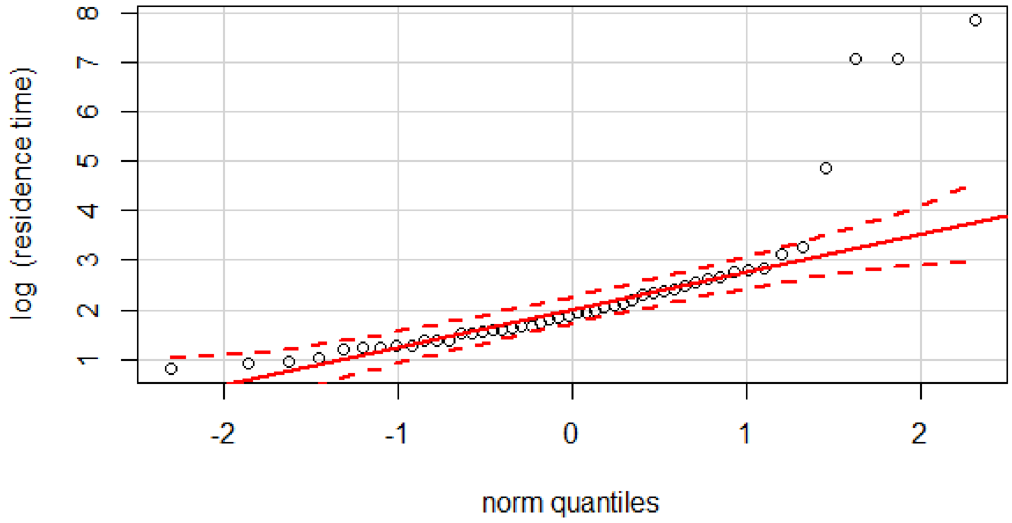


Figure 2.11. Quantile comparison plot for $\log_{10}$ smolt residence times in the turbine channel.

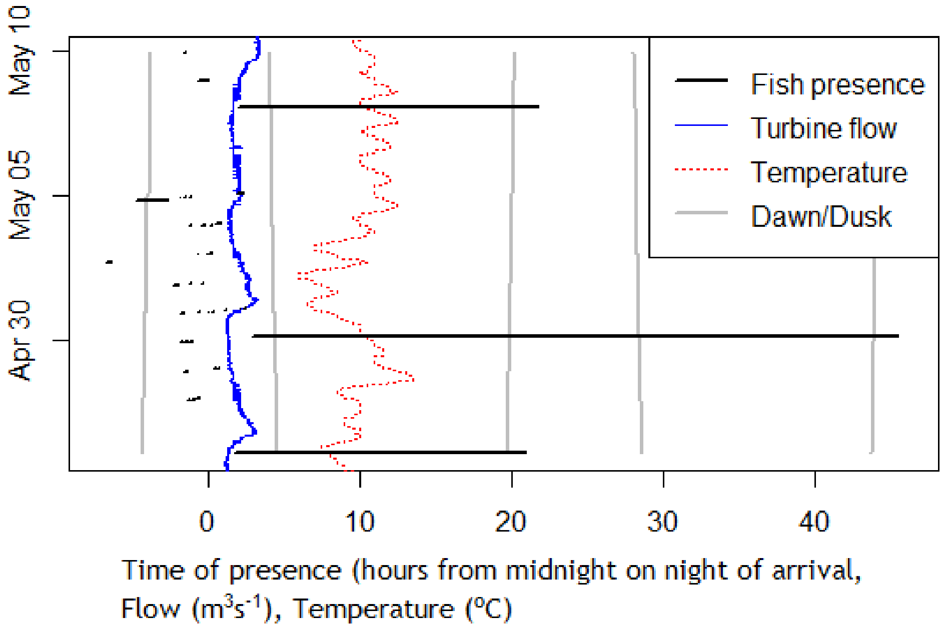


Figure 2.12. Duration and timing of PIT tagged smolt presences (thick black lines and points) in the turbine channel, for those fish detected at both the entrance and exit antennas. Each horizontal series represents an individual smolt.

Residence time was significantly related to species and turbine flow (log-linear regression, $p<0.05$, Table 2.8 and Figure 2.13). A single regression slope was used for Atlantic salmon and trout, since allowing slope to vary with species did not improve model fit (LRT between models with and without the additional parameter, $p>0.1$) whilst diel timing, length and condition factor were not ($p>0.05$), and were removed from the model since they did not improve model fit (*LRT*, $p<0.1$).

Table 2.8. Smolt residence time (mean and SE $\log_{10}$ minutes) in the turbine channel, estimated by a linear model ($F_{3,43}=5.48$, $r^2=0.28$, $p=0.003$).

Predictor	Estimate	SE	*p*
Intercept	12.74	3.78	0.0016
Turbine flow	-1.18	0.43	0.0090
Length	-0.04	0.02	0.056
Species (Atlantic salmon)	-2.66	0.79	0.017

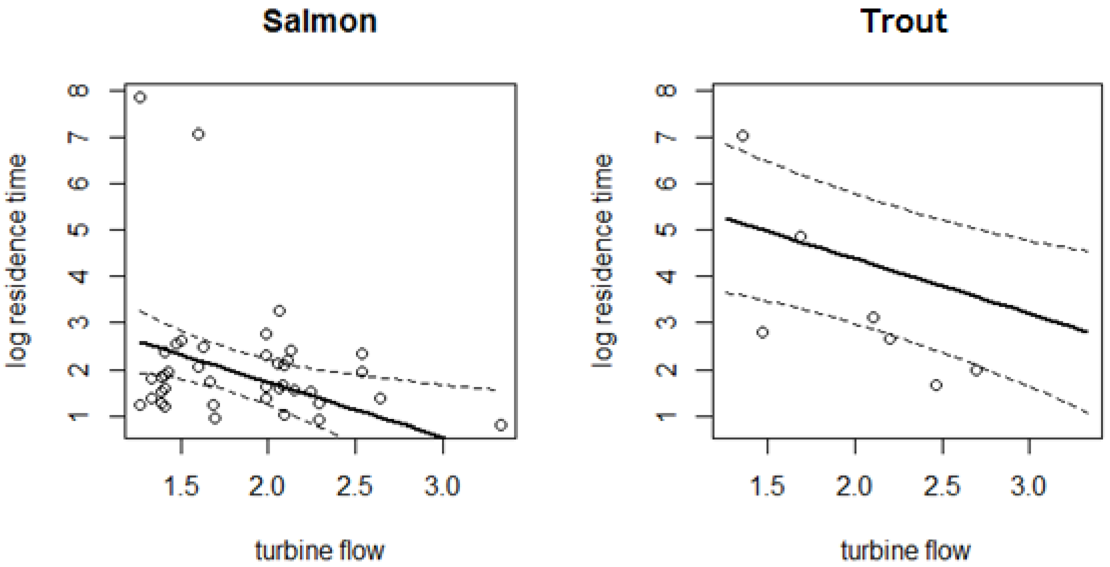


Figure 2.13. Relation between turbine channel residence time (mins) and turbine flow (m^3s^{-1}) for Atlantic salmon and trout smolts. Solid lines represent the fitted regression line, and dashed lines are 95% confidence intervals.

There were no significant differences in the covariates turbine flow, weir flow, condition factor, diel timing and species between those fish which were detected at both P1 and P2, and those which were detected at just one, or any another antenna (binomial GLM, $p>0.05$). There was a significant association in detection at both P1 then P2 with the larger tag size (binomial GLM, *odds ratio=2.84 se=0.97 p=0.03*).

2.3.6 Visual and video observations of smolt behaviour

Several hundred wild smolts were seen holding station along the entire length of the turbine intake channel in the days leading up to this study. These incidental observations were made during the investigation of potential damage to smolts from turbine passage described in Chapter Three, but are presented as further evidence that wild fish are entering the turbine channel. Further to these observations, several hundred of these smolts were captured below the turbine after having passed through it (Appendix A3.2), providing more evidence that wild smolts naturally pass through the turbine channel. During this study smolts were observed aggregating and schooling in front of the trash rack during the day, and this behaviour was recorded using underwater CCTV and a (GoPro) video camera. Groups of smolts were seen moving as a shoal from one side of the trash rack to the other, varying their depth as they did so. When fish did pass into the intake basin during these observations they utilized the horizontal gap under the screen at the bed of the channel.

2.3.7 Survival

Four out of 28 radio tagged Atlantic salmon smolts arrived at the receiver at Grandhome, equating to a mean per-kilometre mortality rate over the 52 km distance from the trap of 0.037 km^{-1} (survival over 50 km = 4/28 = $[1-0.037]^{52\ km}$). One of the surviving fish had taken the turbine route at Craigpot, with the remaining three having passed over the weir and depleted stretch. There was no association between passage route at the scheme and survival to Aberdeen (Fisher's exact test, odds ratio= 0.82, $p>0.1$). Assuming the observed survival to Aberdeen of weir-passed fish of 17.6%, and the observed proportion of turbine passed fish (29%), a sample size in excess of 1660 would be necessary to detect a 5% decrease in survival to Aberdeen in turbine-passed fish, with a power of 80%.

Figure 2.14 shows the proportion of Atlantic salmon smolts known to have survived beyond sequential distance thresholds at 10, 20, 30 and 50 km (see Methods Section 2.2.6.2), as determined by the fixed receivers or opportunistic manual tracking. There was no association between passage route and number of smolts surviving beyond these thresholds as tested by Fisher's exact tests (all $p>0.1$, Table 2.8).

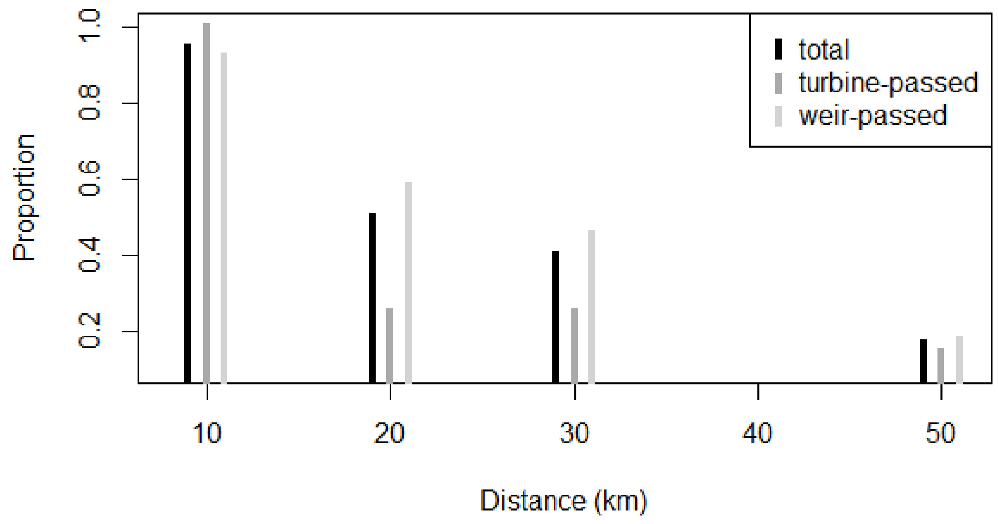


Figure 2.14. Proportions of total, turbine-passed and weir-passed smolts known to have survived beyond sequential distance thresholds by detection at fixed receivers (0 km [total survival, not shown] and 50 km) or by manual tracking (see Section 2.2.6.2). Fish which were not observed beyond the threshold but for which distance at death was unknown were omitted from the denominator in the calculation of surviving proportion.

Table 2.9. Results of Fisher's exact tests (odds ratio and 95% CI) of independence between passage route and survival beyond distance thresholds.

Distance threshold (km from Craigpot)	Odds ratio	95% CI	*p*
20	0.26	0.004-4.4	0.57
30	0.42	0.006 -7.47	0.6
50 (Aberdeen receiver)	0.78	0.012-12.39	1

The greatest observed decrease in proportional survival occurred at the 20 km threshold (Figure 2.14), and so observed survival beyond this interval was tested against covariates. Condition factor was the most highly significant variable (binomial GLM, *odds ratio=69.08, se=39.94, p=0.084*). The variables route and length contributed to model fit (LRT, *p<0.1*) and were retained in the model. Passage through the turbine was negatively but non-significantly correlated with survival (logit scale estimate=-7.02, se=5.67, *p=0.21* whilst survival was greater, but not significantly so, with increasing length (logit scale estimate=0.6371, se=0.5138, *p=0.2150*). Date and time of passage, passage duration, total flow and diel factor did not contribute significantly to model fit (LRT *p>0.1*) and were dropped from the model.

The farthest downstream distance (m) by manual tracking was significantly related to condition factor (linear regression, estimate = 1.980e+05 se=6.008e+04 *p=0.00361*, Figure 2.15). Turbine passage was not a significant predictor of this survival distance (*p>0.1*, Figure 2.16), nor were length, holding, datetime of passage, passage duration, total flow or diel factor (*p>0.1*), although length and passage duration contributed to model fit (LRT, *p<0.1*).

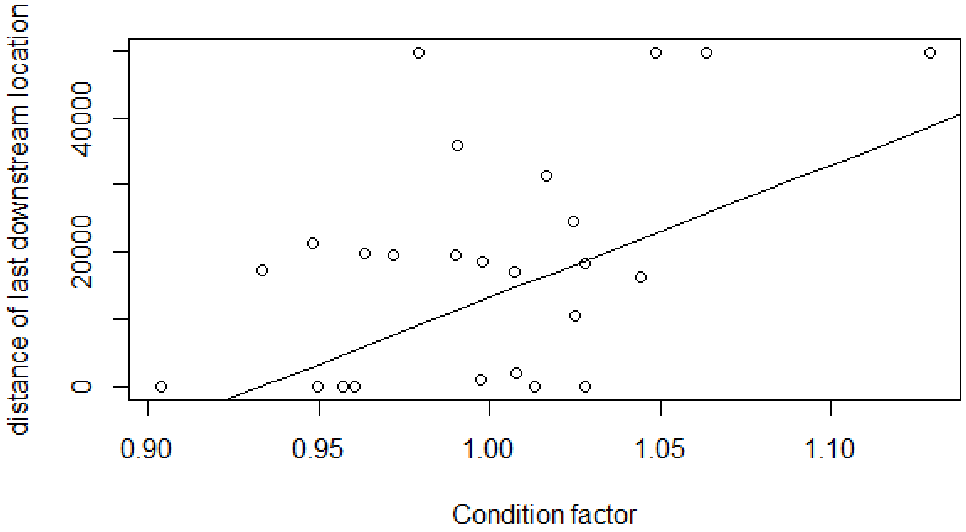


Figure 2.15. Observed (points) and fitted (line) last downstream locations (metres downstream from Craigpot) of smolts against condition factor. The predicted relationship shown uses the mean values for the other covariates retained in the final model (length and duration of passage).

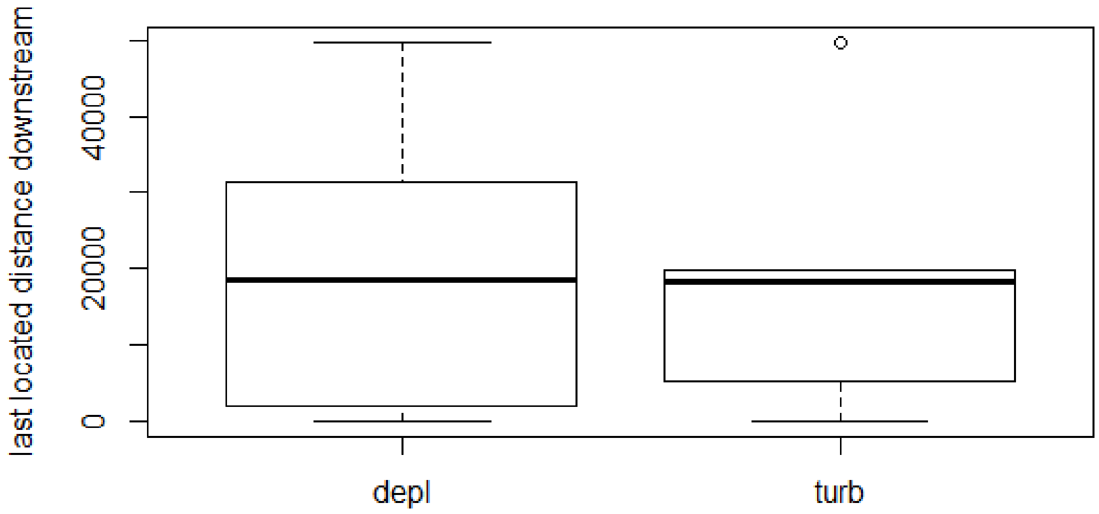


Figure 2.16. Last located distance downstream against passage route at the hydropower scheme. Turbine passage was not a significant predictor of survival (*p>0.1*).

2.4 Discussion

2.4.1 Capture success

There are several possible explanations for the very low capture success in 2013 compared with 2014. First it should be noted that smolt trapping capture success was low throughout Scotland in spring 2013. For example on the nearby river Spey, this was attributed to lower capture efficiency of rotary screw traps during several high water events (B. Shaw, Spey Research Trust, 2013, pers. comm.). On the River Don, peak flows were much greater during trapping in 2013 than in 2014 (Figure 2.6). The 2013 high flow events may have allowed the majority of smolts to pass the trap when it was covering relatively little cross-sectional flow area, whilst the low flow conditions in 2014 were more conducive to efficient trap operation.

The two years of trapping differed substantially in weather patterns. Spring 2013 was colder in the lead up to trapping, followed by rapid warming and high river flow. In 2014 temperatures were relatively high from March onwards, with generally lower river levels during smolt trapping. Additionally, there was high variability in temperature and rainfall between preceding years (mean daily flow at Parkhill in 2013 and 2014 was 25 m^3s^{-1} and 18 m^3s^{-1} respectively). The migration and spawning success of adult fish, the survival of eggs, fry and parr, the feeding and maturation of parr and smolting are all flow and temperature dependent processes, either directly, or indirectly through their influence on feeding (Jonsson & Jonsson, 2011). Thus the differences in annual temperature and rainfall may have acted to alter the magnitude and timing of smolt emigration. Rod catch data for the Don (Figure2.1), also suggests high variability in the abundance of adults between years, with relatively lower and higher catches respectively in 2011 and 2012. Since most River Don Atlantic salmon smolt after two years, this may explain the smolt capture success in 2013 and 2014 respectively.

The change in trap location between 2013 and 2014 may also have increased capture success. This was the first smolt trapping on the river, and so the trapping site was selected from intuitively suitable locations (constrictions of the river channel, or the outer edges of river bends, scouted and chosen in consultation with the Don and District Salmon Fishery Board, and experienced Fishery Trust biologists). The chosen site was a compromise between security considerations and a distance from the hydropower scheme that would allow resumption of normal behaviour of smolts after tagging and before scheme arrival, but that would minimize mortality from natural causes in the intervening river stretch.

2.4.2 Migratory behaviour

In this study, the arrival and passage of smolts at the scheme occurred mostly at night, which is in agreement with the general view of a predominantly nocturnal migration (Jonsson & Jonsson, 2011). There is a possibility that natural behaviour was disrupted by capture and tagging, and that by releasing smolts near dusk, arrivals to the scheme were synchronized at night. However even those fish that did not arrive at the scheme on the night of release were aggregated in the following nights (Figure 2.12 and Appendix A2.4). This indicates that the onset of darkness was initiating migratory movements (Thorpe & Morgan, 1988, Riley, 2012; 2014). This pattern, of movement at night and halting in the day, was also seen within the monitored areas at Craigpot, both in the depleted stretch (Figure 2.9) and in the turbine channel (Figure 2.12) Interestingly, three of the longest radio-tagged smolt passage presences included the crepuscule period (Figure 2.5), but then completed passage in daylight (or just before civil dawn, in one case). This suggests an absence of crepuscular migration as seen by Ibbotson *et al.* (2006), although there were two short passages during dusk.

2.4.3 Passage through the hydropower scheme

A total of 29% of radio tagged, and an estimated 22% of 23 mm PIT tagged Atlantic salmon smolts passed through the turbine channel. This is not dissimilar to the average apportionment of 24% of flow through this channel during tagged fish arrivals. Moreover, there was no evidence that passage route was not simply determined by flow apportionment. These results are consistent with the general view that salmonid smolts tend to migrate with the current (Johnsson and Johnsson, 2011). There is a paucity of information pertaining to the effect of flow apportionment on passage route of juvenile salmonids at small low-head hydropower schemes. In their synthesis of studies relating proportion of flow spilled over dams to proportion of smolts passed by spill in the Columbia river basin, Coutant & Whitney (2000) conclude that the basic flow-following response of juvenile salmonids is generally upheld, with spill effectiveness in passing fish being curvilinear for any particular site, but that this is dependent on physical configuration and conditions during spill.

It is known that migrating juvenile salmonids depart from flow-following behaviour under certain stimuli. Smolts avoid passively drifting through abrupt accelerations in flow (Enders *et al.*, 2009; Haro *et al.*, 1998; Kemp *et al.*, 2008), overhead cover (Kemp *et al.*, 2005; 2008), illuminated areas at night (Fjelstad *et al.*, 2012) and several studies have reported the effectiveness of structures designed to guide smolts away from turbine intakes using hydraulic stimuli (Larinier & Travade, 2002).

At Craigpot, the first of such stimuli that a smolt is likely to encounter whilst passing downstream through either the main river channel or the turbine channel, are the hydraulic (accelerating flow and turbulence) and visual obstacles presented by the weir, or trash rack, respectively. On encountering these, it might be expected that smolts would slow their movements, and potentially initiate oscillatory behaviour, as has been seen at such obstacles elsewhere in the field (Johnson & Morsund, 2000 for Pacific salmon smolts at a surface bypass, and Svendsen *et al.*, 2010, for Atlantic salmon smolts at water abstraction sites). This milling behaviour may result in exploration of the available passage routes. At the weir, depending on flow, the entire upstream edge of the weir face may be available for exploration, as well as the turbine channel route. However once a smolt has reached the trash rack within the turbine channel, it would need to swim 98 m upstream in order to re-access the weir route, a behaviour that was not seen here. Thus it is conceivable that the hydraulic stimuli at the weir may act to divert smolts into the more gentle, laminar flow of turbine channel, but not *vice-versa*, resulting in higher turbine passage rates than expected from the flow-apportionment model. The results do not support this conjecture, but indicate instead that there is no active route selection.

Trash racks at turbine intakes may act to deter smolts from entry, and even to divert them towards bypass systems. In their review of downstream bypasses at small hydropower plants in France, Larinier and Travade (1999) conclude that the spacing of bars and hydrodynamic factors are the greatest influence on the efficacy of these structures as guidance systems. In this study, smolts were observed aggregating and schooling in front of the trash rack during the day. As well as the potential behavioural barrier presented by the trash rack, the channel immediately beyond the trash rack was covered over, which is known to illicit avoidance behaviour in juveniles of other salmonid species (Kemp *et al.*, 2005). When fish did pass into the intake basin during these observations they utilized the horizontal gap under the screen at the bed of the channel. Nevertheless, transit times of PIT tagged fish through the turbine channel were generally low (under 27 minutes for 44 out of 48 smolts). The longer residences of four PIT-only-tagged fish in the turbine channel, extended from the latter part of the night to day-time, with fish moving on again at night (Figure 2.11). For fish which passed over the depleted stretch, three extended passage times observed by radio monitoring also spanned the crepuscular period. One of these began during the day, and passed at night, whilst two arrived at night; one of which then passed the next day, and the other the next night. The general absence of crepuscular movements is in agreement with Ibbotson (2006), who found movement to occur both in the day and night, but less frequently during dawn and dusk.

The significant interaction between route and daytime presence for radio tagged fish suggests the turbine channel to be a more efficient passage route in the day, but the results from PIT tagged fish in this channel do not support this. The extended passage times in both channels during daytime are suggestive rather of a diel pattern of migration, which is independent of passage route: these smolts may simply be halting migration in suitable holding habitat at the onset of daytime, regardless of which route they have taken. Also possible is a differential degree of avoidance of obstacles between day and night. This differential avoidance has been seen for Chinook salmon (*Oncorhynchus* *tshawytscha* Walbaum) smolts in a flume experiment (Vowles *et al.*, 2014a), where a greater proportion of smolts avoided passing accelerating flow over a weir under light conditions than in darkness. Here passage times were increased eight-fold under illumination, presumably because the presence of both visual and mechanosensory cues stimulated avoidance to a greater extent than mechanosensory cues alone. Longer delay during the day than in the night has also been observed at a large hydropower dam for Chinook salmon smolts (Beeman & Maule, 2001).

The few trout observed passing through the turbine channel were significantly slower in passing than the salmon, with other covariates (flow) accounted for. Possible explanations are that this species are generally slower migrants, that some stimulus acted differently on their behaviour in the turbine channel, or that the small sample of trout smolts was not representative of speeds generally. Whilst it is not possible to tease these explanations apart, a difference in migration speed or population frequency of halting behaviour is intriguing and has implications for multi-species management. There is a lack of studies comparing the in-river movement rates and behaviours of wild smolts of these species. Aarestrup *et al.*, (2002) found wild trout smolts to migrate at faster ground speeds than introduced hatchery Atlantic salmon smolts. Thorstad *et al.*, (2004) tracked Atlantic salmon and trout post-smolts in a fjord system, and found swimming speeds of Atlantic salmon to be on average around twice that of trout. These previously demonstrated differences between these species and rearing origins highlight the need for more understanding of the migration behaviour of lesser studied species in relation to riverine obstacles and environmental stimuli (Vowles *et al.*, 2014a). There is also sometimes high variability in migration behaviour within populations (as seen with the few, extended passage times seen in this study), and potentially between life history phenotypes. From a biodiversity conservation perspective, it is desirable to minimize anthropogenic influences on phenotypic expression, and evolutionary selection, over time. A lack of understanding of the variability and dynamics of migration behaviour could constrain these ideals.

2.4.4 Onward migration success

Migration success (measured as proportion of fish surviving beyond defined distance thresholds, and also as furthest observed distance downstream) was not associated with turbine or depleted stretch passage. However this result is limited in its power because of the small sample size of radio tagged fish. It is however, an indication that there were no high levels of severe or indirect mortality resulting from turbine passage. Only four out of 28 radio tagged fish arrived at Grandhome, 50 km downstream; a mean per-kilometre mortality rate of 0.37%. The detection efficiency of the receiver at Grandhome could not be estimated empirically from tagged smolts passing through it, since there were no further receivers downstream. However, the operation of this receiver throughout the study, and the range tests described in section 2.2.2.2 indicate that complete efficiency would be expected, as was seen for the radio equipment at Craigpot (where empirical estimates of efficiency were 100%), and this is the assumption made. The mortality observed is within the bounds of in-river mortality rates for wild Atlantic salmon smolts collated by Thorstad *et al.* (2012), of 0.3-7% km^{-1}. A study of trout smolt emigration on another east coast river system in Scotland (the river Tweed, Gauld *et al.*, 2014) found per-kilometre mortality rates of 0.88% and 0.55% in successive years. In their two-year study, they attributed higher losses of acoustically tagged trout in a low flow year to flow mediated delay at small barriers, leading to greater predation.

In-river smolt mortality in other studies has been attributed to predation (Aarestrup & Koed, 2003, Gauld *et al.*, *op. cit.*), by piscivorous birds (Harris *et al.*, 2008), mammals (Heggenes & Bogstrom, 1988), and fish (Jepsen *et al.*, 2000). On the river Don, red-breasted mergansers (*Mergus serrator* L.), goosanders (*Mergus merganser* L.), cormorants (*Phalacrocorax carbo* L.) grey herons (*Ardea cinerea* L.) otters (*Lutra lutra* L.), American mink (*Neovison vison*, Schreber), and northern pike (*Esox Lucius* L.) are all present and assumed to predate upon smolts. The River Don from Craigpot to Grandhome has relatively few obstructions, and the few that there are, are broken with a clear flow route through them (personal observations whilst kayaking this reach of river during manual tracking for this study). However the low spring river levels in 2014 may have contributed to predation levels, since the time taken to reach the estuary would likely be longer, with associated increased exposure to avian and terrestrial predators in shallow, clear water, and increased energy expenditure if active swimming occurred in the lower, slow flowing reaches of the river.

Another potential cause of mortality is disease (Garcia de Leaniz, 2008). Infection by *Saprolegnia*, for example, is associated with elevated temperatures in spring (see Chapter 3). Any of these stressors could act in combination to increase the likelihood of mortality, and it is recognized that the capture and tagging process may also have contributed. Accounting for tagging effects is notoriously problematic in field studies on fish which cannot be observed without telemetry tagging (*sensu* Cooke et al., 2010). The effects of tagging were minimised as far as possible by following best practice and ensuring the healthy swimming condition of tagged fish before release, with adequate recovery period.

Both survival beyond 20 km and the furthest downstream tracked distance of Atlantic salmon smolts was correlated with condition factor. This result should be treated with caution, due to the small and diminishing sample size with downstream distance, and the weak correlation in the case of survival beyond 20km. The implication; that mortality occurs differentially on the population, with condition factor as a correlate for survival, is intuitive, since condition factor is related to energy reserves (Herbinger & Friars, 2008). It would be expected that smolts in better condition travel further and evade mortality from various sources for longer. Body morphology and migration timing can vary between sub-catchments (Ridell & Legget, 1981; Stewart *et al.*, 2006), and condition factor seems likely to decrease with time since smoltification and initiation of migration (although no literature has been found to support this). If this is the case, this result has implications for the selective adaptation of the phenology of migration. Although not directly comparable, a mechanism of transgenerational phenotypic influence has been proposed by Todd *et al.* (2012), who found the size of smolts leaving the North Esk to be related to maternal condition factor. No similar reports of reduced in-river emigration success with decreasing condition have been found elsewhere, although Tipping & Blankenship (1993), found smolt condition factor not to influence smolt-to-adult survival in Cutthroat trout (*Oncorhynchus clarki*). Whilst an interesting avenue for further research, it should also be noted that furthest tracked distance downstream as a measure of migration success was limited in its accuracy because of sporadic tracking coverage.

In this study, greater mortality was not associated with either the turbine or weir passage route. This is consistent with a perspective of low risk to Atlantic salmon smolts from passage through ASHTs (Kibel, 2007), in contrast to the generally high mortality rates of 5-100% for fish passing through conventional turbines, summarized by Larinier & Travade (2002). However his result should be interpreted with reference to the limited sample size, and hence the limited statistical power to detect differential survival between turbine passed and non-turbine passed smolts (see Section 2.2.7). It should also be acknowledged that any low-frequency impact may have been masked by relatively high mortality from other sources. It is possible that there could be a different weighting of any turbine effect under differing environmental circumstances. All of the potential contributors to mortality discussed above have been raised in the context of latent or cumulative effects on smolts from hydropower scheme passage (Coutant and Whitney, 2000), and this highlights the need for a holistic approach to assessing these effects. Both natural and anthropogenic influences should be considered at geographic and temporal scales that influence populations. When applying the outcomes of this study, the specificity of the study situation, in both space and time, should be remembered.

2.4.5 Management implications and conclusions

This study has demonstrated that wild migrating Atlantic salmon and trout smolts pass naturally through an open offtake channel and Archimedean screw turbine, with moderate water velocities and under a variable abstraction regime designed to take approximately one quarter to one third of the flow. The apportionment of wild migrating smolts through alternative passage routes was not significantly different to flow apportionment through these routes. This is of value in designing abstraction regimes at other sites, particularly where concerns over the hazards of turbine passage are greater.

In general, under the conditions observed, there was no undue delay of smolts at the scheme, although passage times were much higher for a few cases. These longer passage times occurred with similar frequency and lasted similar duration between the turbine and weir passage routes, and are associated with a diel halting in migration during daytime.

There was no evidence to suggest that onward survival or migration success was hampered by turbine passage as contrasted with passage over the weir and depleted stretch, but this outcome is limited in power by the low initial sample sizes and generally high (but not unusual) levels of mortality seen downstream of the scheme. This, in combination with the generally short passage times observed, supports the decision by regulating authorities to allow such turbines to operate without the need to prevent smolts from entering them. Again consideration should be given to the site- and circumstance-specificity of the study in interpreting this outcome, and also to the potentially different effects on other species and life stages.

These findings suggest that passage through the intake channel and turbine at this site are viable routes for wild migrating smolts, and that the scheme generally provides a safe and efficient fish passage situation. Application of this interpretation should be made with careful reference to the circumstances of the study and management requirements. The migration pathways of Atlantic salmon are becoming increasingly modified throughout their range. It is vital for the continuity of salmon populations to ensure that these modifications – such as low-head hydropower – do not inhibit their migrations. Prior research on smolt responses to hydraulic stimuli (Enders *et al.*, 2009; Haro *et al.*, 1998; Kemp *et al.*, 2008), and experience of the variable success of downstream guidance systems (Larinier & Travade, 2002) suggests that site configuration and hydraulics are key factors in influencing smolt passage. It is recommended that further studies on low-head hydropower focus on relating these factors to smolt passage behaviour. Given the limited evidence on the effects of low-head hydropower passage on survival of downstream migrants, it is recommended that more data be gathered from sites with varying configurations, abstraction regimes and turbine specifications.

Appendix A2.1: Salmon and trout parr PIT tagged in Autumn 2013

Table A2.1. Juvenile salmon and trout PIT tagged in Autumn 2013, captured by electrofishing in tributaries (Bandley burn, Cushnie burn, Deskry burn, Nochty burn), or by the rotary screw trap (RST).

Tagging site	Bandley	Cushnie	Deskry	Nochty	RST	total
15/05/2013 - 14/06/2013 (spring)						
sa					18	**18**
bt					6	**6**
total					**24**	**24**
09/09/2013 -21/10/2013 (autumn)						
sa	5		12	18	137	**172**
bt	30	35	7		14	**86**
total	**35**	**35**	**19**	**18**	**151**	**258**

Appendix A2.2: PIT antenna efficiencies

Table A2.2. Efficiency estimates, based on PIT tagged fish detections for each PIT antenna (Pdepl, P1, PLtop, PLmid, PLbot, P2A1 P2A2) and composite antennas Pturb (all turbine-channel antennas), and P2 (two antennas operated across the same channel a meter apart by a multiplexing PIT reader). Estimates for P2 are given based on detections of fish also tagged with radio tags, which were detected downstream, and for each antenna, based on the number of fish detected at the other of the two. *Estimates for P2A2 are made using the number of fish detected at P2A1, rather than downstream antennas.

	23mm PIT tags			12mm PIT tags		
i	p̂i	N, i	N, downstream of i	p̂i	N, i	N, downstream of i
Pdepl	0.47	8	17	-	-	-
P1	0.86	54	63	0.20	5	25
Pltop	0.26	16	61	0.04	1	24
Plmid	0.26	16	61	0.04	1	23
Plbot	0.69	42	61	0.09	2	23
P2(radio)	0.71	5	7	-	-	-
P2A1	0.74	31	42	0.00	0.00	13.00
P2A2	0.84	31	37*	0.00	0.00	2*
P2comp	0.96	-	-	0.00	-	-
Pturb	1.00	-	-	0.33	-	-

Appendix A2.3: Justification for log-transforming turbine and weir discharge as covariates for binomial regression of passage route

The binomial regression model is formulated to test the relation of passage route to proportional take through the turbine using turbine and weir flows as its inputs.

Total river discharge is given by:

$$Q_{tot} = Q_{turb} + Q_{weir}$$

Where:
Q_{tot} = total discharge,
Q_{turb} = discharge through the turbine, and
Q_{weir} = discharge over the weir

The null hypothesis is that the probability of passage through the turbine route is equal to the proportion of total discharge passing that route.

H_0:

$$P = \frac{Q_{turb}}{Q_{turb} + Q_{weir}}$$

The odds ratio under this hypothesis is:

$$\frac{P}{1-P} = \frac{Q_{turb}}{Q_{weir}}$$

since

$$1 - P = \frac{Q_{weir}}{Q_{turb} + Q_{weir}}$$

Then the log odds (the response of the binomial GLM) are:

$$log\left(\frac{P}{1-P}\right) = log\left(\frac{Q_{turb}}{Q_{weir}}\right) = \log(Q_{turb}) - \log(Q_{weir})$$

a linear relationship with intercept=0, and a coefficient of 1 for the logged ratio of turbine to weir flow, or coefficients of 1 and-1 for the log transformed turbine and weir flows respectively.

Hence firstly a binomial regression including the logged ratio of turbine and weir flows is performed to test the hypothesis of zero intercept. Then the model is repeated without the intercept to estimate the dependence of passage route on proportional take. If the coefficient is unity, then the hypothesis that passage route is equal to proportional flow is accepted. Lastly the dependence of passage route on the two log transformed flows as separate terms is tested to assess dependence of passage route probability on turbine flow given the observed combinations of turbine and weir flows.

Appendix A2.4: Transition from release to detection at the hydropower scheme, for PIT tagged Atlantic salmon and trout smolts.

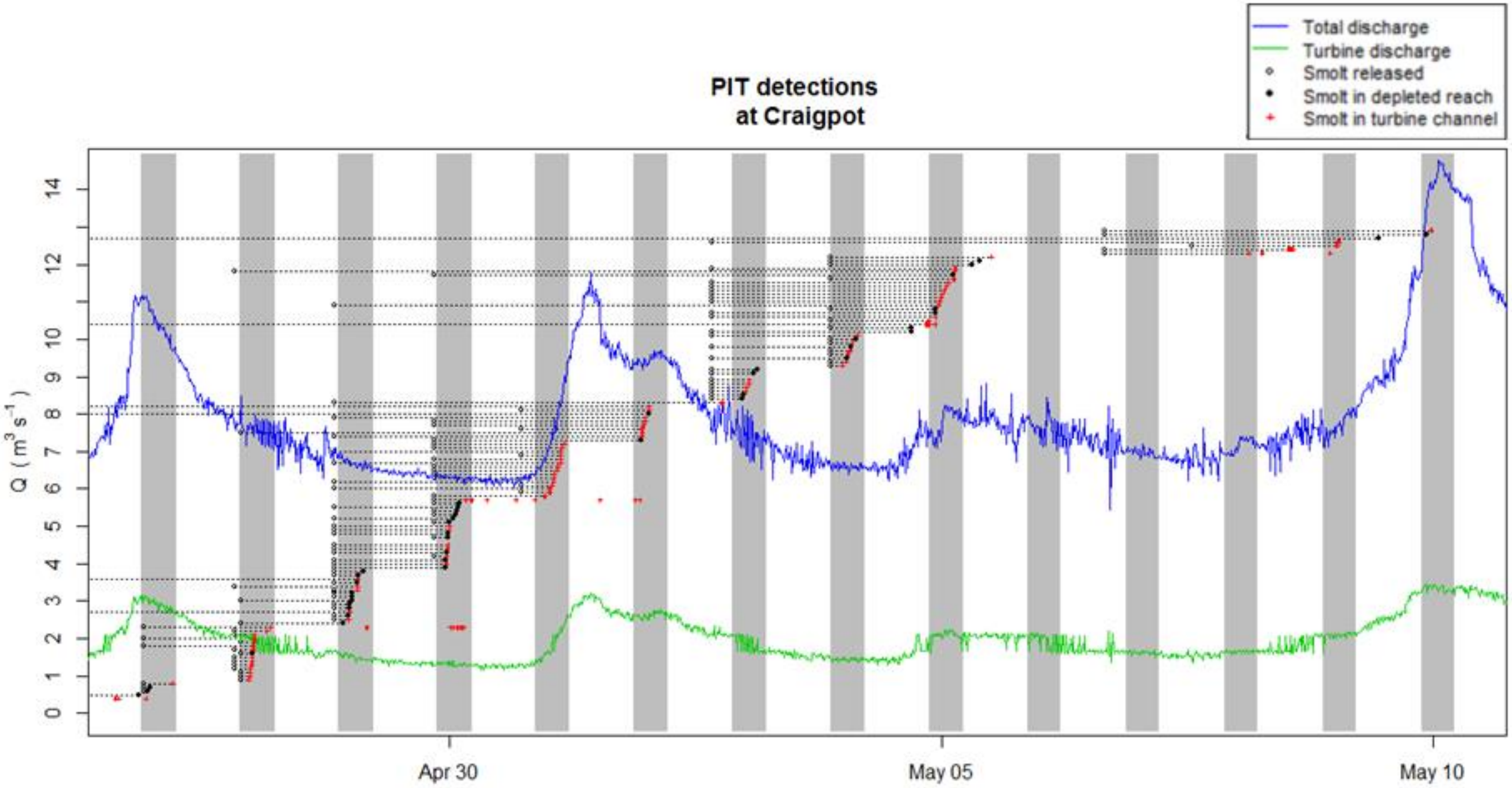


Figure A2.4. PIT detections at Craigpot, showing time of release at trap location, diel timing (grey shaded bars are night time) total discharge (blue upper line) and turbine discharge (green lower line).

Chapter Three

Assessment of the risk of physical trauma to Atlantic salmon (*Salmo salar*) smolts resulting from passage through an Archimedean screw turbine

3.1 Introduction

With the recently renewed interest in small-scale hydropower development, ASHTs have been increasingly favoured for the retrofitting of existing low-head historic barriers with modern turbines. Although there is a well-developed body of research concerning the effects on fish of passage through conventional turbines at large-scale hydropower schemes, there is limited information on the effects of these low-head, ASHT schemes. At present, the majority of evidence is limited to commercial consultancy reports (e.g. Kibel, 2007; 2008; 2009; 2011; Spah, 2001; Merkx & Vries, 2007), and these support a perspective of low-risk to fish which pass through these turbines. The only study in the peer-reviewed scientific literature that has attempted to quantify damage rates from ASHTs (Bracken & Lucas 2013) found a damage rate of 1.5% for downstream migrating larval and juvenile lampreys (*Lampetra sp.)* passing through through an ASHT. The objective of this chapter is to test the potential for an ASHT to cause damage to Atlantic salmon smolts that pass through it. The overall aim was to estimate the prevalence and severity of damage to Atlantic salmon smolts resulting from passage through an ASHT.

ASHTs lack many characteristics associated with damage to fish by conventional turbines: they operate at low rotational speeds (up to 30RPM), and there are no rapid or extreme changes in water pressure and velocity or high shear stresses between bodies of water moving at differing velocities. However, several mechanisms for damage by ASHTs to fish have been identified (Potter *et al.*, 2012) namely: impact by the leading edges of the turbine; grinding between moving and stationary turbine parts; or abrasion. The regulatory authorities responsible for licensing new installations in the UK have adopted guidelines (EA 2013, SEPA 2014) for the operation of these turbines based on the outcomes of a series of investigations by a commercial consultancy (Kibel, 2007; 2008; 2009;

2011). Thus there is no requirement to prevent fish from entering these turbines, provided that turbines operate within certain limits of rotational speed, and that appropriate protection is fitted to the leading edges of the turbine blades in order to mitigate strike to fish. Similarly in Europe, there is no general requirement to screen fish from entering ASHTs (D. Mann, Mannpower, May 2016, pers.comm.).

Although the available evidence points towards ASHTs being low impact, there is a need to corroborate this evidence, and that is the objective of this chapter. Atlantic salmon and sea trout smolts are of particular concern since they are a vulnerable life stage of a species which is economically important and of high conservation interest. Several authors have discussed the potential for subtle turbine-induced effects to lead to indirect (Travade *et al.*, 2010; Cada 2003; Colotelo *et al.*, 2009; Hasler *et al.*, 2009; Schweizer *et al.*, 2011 or delayed (Budy *et al.*, 2002; Cooke *et al.*, 2011; Thorstad *et al.*, 2012) mortality, as has been demonstrated for large-scale hydropower systems (Ferguson *et al.*, 2006). There may also be an accumulation of such effects on the onward performance and survival of smolts.

3.1.1 Methodologies for investigating the potential for subtle damage to fish from turbine passage

The concerns regarding the potential for subtle or delayed effects on smolts from turbine passage highlight a need for methodologies which can detect such subtle or chronic effects, and more information on their significance for onward survival.

Most studies on downstream fish passage through conventional hydropower schemes have been designed to estimate direct mortality (e.g. Bell & Kynard, 1985; Mathur *et al.*, 1995; Normandeau Associates, 2009). However, there may be a spectrum of severity of injuries which could disadvantage fish and decrease the likelihood of survival. To assess such sub-lethal effects, some studies have captured fish at the outflow of turbines for visual inspection for signs of external injury (e.g. Bracken & Lucas, 2013; Kibel *et al.*, 2009). This may be extended to gross pathological examination and histopathology of potentially affected tissues or organs.

Damage is most frequently categorized as several types (for example descaling, laceration, beheading, haemorrhage, haematoma, eye damage, split fins, or spinal fracture, although this is mostly limited to the assessment of mortalities and not sensitive examination of subtle effects to live fish (see reviews by Monten (1985) and Larinier & Travade (2002)). These categories lack information on the severity of injury, which becomes important when considering novel technologies which involve more slowly moving parts. In their studies, Kibel *et al.* (2009) used a scoring system to rate the severity of injury, with the following categories:

1 - Death or serious injury likely to cause death within 24 hours. Deep wounding exposing internal organs;
2 - Moderate damage, including abrasions to skin. Fin damage and significant scale loss above 15%;
3 - Very little damage. Limited if any fin damage. Between 1% and 15% scale loss;
4- No damage.

Some studies have kept turbine passed fish under observation following turbine passage either to detect delayed mortality or to assess behaviour for subtle effects which may affect survival. Cada *et al.* (2003), for example, examined startle response following turbine passage, and Bracken & Lucas (2013) assayed symetrical swimming motion in turbine-passed lampreys.

Serum biochemistry may be a useful tool for identifying subtle effects which are not readily apparent on visual examination. There is an extensive literature on the use of endocrine measures of stress in fish. These are reviewed in relation to potential turbine passage applications by Hasler *et al.* (2009). The difficulty with this approach for assessing stress to fish from hydropower turbine passage is the need for strict time control, as the response of some of these hormones (cortisol, for example) is rapid. Capture methods, and any pre- or post- trial handling of fish may confound the hormone response, and control groups (for example, sampled from a bypass channel) may be similarly stressed to turbine-passed fish. Mauls & Mesa (1994) measured cortisol in fish which were electricfished and immediately sacrificed after passage through a large-scale hydropower system, but found that there was no significant effect when compared to fish which had passed through a bypass system. The relative effects of passage experience and sampling technique were not separated in that case.

A more promising avenue for identifying tissue damage is the measurement of intracellular enzymes in the blood serum. When cells are damaged or die, these enzymes are released into the blood. Measuring the levels of these enzymes in collected serum samples can allow inferences about the magnitude and type of tissue damage (Hasler *et al.*, 2009). This type of clinical pathology is routine for domesticated animals, and is becoming an established tool in aquaculture. The enzymes Creatine Kinase (CK), Aspartate Aminotransferase (AST), Lactate Dehydrogenase (LDH) and Alanine Aminotransferase (ALT) in particular, but others also, have received research attention (e.g. Rodger *et al.*, 1991; Yousaf & Powell, 2012). For the most part, these attentions have focussed on the response of these enzymes after a disease or chemical challenge, or with differing dietary constituents for the purposes of aquaculture management, but some studies have examined their usefulness for detecting mechanical trauma to fish.

Congleton & Wagner (2004) measured serum constituents of naturally migrating wild and stocked Chinook salmon smolts, and considered ALT, AST and LDH to be general indicators of tissue damage, and CK to indicate damage to the muscle or heart, where it is most concentrated. In a study on damage caused by handling methods for farmed channel catfish, Grizzle *et al.* (1992) found the highest plasma activities of AST and LDH in the group with the highest incidence of external injuries from handling methods. Dobšíková *et al.* (2006) and Dobšíková & Svobodova (2009) found AST, CK and LDH levels in common carp (*Cyprinus carpio* L.) to be significantly influenced by handling and transportation. The use of such assays are also supported by successful application in measuring angling stress (Butcher *et al.*, 2011; Cooke *et al*, 2013; Killen *et al.*, 2003; Morrissey et al., 2005; Rapp et al., 2012; Wells et al., 1986), and the effects of pollution (e.g. Escher et al., 1999).

A difficulty with using these biochemical approaches is that there is currently a lack of information in the literature base on normal reference ranges, and expected effect size. Sandnes *et al.* (1988), and very recently Braceland *et al.* (2016) published normal ranges for these enzymes in adult farmed Atlantic salmon, but other studies have found values outwith these ranges in control groups for adults (Vangen & Hemre 2003; Hemre *et al.*, 2007), pre-smolts (Petri *et al.*, 2006), and Atlantic salmon smolts (Hevrøy *et al.*, 2011). A review of studies which used these enzymes in Atlantic salmon alone reveals very variable ranges of mean activities and standard deviations in control groups (Table 3.1). This lack of consistent ranges in the literature suggests that activities of these enzymes in the blood may vary widely between species, stages and individuals; with condition and environmental conditions. Therefore any attempt to identify subtle damage by using these measures should incorporate appropriate controls for comparison with the challenged group.

Table 3.1. The range of mean enzyme activities, and standard deviations within and between published studies for control groups, from studies measuring the activities of AST, ALT, CK and LDH in Atlantic salmon. For studies where no mean concentrations were given but range was present, mean is taken as the mean of the range. The unit (U) of enzymatic activity is the amount of enzyme that catalyzes the conversion of one micromole of substrate per minute under standard conditions (NC-IUB, 1978).

Enzyme	Range of mean activities (U/l), between groups and studies	Range of within-study standard deviations	Standard deviations of the means between-studies	References
AST	37-616	9-121	211.1	Hemre, *et al.*, 2007; Hevrøy *et al.*, 2011; Petri *et al.*, 2006; Sandnes *et al.*, 1988; Vangen & Hemre, 2003; Wagner & Congleton, 2004
ALT	4-50	1-9.2	14.87	Hemre, *et al.*, 2007; Hevrøy *et al.*, 2011; Petri *et al.*, 2006; Sandnes, *et al.*, 1988; Vangen & Hemre, 2003; Wagner & Congleton, 2004
CK	1582-10297	425-6277	4408.5	Rodger *et al.*, 1991; Wagner & Congleton, 2004; Yousaf & Powell, 2012
LDH	235-1757	53-781	686.6	Hemre *et al.*, 2007; Wagner & Congleton, 2004; Yousaf & Powell, 2012

A recent and promising assay with high specificity for skeletal muscle damage measures enolase 3 enzyme (Braceland *et al.*, 2014). Enolase 3 is defined classically as a glycolytic enzyme catalysing the conversion of 2-phosphoglycerate to phosphoenolpyruvate in the ninth and penultimate step of glycolysis (Panchioli,2001). Three isoforms of enolase, which have differing distributions in body tissues, have been identified in mammals (Tracy & Hedges, 2000). Braceland *et al.* (2014) showed a significant relationship between white muscle pathology and serum enolase 3 (hereafter referred to simply as enolase, since no other form is discussed further in this thesis) content through the use of histopathology and serum protein analysis in adult Atlantic salmon challenged with pancreatic disease. The output of the assay is a semi quantitative measure of enolase activity in the serum, and appears to lack the problem of high variability in apparently healthy fish that the other analytes (AST, ALT, CK and LDH) exhibit. Whilst the enolase assay has demonstrated effectiveness for identifying chronic, disease induced damage to skeletal muscle, the response of this enzyme to acute trauma is untested.

3.2 Methods

There are already many ASHT schemes operational, hence, in order to attain results with the greatest applied value, controlled field tests were carried out at a full-scale, commercially operating ASHT. These tests involved the assessment of turbine exposed and control groups of Atlantic salmon smolts. Hatchery origin Atlantic salmon smolts were used in order to attain predictably sufficient sample sizes during the planned period for the experiments. Two approaches were used to assess damage:

> Firstly, visual inspection of fish and post-hoc analysis of photographs were used to identify and measure external signs of damage.
>
> Secondly, to attempt to detect subtle damage which may not be visually apparent, levels of activity of the enzymes AST, CK, LDH and enolase were measured and compared between turbine-passed and control groups

of fish. This is a novel application of serum chemistry techniques for the assessment of mechanical trauma from hydropower turbine passage.

3.2.1 Hydropower site and turbine specifications

These experiments were carried out at Craigpot hydropower scheme, between 10 and 23 April 2014. The location of the scheme is shown in Figure 2.1, Chapter 2, and scheme layout is detailed in Figure 3.1. A full site description and explanation of the flow management regime is given in Chapter 2, Section 2.2.1, but the turbine specifications are re-iterated here.

The scheme uses a 4-bladed Archimedean screw turbine (Landustrie, Sneek, the Netherlands) connected to a generator, to convert the movement of water over the available head of 2.2 m to electricity, up to a maximum of 60 kW at its full capacity of 4 m^3s^{-1}. The length of bladed screw is 5.4 m, and the diameter is 2.9 m. The screw is mounted in a steel trough set at 22° to horizontal, through which the water flows, driving the screw. The upstream leading edges of the turbine blades are fitted with rubber bumpers with 35 mm of compression to mitigate the physical impact of blade strike to fish (EA, 2009; SEPA, 2014). The maximum gap between the screw blades and trough is 5 mm.

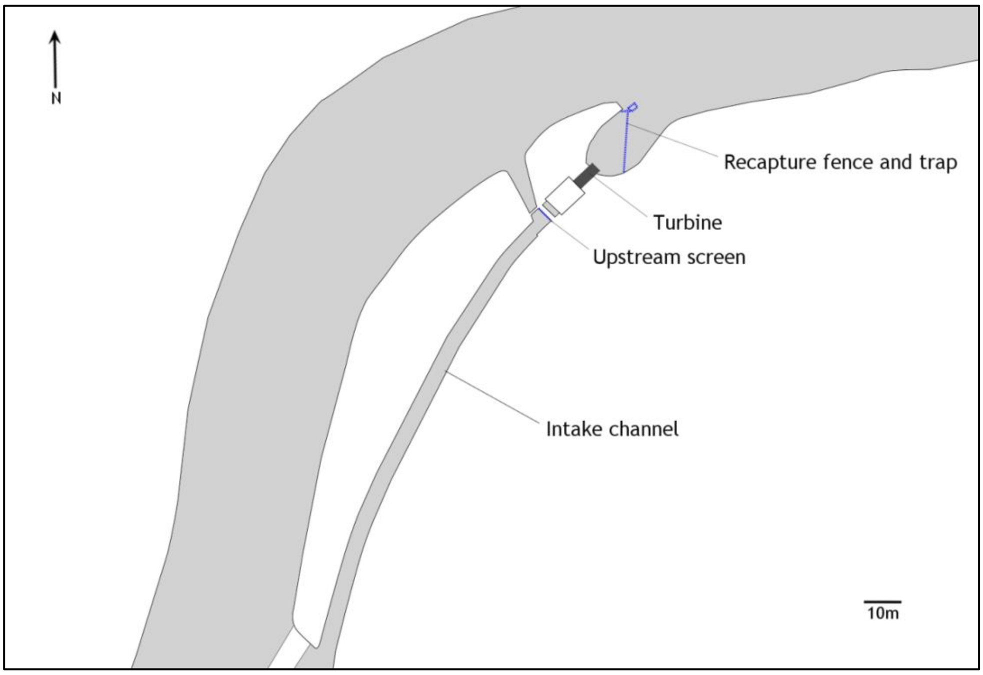


Figure 3.1. Site layout and location of fenced area around the turbine within which the trials were carried out.

During the turbine passage trials, the control system was altered in order to control turbine speed. This was achieved by modifying the target weir crest depth for the control system to achieve the desired speed. The flow under manipulated turbine conditions is the ideal flow (calculated using Equation 2.3, where it is designated q_{turb}), multiplied by the fraction of the ideal flow cross sectional area which is filled under the manipulated condition.

$$q_{trial} = q_{ideal}\,\frac{A_{trial}}{A_{ideal}}$$

Equation 3.1

Where

A_{trial} = cross sectional area under manipulated conditions,

A_{ideal} = cross sectional area under the normal operational regime,

q_{trial} = flow under manipulated conditions

q_{ideal} = turbine flow under normal conditions.

This relationship (Equation 3.1) makes the assumption that the control system maintains a constant weir-crest depth, and that this results in a constant depth and cross-sectional flow area at the turbine intake. Average water velocity is given by flow divided by cross-sectional area:

$$v = \frac{q}{A}$$

Equation 3.2

Since flow is assumed to be linear with RPM, if the cross-sectional area for flow is fixed, it follows that water velocity is also linear with RPM.

Combining Equations 2.3, 3.1 and 3.2 yields the result that water velocity under manipulated conditions is independent of actual cross-sectional flow area:

$$v_{trial} = \frac{q_{trial}}{A_{trial}} = \frac{q_{ideal}\,A_{trial}/A_{ideal}}{A_{trial}}$$

$$v_{trial} = \frac{r}{r_{max}}\,\frac{q_{max}}{A_{ideal}}$$

Equation 3.3

Velocities estimated using this relationship are given in Results section 3.3.1.

3.2.2 Turbine and river state

The turbine passage trials were carried out at two turbine speeds corresponding to operation at maximum capacity (FAST, 26RPM), and near to the lower limit of generation (SLOW, 8RPM). These speeds correspond to maximum blade tip velocities of 3.95 $ms^{-1}$and 1.21 ms^{-1} respectively (circumference x rotational speed = π x 2.9 m x 8 RPM / 60 s). In the original design, fish releases were to be carried out with turbine speed alternating each day. As the experiments progressed and the difficulty of recapturing substantial numbers of released fish within a day became apparent, the decision was made to focus on the FAST speed after an initial trial with the SLOW speed, so as to reduce the potential for release batches to encounter multiple speeds. A release at the SLOW speed was again made at the end of the trials to increase the sample at that speed. River discharge was inadequate during the trials for FAST turbine generation under the regulated abstraction regime, hence permission was granted by the Scottish Environment Protection Agency for over-abstraction during the trials. In order to estimate water velocity entering the turbine, measurements were taken immediately upstream of the turbine using a current meter (Marsh McBirney Flo-Mate 2000, Hatch, USA), for FAST and SLOW trial speeds. Velocity was then estimated as the flow calculated from these measurements divided by the cross sectional area of the filled turbine trough, as calculated from measured depth. Velocities and water depths were measured 3 m directly upstream from the turbine through a gap in the floor of the turbine house on three occasions during the turbine trials. Point velocity was measured at half depth on the first two occasions (at SLOW and FAST speeds) and a velocity profile taken on the third (FAST speed). Velocities were measured at 0.2, 0.4 and 0.8 depth at five points across the 3.07m channel width.

Temperature data (in degrees Celsius at 15-minute intervals) were collected using a temperature logger (Fourtek Picolite, USA) installed at mid depth in the turbine intake channel. Turbine operational data (weir crest depth, rotational speed and power output at fifteen minute intervals) were provided by Highland Ecodesign, Perth.

3.2.3 Blade strike model

The theoretical probability of strike to a fish by the leading edges of the turbine was calculated from Equation 3.4, with the assumption that fish drifted passively at the mean water velocity (estimated from equation 3.3 and the empirical measurements described in Section 3.2.1, separately, for comparison), and are oriented perpendicular to the plane of the leading edge swept area. This follows the relationship first proposed by Von Raben (1958), and used by many others since. It does not at this stage incorporate a correction factor for the ratio of actual strikes (or mortalities, or injuries) to theoretical strikes.

$$P = \frac{l}{w}$$

Equation 3.4

Where:
P = the probability of strike,
l = the length of fish,
w = the 'water length': the distance along the turbine axis that a point in the water has moved between successive blade passes (Turnpenny 1998), equal to $\frac{\mathrm{u}}{4R}$ for a 4-bladed turbine, where R is the rotational speed of the turbine, and u is the water velocity entering the turbine, taken as the mean water velocity in this case.

This model for strike probability does not address the risk of contact by fish with moving or stationary parts once inside the turbine trough. Whilst this is likely to depend on hydraulics and fish behaviour within the turbine, no analytical relationship has yet been defined.

3.2.4 Experimental fish

These turbine passage experiments were carried out under UK Home Office Licence (project licence number PPL 40/3425) and complied with the UK Animals (Scientific Procedures) Act 1986. It was decided to use hatchery origin Atlantic salmon smolts. The use of wild fish would have been the first preference, since the aim of the trials was to test for effects on the naturally migrating stock. Whilst it was accepted that turbine passage effects may differ between wild and cultivated smolts, these experiments required predictably sufficient sample sizes during the planned period for the experiments, a criterion which could not be relied upon from the collection of wild fish within the River Don system. Furthermore, and particularly with reference to the blood chemistry outcomes, hatchery fish could be sourced from common genetic and environment stock, and so could be expected to have less variable baseline values and more consistent experimental responses than wild fish, which may vary a great deal in their origin and prior history.

Atlantic salmon smolts were sourced from Howietoun Hatchery, Stirlingshire, Scotland, and transported by trailer tank to Craigpot hydropower scheme on 8 April 2014. The fish were transferred to a two metre square holding tank by hand net and bucket. The holding tank was supplied with fresh water from an immersion pump in the river. Smolts were acclimated to ambient water temperatures over several minutes by gradually mixing the water they had been transported in with additions of river water. Once in the holding tank, smolts were at ambient river temperature and experienced natural photoperiod for the time of year when natural smolt migration takes place.

Two experimental groups were used to assess change in visible condition of fish from passage through the turbine: a turbine treatment group was released above the turbine and recaptured below (TREATMENT); and a recapture control group was released below the turbine and recaptured as a control for possible change to fish condition resulting from recapture (CONTROL). As well as visual and photographic assessment of these fish (see below), blood samples were taken from recaptured fish. An additional, unexposed control group was sampled for blood directly from the holding tank to provide a baseline for blood biochemistry parameters (UNHANDLED). Ten UNHANDLED samples were also taken on 7 April before transport from the hatchery. This initial sample was to establish the variability of the blood chemistry parameters, and also as a check for any difference in the range of UNHANDLED samples during the trials due to transporting fish from the hatchery to the test site. The results from this group are presented for comparison with the experimental groups because of reduced sample sizes in the event of the trials and analysis (see Section 3.2.8.2).

A small number of fish which had been euthanized for the UNHANDLED control blood samples were also used to test the effects of turbine passage for physical damage on dead, passively drifting fish. The results of these tests are presented in Appendix A3.1. A limited sample of wild Atlantic salmon smolts and brown trout were also used in the experiments, and the results from these (physical damage and blood biochemistry) are presented in Appendix A3.2. Table 3.2 shows the numbers of fish used by date.

3.2.5 Fish introductions and recapture

3.2.5.1 Release

Release times varied from 11:35 to 22:45 but were more frequently in the late afternoon to evening (Table 3.2), with recaptures extending into the night and resuming the following day. This was to replicate the natural tendency for smolts to migrate at night. Diel timing of turbine passage may be an important risk factor due to visual cues for avoidance and evasion behaviour. TREATMENT fish were released from a bucket of water through a 15 cm diameter plastic pipe with its exit directly into the turbine intake basin, 2 m downstream of the trash rack and 4.5 m upstream from the turbine mouth. The intent was to allow the fish sufficient time to orient with the prevailing flow before entering the turbine. In order to prevent fish from escaping upstream, a fence of 10 mm smooth plastic mesh was fitted across the trash rack (Figure 3.2) and remained in place for the duration of the experimental period (7 April to 21 April). This fence may have altered local intake basin velocities slightly. However it is expected that overall mean intake basin velocity would be unaltered, as it is fixed primarily by turbine speed. Hence it is felt that strike probabilities and fish behaviour would have been minimally affected, but no empirical comparison was made between the screen-installed and screen-not-installed condition. CONTROL fish were released simultaneously with, and in the same way as the treatment fish, but 2 m downstream of the turbine. The release equipment was carefully checked to ensure that it would cause no damage to fish as a result of the release process.

Table 3.2. Experimental releases (or sampling, in the case of the UNHANDLED control fish) by date.

Date	Turbine speed	CONTROL	TREATMENT	UNHANDLED	Release time
2014-04-07	-	-	-	10	-
2014-04-10	SLOW	14	16	-	19:20
2014-04-11	FAST	14	16	-	17:45
2014-04-15	FAST	18	27	-	19:20
2014-04-16	-	-	-	19	-
2014-04-17	FAST	18	24	-	15:51, 17:35, 19:06
2014-04-18	FAST	19	28	-	11:35, 12:51, 14:22, 22:10, 22:45
2014-04-20	-	-	-	30	-
2014-04-21	SLOW	19	25	-	20:15

Figure 3.2. View of the upstream fence laid over the trash rack at the turbine intake in order to prevent fish escapement upstream and the entrance of wild fish during the trials.

3.2.5.2 Underwater CCTV monitoring

Two underwater cameras (Sony model IR 37CSHR-IR) were installed in the intake basin, connected to a digital video recorder (Avtech model KPD674 4-channel CCTV DVR H.264). The first was 2.5 m directly upstream of the turbine *ca.* 0.5 m below the surface and 1 m from the floor of the basin with the entire submerged part of the turbine mouth in the field of view. The second was on the true right wall of the basin, in line with the mouth of the turbine and pointing across it, *ca*. 0.2 m from the floor of the basin. The intention was to quantify rate of contact between the leading edges of the turbine and introduced fish, and characterise approach behaviour. The cameras were sensitive to infrared (IR) light and equipped with IR light emitting diodes, which were also supplemented with additional small external IR lights. However the video record during darkness was completely obscured by entrained bubbles and particles lit by these lights, and so no observation was possible at night. During the day, although useful for identifying and qualitatively describing fish behaviour, the clarity and contrast of video in the shaded conditions in the basin prevented any potential observation of leading edge contact.

3.2.5.3 Recapture

A diagonal fence was installed below the turbine to guide fish into a funnel net with a mesh box at its end (Figure 3.3). The fence was constructed in several panels using metal frames and welded mesh, covered with 10 mm smooth plastic mesh and each panel shaped to fit the natural shape of the outflow basin bed. After installation the surface of the fence was checked to ensure that any potential damage to fish from contact with it was at a minimum and that there were no gaps through which fish could escape. The fence was set at an angle of 45 degrees to the flow (plan view), and inclined at a 40 degree angle to the bed. This provided a large surface area of mesh for the bulk of water to shed through, whilst guiding fish towards the recapture box. A flap of additional plastic mesh was included at the base of the fence and was covered with substrate material to ensure no gaps between the fence and the substrate. This recapture system remained in place for the duration of the experimental period.

Figure 3.3. View of the downstream fence and recapture net and box, during installation (left panel). Crowding fish towards the recapture net and box during the trials (right panel).

Not all fish arrived in the recapture system naturally and instead held station in the turbine outflow basin. These fish were encouraged into the recapture box using mesh crowding panels (Figure 3.3), and any fish remaining uncaught were carefully corralled using a seine net. In all cases the method of capture was recorded. A number of wild salmon smolts and brown trout were also captured during the trials. These must have been present within the screened off area (either above or below the turbine), and were captured and photographed. On 24 April 2014, the screen above the turbine was removed, allowing a large number of wild smolts to pass through the turbine into the recapture area. A sample of these fish were captured and processed. Results pertaining to wild fish captured below the turbine are included in Appendix A3.2.

3.2.6 Fish processing

3.2.6.1 Fish assessment prior to passage trials

Prior to the trials, fish were individually visually assessed for damage, photographed and marked using elastomer visible implants (EVI) (Northwest Marine Technology, USA) to distinguish between TREATMENT and CONTROL groups, and release batch. EVI was chosen, rather than the use of surgically implanted PIT tags, for example, because of the possibility of a more invasive procedure affecting fish condition, behaviour or measured responses to a greater extent. Fish were caught and assigned to TREATMENT and CONTROL groups alternately in the sequence that they were captured from the holding tank in order to avoid any bias in the groups due to catchability. Fish were kept wet and immersed in water between each operation, with no longer than 1 minute air exposure at a time. Total time from anaesthetic induction to end of processing averaged 154 seconds.

Figure 3.4. Example of elastomer visible implant on a wild Atlantic salmon smolt.

Each fish was anaesthetised using 50 ppm benzocaine solution before marking with EVI. The EVI mark was placed under the skin behind or above the eye on the left side of the fish for control fish, and on the right side of the fish for treatment fish (see Figure 3.4 for an example). Different colours or positioning of the EVI mark were used to distinguish separate release batches of fish. After measurement of length and mass the fish was placed on laminated graph paper and photographed 12 times (for an example, see Figure 3.5) in order to gain a variety of shading conditions and angles for later image analysis. All fish were photographed on each flank from directly above and also at an angle closer to a dorsal view, this was then repeated with the fish rotated end for end, and finally a photograph was taken once each from a dorsal and from a ventral aspect. In order to assess potential damage to the pectoral fins, these were splayed against graph paper and photographed. The fish was concurrently assessed by marking visible scale loss or injury on printed templates (Figure 3.6). In the final analysis, these marked sheets were not used except for identification purposes, rather basing assessment on the photographs. A photograph of the assessment sheet was taken at the end of each set of fish photographs, so that the photos could be related to individual fish data later. The photography and assessment element of processing lasted under 20 seconds per fish. Fish were allowed to recover in a tank supplied with fresh river water for at least 30 minutes from the time that the last fish was processed. All fish recovered and were seen to swim normally, with good balance and no signs of distress. This recovery period was chosen in order to minimize change to external condition prior to exposure to the treatment, for the assessment of external damage.

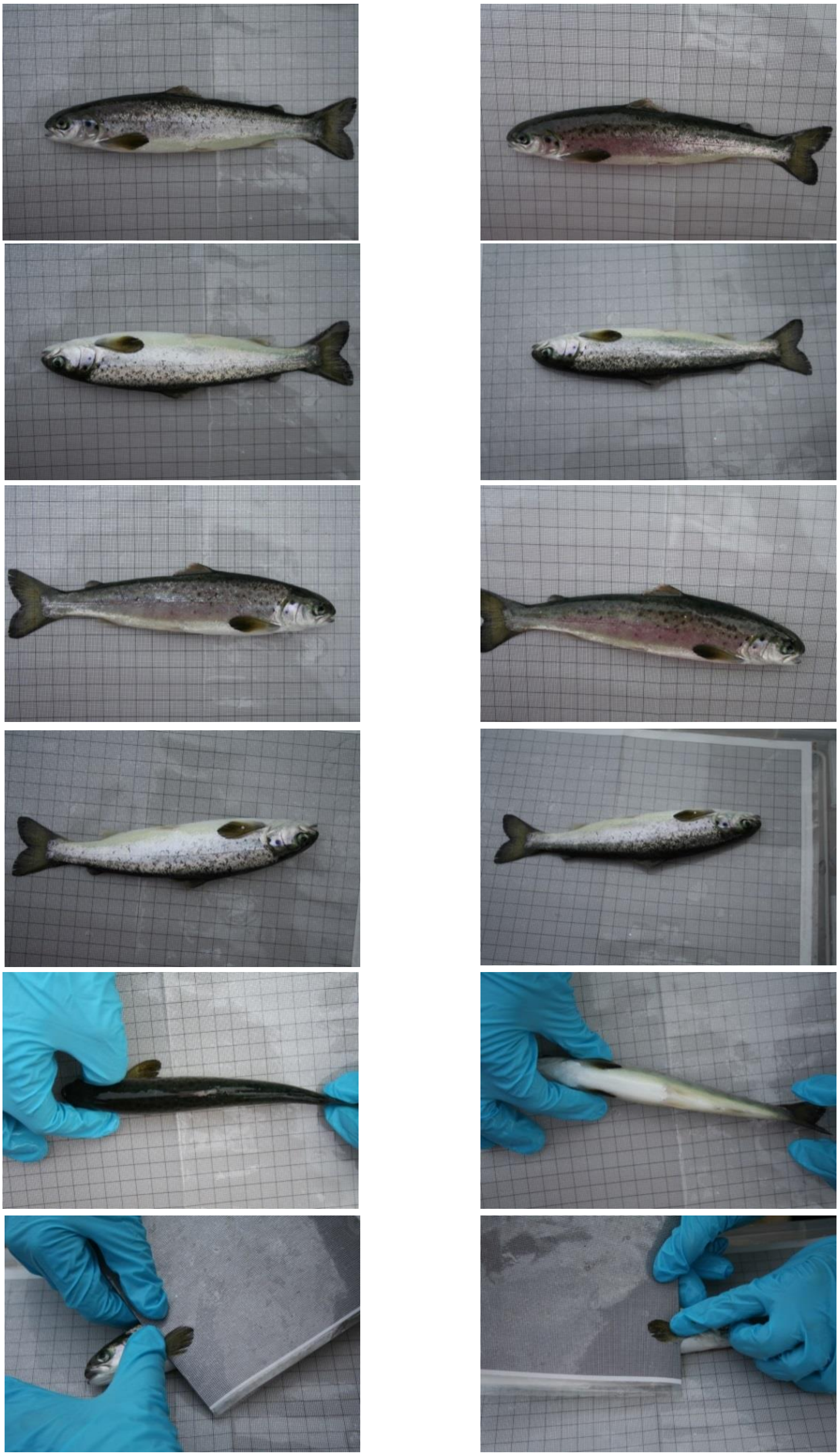
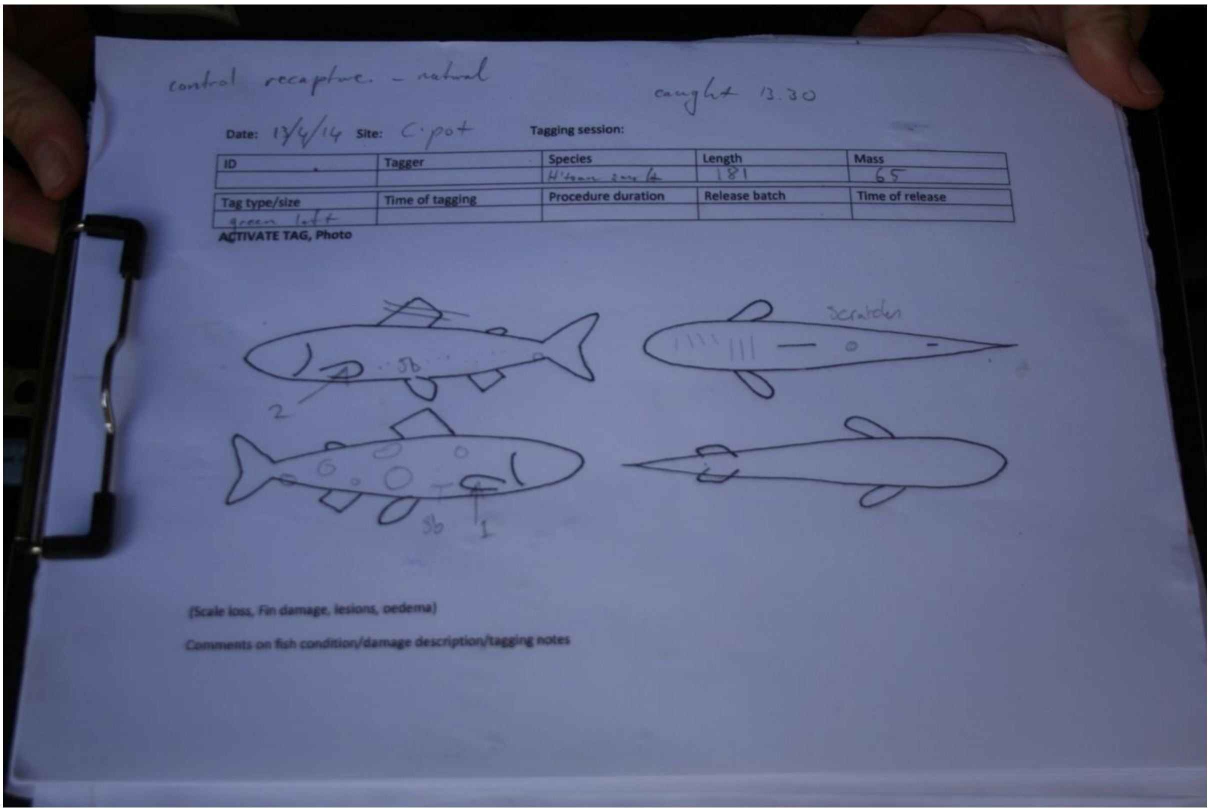


Figure 3.5. Example of photographs taken for each fish.



control recapture. – natural

caught 13.30

Date: 13/4/14 **Site:** C.pot **Tagging session:**

ID	Tagger	Species	Length	Mass
		H'town [illegible]	181	65
Tag type/size	Time of tagging	Procedure duration	Release batch	Time of release
green left				

ACTIVATE TAG, Photo

(Scale loss, Fin damage, lesions, oedema)

Comments on fish condition/damage description/tagging notes

Figure 3.6. Example of an assessment sheet from a recaptured fish. Photo taken during the assessment.

3.2.6.2 Post-trial assessment of fish

Recaptured fish were euthanized using an overdose of benzocaine and pithing before the assessment process was repeated as for prior to release. Care was taken to ensure that handling-induced damage was kept to a minimum and consistent across all fish.

A sample of blood was taken immediately after capture and euthanasia by caudal puncture with a 19 gauge needle and 1 ml syringe. Blood samples were centrifuged and stored on ice until they could be frozen at -20°C at the end of that day’s sampling period. A limited number of TREATMENT and CONTROL samples were analysed during the trials for AST, CK and LDH, on 15 April 2014, and 10 samples had been taken and analysed on 7 April 2014 before the fish were transported from the hatchery as an initial control. The remainder of samples were kept frozen at -20 °C until more permanent storage at -80 °C after the trials were complete. Samples were kept frozen and freeze-thaw kept to a minimum and consistent across all samples (except for the aforementioned pilot samples) until analysis (but see Section 3.2.8.2).

3.2.6.3 Scale-loss assessment

Scale-loss was assessed *post-hoc* from the photographs taken during the fish assessment. Photos were scored in random order without reference to experimental treatment data – that is the scorer did not know if a photo was of a TREATMENT or CONTROL fish, nor whether it was from before or after exposure to either treatment. A score from one to four was assigned to each fish according to the following grading system, and by comparison with Figure 3.7.

> **Grade 1**: 0-1%; negligible scale loss, scattered and isolated scale loss across the fish's body;
> **Grade 2**: 2-4%; low scale loss, scattered across the body but with multiple groups of scale loss several scales high and wide;
> **Grade 3**: 5-9%; moderate scale loss, mostly small patches scattered across the body but with at least one larger patch, the height and width of which approximates the width of the wrist of the tail; and
> **Grade 4**: 10-30%; extensive scale loss comprising multiple patches, with at least one patch with both dimensions exceeding the width of the wrist of the tail.

The reference diagrams shown in Figure 3.7 were designed to be typical of the grade and aid scoring, though considerable variation in patterns of scale loss distribution occurred. This grading system was arrived at with prior knowledge of the range and variety of scale loss extent and patterning, the clarity of the photographs and the presence of glare and shading on the fish surface making more precise measurement difficult. It was assessed that visual grading of the set of photographs for each fish was a more appropriate and efficient method than the manual or semi-automated delimiting of scale loss area. Scale loss area was

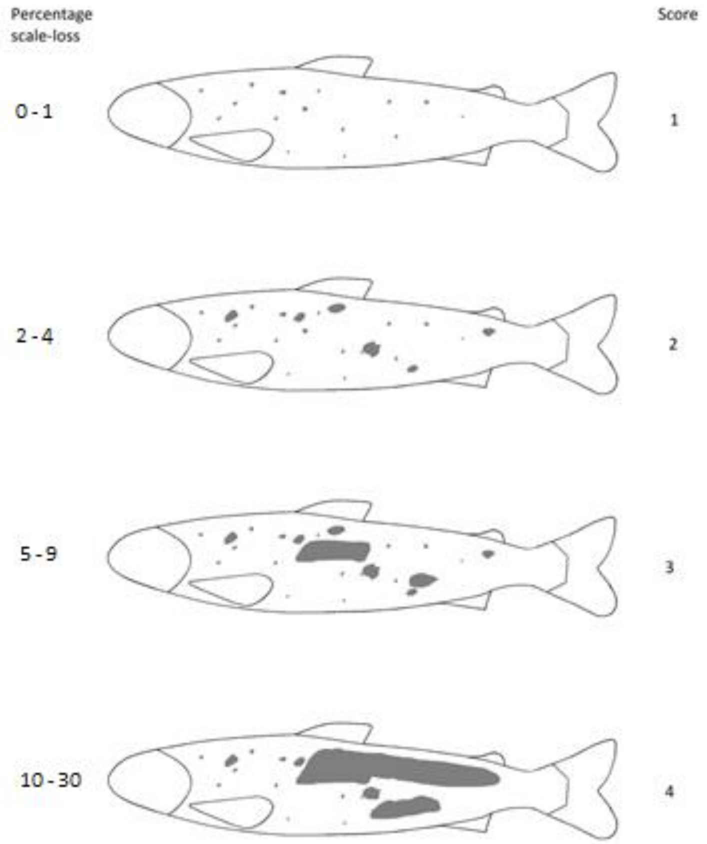


Figure 3.7. Scoring categories for scale-loss. Each picture represents the upper limit of scale-loss for inclusion in the scoring category. Fish with scale-loss exceeding a score of four were scored as four.

measured on a small number of photographs using 'ImageJ' image analysis software. Automated delimiting of scale-loss area was not possible because of variability in clarity, shading and glare within and between images. Manual measurement using the same software was unreliable for the same reasons.

Pictures of recaptured fish were then matched manually with those taken of the same individual before release: first by narrowing the number of fish using the batch EVI mark, then using length and weight data to filter individuals of similar size, and then matching individuals using distinctive markings. In the first instance spots on the gill cover and distinctive fin shapes (deformities of the dorsal fin proved the most useful distinguishing feature for hatchery fish) were used to match individuals. Where these identifiers were not adequate, patterns of pre-existing scale loss and fin damage were used. It is recognized that these identifiers may have changed as a result of the trials but where matches were made, the patterns used were corroborated with at least two other identifiers on separate areas of the fish. In practice this proved an effective method of identification. The sample size allowed efficient identification and matching of photographs without the time cost of validating appropriate automated image recognition software (for example, within ImageJ) on images of variable quality and lighting conditions. Of two-hundred and thirty-nine fish that were assessed, four could not be identified and matched, and these were excluded from the analysis.

3.2.6.4 Assessment of fin damage

Pectoral fins were assigned a score from zero to four according to the following categories:

0 – fin entirely intact, with no splits between fin rays

1 – splits present between fin rays

2 – surface area of fin reduced, but more than 75% remaining

3 – surface area of fin reduced, with 50% to 75% remaining

4 – surface area of fin reduced by more than 50%

3.2.6.5 Presence of *Saprolegnia* infection

During the trials, a number of fish which were held in tanks at the trial site were seen to be infected with *Saprolegnia spp*. a freshwater fungus. *Saprolegnia* outbreaks are common in freshwater fish, especially in association with warming water temperatures (Roberts, 1978, and personal observations of wild juvenile salmonids captured on the River Don in 2013 and 2014). Saprolegniasis in salmonids is also generally considered to be secondary to stress (Richards & Pickering, 1978), such as may be caused by handling and transport.

There is the possibility of this infection influencing the measured responses in several ways: 1) by directly altering the blood chemistry through tissue necrosis or immune response; 2) by altering hazard avoidance behaviour (infected fish were observed to be less active, and less likely to exhibit the startle response) and hence the likelihood of injury occurring, with resultant effects on blood chemistry or visible measures of damage; or 3) by affecting the measures of visible damage used – areas of saprolegnia infected skin cannot be assessed for scale loss, since the fungus covers the scales.

Therefore only those fish with no visible sign of infection were included in the analysis. Appendix A3.3 describes a grading method for the degree of *Saprolegnia* infection, the results of the full set of fish, and the relation of responses measured with degree of infection.

3.2.7 Metrics for change in visible fish condition

To test whether treatment had an effect on scale-loss, the numbers of fish with differences in scoring category from before release to after recapture (score-change) was compared between treatment and CONTROL groups. Since each side of each fish was scored separately, the side with the higher value of score-change was used as the response tested in order to detect new scale-loss. Score-change was tested at two thresholds of severity: first for any positive change (i.e. a move from any scoring category to a higher one, labelled condition α) and second for any change greater than one scoring category (condition β). To detect overall changes to both sides of the fish, the summed score-changes of both sides of each fish were tested for any positive change greater than one (i.e. a move from any scoring category to more than one higher, or a move by one on both sides of the fish, labelled condition γ).

Change to pectoral fin condition was identified as any positive change in score of each fin between pre- and post-trial assessment. The numbers of each fin with a change were then compared between TREATMENT and CONTROL groups.

3.2.8 Serum chemistry analysis

3.2.8.1 Enzyme-linked immunosorbent assay (ELISA) for enolase

ELISA (Engvall & Perlmann, 1971) is a test that uses antibodies and a colour change reaction to detect an enzyme or antigen. Proteins from a sample are allowed to bind to a surface (the wells of an assay plate). A specific antibody is applied so it can bind to the substance of interest. This antibody is linked to an enzyme. When the substrate of the enzyme is added, the subsequent reaction produces a detectable signal: most commonly a colour change in the substrate.

Muscle lysate (the protein extract from muscle) known to contain enolase at a high concentration was used as a standard on each assay plate from which to derive arbitrary units (AU) for enolase activity. The standard was serially diluted (1:2500, 1:5000, 1:10000, 1:20000, 1:40000, 1:80000) on each plate using an ELISA plate coating buffer (0.2 M sodium carbonate/bicarbonate pH 9.4), and assigned as 100 AU down to 6.25 AU. In addition, a low and high standard sample was added to each assay plate in order to assess inter-assay variability. All samples were duplicated on the assay plate in order to assess intra-assay variability.

ELISA for enolase was carried out following the methods of Braceland *et al.* (2014). To each well, 100 µl of either diluted sample (1:2500), serially diluted standard Atlantic salmon serum, diluted low and high standard (1:2500), or blank (buffer alone) was added and the plate left overnight at 4^{o}C. Wells were emptied and then washed three times using 250 µl of a buffer solution comprising 50 mM Tris, 150 mM NaCl and 1% polyethylene glycol sorbitan monolaurate (TTBS), blocked with 10% weight by volume (in TTBS) powdered skimmed milk and left on a shaker for 1 h at room temperature. The plate was washed again three times, and 100 µl of primary antibody (ENO3 diluted to 1:1000 in TTBS) added. The plate was left on a shaker for one hour, washed again three times and 100µl of an HRP linked donkey polyclonal secondary antibody to rabbit (Abcam, Cambridge, UK) at a 1:10000 dilution added for one hour on a shaker. A final three washes with TTBS was performed before developing using a 3,3’,5,5’-tetramethybenzidine (TMB) Microwell Peroxidase Substrate Kit (Insight Biotechnology) which by peroxidase reaction catalysed by HRP forms a blue by-product. After 10 minutes incubation on a shaker, the reaction was halted using 50µl of 1M hydrochloric acid, causing the colour of the solution to change to yellow. Light intensity was measured at 450 nm using a plate reader (FLUOstar OPTIMA, BMG Labtech, Ortenberg, Germany).

Accuracy was determined by parallel curves of dilutions of serum sample with a high enolase content versus the calibrator (Braceland *et al.*, 2014). Precision was established by calculating the intra-assay coefficient of variance of all the paired samples, and the inter-assay coefficients of variance of the low and high standards. The limit of detection was assessed as the amount in AU of enolase detectable at 3 *sd* from the mean of the blanks.

3.2.8.2 Determination of AST, CK and LDH

Whereas the ELISA for enolase results in a measure in AU relative to a standard, determination of AST, CK and LDH use methods which give the internationally recognized unit (U) of enzymatic activity. This is the amount of enzyme that catalyzes the conversion of one micromole of substrate per minute under standard conditions (NC-IUB, 1978). A limited number of treatment and CONTROL samples were processed during the trials for AST, CK and LDH, on 15 April 2014, and 10 samples were processed before the fish were transported from the hatchery as an initial UNHANDLED control. The sample numbers in each of these groups (UNHANDLED control, turbine TREATMENT, recapture CONTROL) were low; however, these data were considered more reliable than those from the later analysis, which exhibited far reduced activity levels. The apparent differences in activity ranges between the two sets of data, and full results for the later set are presented and discussed in Appendix A3.4. Although no clear reason to explain this difference could be identified, the second set of results have been excluded from the current analyses due to suspected storage effects.

Determination of AST, CK and LDH activities was carried out by Glasgow University Veterinary Diagnostics using an autoanalyser (AU640, Olympus, Japan). Where necessary, samples were diluted in order to meet minimum sample requirements. The methods and quality assurance procedures used by the laboratory are included for reference below.

The method for AST is based on the recommendations of the International Federation for Clinical Chemistry (Bergmeyer *et al.*, 1985). In this method, aspartate aminotransferase (AST) catalyses the transamination of aspartate and 2-oxoglutarate, forming L-glutamate and oxalacetate. The oxalacetate is reduced to L-malate by malate dehydrogenase (MDH), while NADH is simultaneously converted to NAD+. The decrease in absorbance due to the consumption of NADH is measured at 340 nm and is proportional to the AST activity in the sample. Endogenous pyruvate is removed by the LDH-reaction during the incubation period. The autoanalyser was calibrated for this test, with a calibrator value traceable to a Beckman Coulter Master Calibrator. The linear range given for this test is from 3-3000 U/l, with the lowest detectable level of AST being 1 U/l in the manufacturer protocol sheet for the reagent (OSR6509).

The measurement of CK was according to manufacturer instructions (OSR6279) and follows the recommendations of the International Federation of Clinical Chemistry (IFCC). CK reversibly catalyses the transfer of a phosphate group from creatine phosphate to adenosine diphosphate (ADP) to give creatine and adenosine triphosphate (ATP) as products. The ATP formed is used to produce glucose-6-phosphate and ADP from glucose. This reaction is catalysed by hexokinase (HK) which requires magnesium ions for maximum activity. The glucose-6-phosphate is oxidised by the action of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) with simultaneous reduction of the coenzyme nicotinamide adenine dinucleotide (NADP) to give NADPH and 6-phosphogluconate. The rate of increase of absorbance at 340/660 nm due to the formation of NADPH is directly proportional to the activity of CK in the sample. The autoanalyser was calibrated with the calibrator value traceable to the IFCC reference method. The test is linear within an enzyme activity range of 10 – 2000 U/l, and with lowest detectable level estimated at 3 U/l

For LDH the analysis method (OSR6126) is based on the recommendations of the Scandinavian Committee on Enzymes. LDH catalyses the reduction of pyruvate to lactate at a neutral pH. This reaction is coupled with the oxidation of NADH to NAD+. The decrease of NADH is measured at 340 nm and is directly proportional to the enzyme activity in the sample. The calibrator value is traceable to a Beckman Coulter Master Calibrator. The test is linear within an enzymatic activity range of 50 - 3000 U/l. The lowest detectable level on an AU600 analyser was estimated at 5 U/l.

Manufacturer sourced quality control standards for AST, CK and LDH were tested to check assay accuracy. The closer the value of the standard is to its historical mean value, the more accurate are the assay results. The acceptable range is the mean ± 2 *sd* (standard deviation). The Glasgow Veterinary School Clinical Pathology laboratory also participates in the UK NEQAS Quality control system.

3.2.9 Statistical analyses

All statistical analyses were carried out using R (R Development Core Team, 2008).

3.2.9.1 Analysis of scale-loss scores

Fisher's exact test was used for comparisons between TREATMENT and CONTROL groups of the frequencies of changes to scale loss scores between release and recapture at the three defined score-change conditions, and for changes in pectoral fin score.

Generalized linear mixed effect models (GLMM) were used to check for potential influences on scale loss by other measured covariates which could not be controlled as part of the experimental design. Logistic GLMMs were performed with each of the three score-change conditions as a response. The covariates included were: treatment group, turbine speed category, lag between release and recapture, method of capture, average scale-loss score over the two sides of the fish before the trial, fork length and Fulton's condition factor (100 x mass (g)/[fork length (cm)]^3. Release batch was included as a random effect. The minimally adequate model was selected by the sequential deletion of covariates which caused no significant decrease in the fit of the model when omitted, as tested by likelihood ratio tests. The threshold for retention of covariates was $p < 0.1$, and the threshold for significance was $p < 0.05$.

Estimates for the probability of each score-change condition were based on the assumption that each fish was an independent trial with a probability of a change in score resulting from the process of the trial. The estimate of that probability is the number of fish with a change in score divided by the number of trials. Ninety-five percent confidence intervals were calculated from the binomial distribution for each probability and sample size.

3.2.9.2 Analyses of blood-chemistry data

Kruskal-Wallis tests were used to check for differences in blood analyte activities between UNHANDLED, CONTROL and TREATMENT groups. Mann-Whitney U-tests were used to check for differences between TREATMENT and CONTROL groups. These non-parametric tests were used because of the non-normal distributions of the data.

During the trials, there were potential influences on blood chemistry which were not part of the experimental design. Additional variation in response may also have arisen from lab analysis techniques. In order to account for these potential sources of systematic variation, generalized linear model regressions were performed which included recorded potential covariates. These regressions were carried out only on the data from the TREATMENT and CONTROL fish, not including the UNHANDLED fish, since these were not exposed to trial conditions.

Potential uncontrolled covariates identified and measured during the trials were: release batch (corresponding to a date and time of release); date and time of recapture (blood sampling time); the lag between release and recapture of each fish; temperature and temperature deviance (calculated for the 24 hour period prior to sampling); fish length; condition factor; method of capture (seine, recapture box, crowding); and actual turbine speed during the release-recapture period for each fish (summarized as mean and range). Assay plate for enolase was identified as a source of systematic variation from the laboratory analysis.

Table 3.3. Thresholds of detection for enolase, and distribution of data over thresholds. These data include *Saprolegnia* infected fish. The lowest detected value was 1.58, but the calculated lower limit of detection was 5.83.

Lowest detected value	1.58
Lower limit of detection	5.83
Proportion of sample below lowest value	0.53
Proportion of sample below limit of detection	0.90
Upper limit of detection	140
Proportion of sample above upper limit	0.01
Total N	223

The enolase data were bounded by limits of detection, were zero inflated and had a left skewed distribution (Table 3.3). Therefore three regressions were carried out for these data: a logistic regression on the binary data dichotomised around the lower limit of detection, an ordinary regression on the continuous part of the data, and a logistic regression on the data dichotomised around a chosen threshold, the median enolase activity for the UNHANDLED group.

Collinearity

Covariates were checked for collinearity using the corvif function from the R library "HighstatLibV6" (Zuur *et al.*, 2009). Sampling time, lag, batch, speed category, and average speed were found to be collinear (variance inflation factor > 3). Release-recapture lag is calculated from batch release time and sampling time, and so these three variables are not independent. Batch was selected for use in the models as the most pertinent potential covariate. Removing sampling time resulted in acceptable levels of collinearity (Variance inflation factor < 3) for lag. Whilst there may have been a temporal trend in blood chemistry, the random effect of batch was deemed more important for the analysis, and since batch is really a temporal categorical variable it would be expected that any underlying temporal differences would be captured. Average turbine speed is clearly not independent of the speed category, and so speed category was selected as the simplest variable.

Model selection

Stepwise model-selection was carried out for each separate regression to reduce the number of coefficients to the minimally adequate model. Covariates were removed on the basis of likelihood ratio tests between the current model and the models reduced by each of the remaining covariates in turn. Covariates resulting in $p<0.1$ on deletion were retained in the model.

3.2.9.3 Correlation between scale loss and blood responses

Once the final regression models for the blood responses had been selected, scale-loss scores were included to test for correlation between these and the blood responses. The average scale-loss score before release, and change to average scores between release and recapture were selected for use.

3.2.9.4 Post-hoc power analysis

Post-hoc power analysis was carried out by simulating data with a range of assumed effect sizes, given the sample sizes attained in the trials, followed by analysis using the statistics described. The power was calculated as the proportion of 1000 simulated datasets at each effect size which yielded a significant treatment effect at the p=0.05 level. The effect size which would be reliably detected was taken as that which resulted in a significant result in 80% of simulations. For the blood chemistry analytes, the following model was used to simulate data: unperturbed enzyme activity levels were assumed to be normally distributed with the mean and standard deviation of the UNHANDLED group. A portion of CONTROL group activities were altered with a binomial probability p_c, at an effect size e_c. A portion of TREATMENT group activities were altered with a binomial probability p_t at an effect size e_t. Data were simulated at four control effect prevalences (0.01, 0.05, 0.1 and 0.5) and two control effect sizes (a factor of 1.5 and 3, corresponding to a low and high effect size). The treatment effect prevalence necessary for 80% power using a t-test for a range of treatment effect sizes was then calculated and graphed. A t-test was used since this is the equivalent of a linear model with only one two-category covariate. Thus the power calculated is that of the model to detect a treatment effect with the partial effects due to other covariates already accounted for.

3.3 Results

3.3.1 Turbine and river state

The overall sample mean of the average turbine speed between introduction and recapture of each fish was 21.88 RPM (*sd* = 3.85, range: 11.68 - 25.60) for the FAST speed, and 9.96 RPM (*sd* = 2.13, range: 7.96 - 14.51) for the SLOW speed. Mid-channel water velocity in the intake basin at the SLOW and FAST speeds were 0.22 ms^{-1} and 0.61 ms^{-1} respectively. Turbine discharge calculated from a velocity-depth profile at the FAST speed was 2.54 m^3s^{-1}, with a mean water velocity in the intake basin of 0.43 ms^{-1}. Assuming maximum flow of 4 m^3s^{-1} at 26 RPM at ideal channel depth, mean velocity entering the turbine mouth calculated from channel cross section (measured depth and known turbine diameter) and fractional filling of the turbine intake was 0.37 ms^{-1} at the SLOW speed and 1.21 ms^{-1} at the FAST speed. Using the measured discharge and fractional filled area resulted in a mean velocity at the turbine mouth of 1.24 ms^{-1} at the FAST speed. Fifteen-minute logged turbine and river discharge data are shown in Appendix A3.5. Average mean daily water temperature during the trials was 8.6 ^oC (range: 7.5-10.2), and average daily temperature deviance was 3.4 ^oC (range: 1.5-6) Fifteen-minute logged temperature data are shown in Appendix A3.5.

3.3.2 Recaptures

During the trials, time from release to recapture varied from three minutes to 10.7 days (Table 3.4). One fish that was recaptured 20 days post-release, after the upper screen was removed, was excluded from the analyses. These lags between release and recapture were a result of fish remaining in the intake basin for long periods before passing through the turbine, and in the outflow basin before arriving in the recapture box. In addition, during the first part of the trials it was discovered that there was a pool underneath the turbine trough itself, which fish may have accessed through small openings on either side at the end of the turbine, hence evading capture for long periods. These openings were blocked, and crowding and corralling methods adopted from April 15 onwards, resulting in shorter release to recapture lags and greater recapture rates.

Table 3.4. Numbers of recaptured fish by release date and number of days lapsed until recapture.

RELEASE DATE	TURBINE SPEED	TREATMENT/ CONTROL	NUMBER OF FISH RECAPTURED PER DAY SINCE RELEASE										
			0	1	2	3	4	5	6	7	9	10	11
2014-04-10	SLOW	CONTROL	2	3							2	2	1
2014-04-10	SLOW	TREATMENT	3		1					1	1		
2014-04-11	FAST	CONTROL	8	2	1				1		1		1
2014-04-11	FAST	TREATMENT	9					1	1		1		1
2014-04-15	FAST	CONTROL	2	14	2								
2014-04-15	FAST	TREATMENT	5	16	4		1						
2014-04-17	FAST	CONTROL	6	1	5			3					
2014-04-17	FAST	TREATMENT	11	1	8	4		3					
2014-04-18	FAST	CONTROL	5	9			5						
2014-04-18	FAST	TREATMENT	4	9	2		5						
2014-04-21	SLOW	CONTROL		11	1								
2014-04-21	SLOW	TREATMENT		15	1								

3.3.3 Avoidance behaviour

Introduced hatchery Atlantic salmon smolts avoided turbine passage by remaining in the intake basin. Whilst it was not possible to separate the total lag from release to recapture into time above and below the turbine, smolts were observed holding station above the turbine for for up to several hours using the installed underwater CCTV cameras. The smolts made use of low water velocity regions below and to either side of the turbine to hold station. These observations were only possible when sufficient daylight illumination allowed, and so were restricted to the remaining hours between release and darkness. In general the majority of recaptures below the turbine were in the day following release.

3.3.4 Change in visible condition

No external signs of severe trauma, such as haemorrhage, cuts or indents were observed, therefore the results focus on identifying differences in subtle damage – scale loss and fin damage – between TREATMENT and CONTROL groups.

3.3.4.1 Change in scale-loss score between release and recapture

There were no significant differences between TREATMENT and CONTROL groups in the proportions of fish with any of the three score-change conditions, for the trials as a whole, nor within the FAST and SLOW turbine speed tests (Fisher's exact tests, $p > 0.1$) (Table 3.5). Figure 3.8 shows the frequencies of changes to score of each side of all fish. Overall, 7.46% of TREATMENT fish had a score-change at condition ß (Figure 3.9), which represents the most severe new scale-loss to one side of a fish, and equates to new scale-loss ranging from 4-30%. Five percent of the CONTROL group was affected at the same condition. At condition α, which represents any new scale-loss to either side, 2.8% of treatment fish were affected over and above the CONTROL group proportion of 37.5%. The proportion of CONTROL fish affected by condition γ, which accounts for any change to both sides exceeded the treatment estimate of 11.9% by 8.1%.

Table 3.5. Results of Fisher's exact tests for differences in frequencies of fish with changes in score between TREATMENT and CONTROL groups, for all smolts, and the smolts sub-setted by turbine speed category. N(C): total (and CONTROL) sample size. The infinite odds ratios (∞) were due to zero CONTROL fish with the score change condition in the SLOW group.

		Any positive change		Summed side changes>1		Maximum change to either side>1	
Group	N(C)	odds	*p*	odds	*p*	odds	*p*
Treatment-Control	107(40)	1.12	0.84	0.55	0.28	1.53	0.71
FAST Treatment-Control	91(30)	0.84	0.82	0.37	0.13	1.02	1
SLOW Treatment-Control	18(10)	2.82	0.56	∞	0.44	∞	0.44

Condition α was correlated with average score before release (GLMM, estimate = -2.7, $p < 0.05$), and lag between release and recapture (GLMM, estimate = 0.335, $p < 0.05$). Condition γ was correlated only with the lag between release and recapture (GLMM, estimate = 0.2883, $p < 0.05$). Treatment group, turbine speed category, method of capture, fork length, and condition factor did not contribute significantly to the fit of the models. It was not computationally possible to determine correlations with covariates by logistic regression for condition ß as only five out of the 86 fish with all covariates measured had changes in score greater than 1.

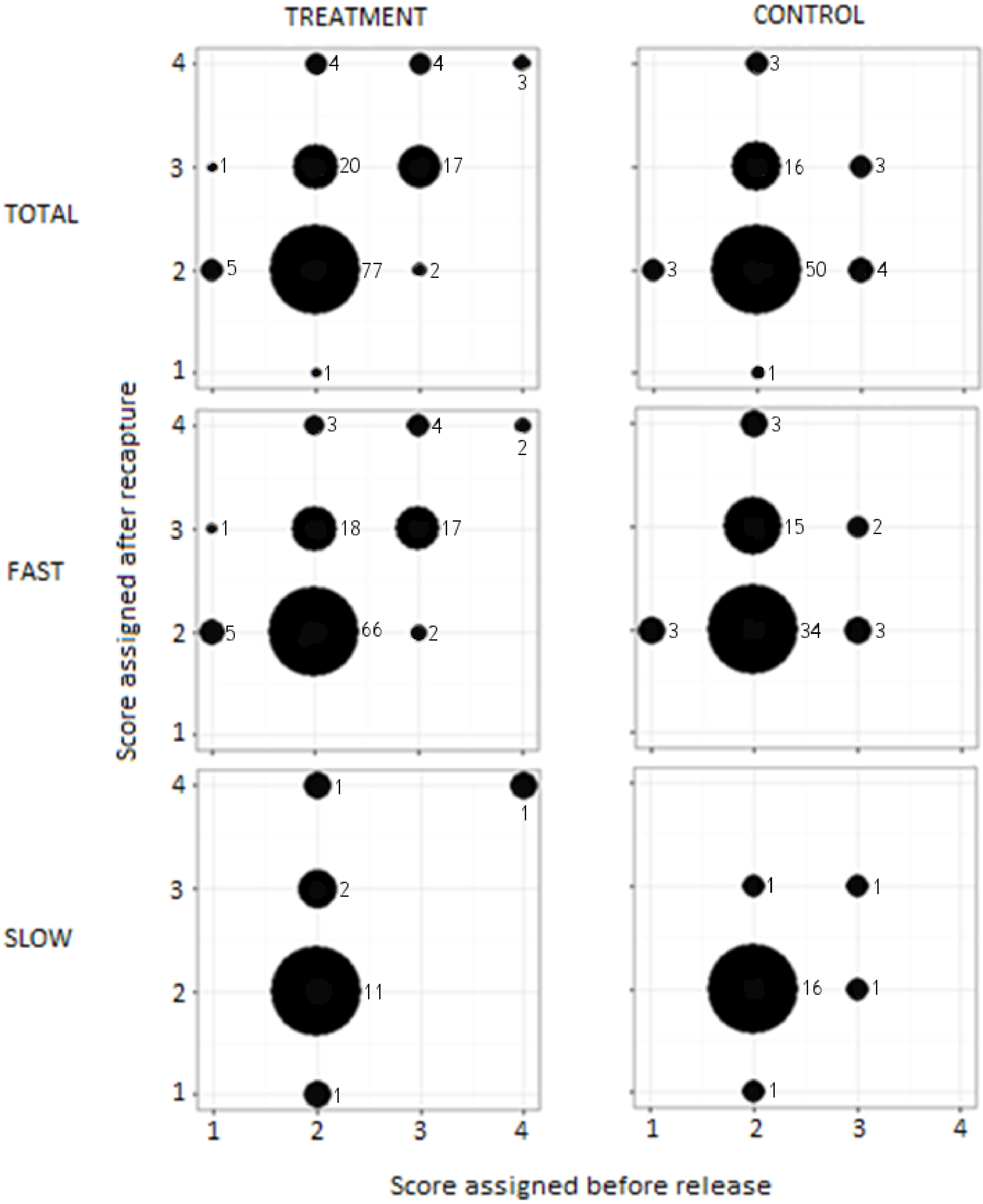


Figure 3.8. Scores assigned to each side of each fish before and after the trials. Each side of the fish is counted separately in order to illustrate changes in score without summarizing scores over both sides. This avoids any masking of effects to just one side of a fish, but effectively doubles the apparent sample size for these plots. Column 1: TREATMENT fish; column 2: CONTROL fish. Row 1: Trials at both FAST and SLOW speeds combined; row 2: FAST trials; row 3: SLOW trials. The area of the points in each panel is proportional to the number of sides with the assigned scores, which are labelled. Points on the diagonal (score before = score after) indicate no change in score. Points above the diagonal indicate an increase in score resulting from the trial. Points below the diagonal indicate a decrease in score (improvement in fish condition) and are a result of scoring errors.

Figure 3.12 shows the power of Fisher's exact test to detect a treatment effect given the sample sizes attained, and assuming a range of effect sizes, calculated from simulations. Taking 0.8 as the acceptable power threshold, the minimum expected detectable treatment prevalence is 0.2 greater than an assumed CONTROL prevalence of 0.05. If we accept the treatment and CONTROL prevalence given by the data, this would result in detection with 0.035 probability. Power decreases with increasing CONTROL prevalence.

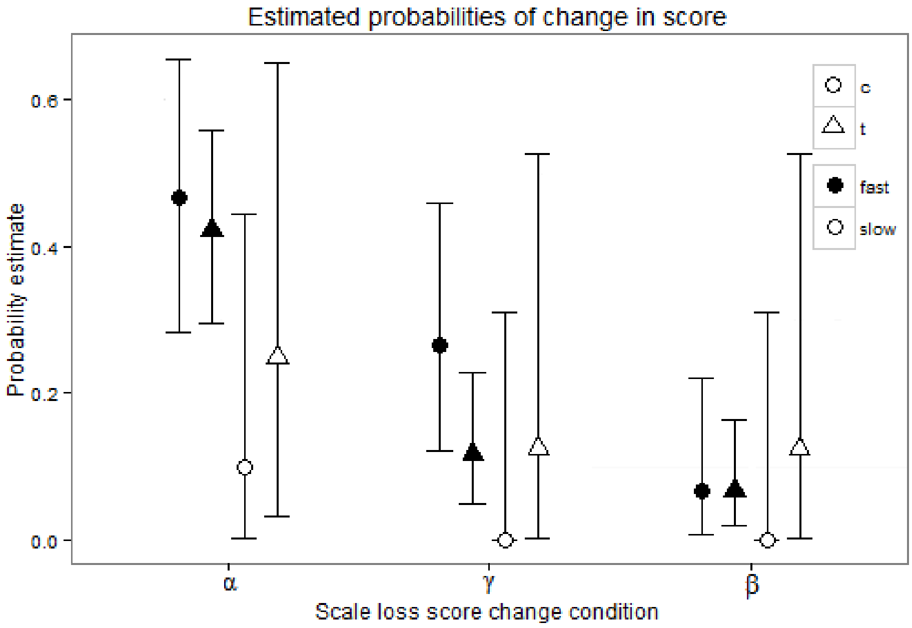


Figure 3.9. Estimated probabilities, based on the data collected, of changes in scale-loss scoring category, at the three conditions for CONTROL and TREATMENT groups for the FAST and SLOW turbine speeds. Solid symbols represent the estimates for the FAST speed. Hollow symbols represent the estimates for the SLOW speed. Circles represent CONTROL fish and triangles represent treatment fish. 95% confidence intervals for the estimates are derived from a binomial distribution and shown with black lines. Score change conditions are: α = any positive change in scale loss score; γ = any positive change greater than 1, when the scores for each side of the fish are summed; β = any positive change greater than 1 for either side of the fish.

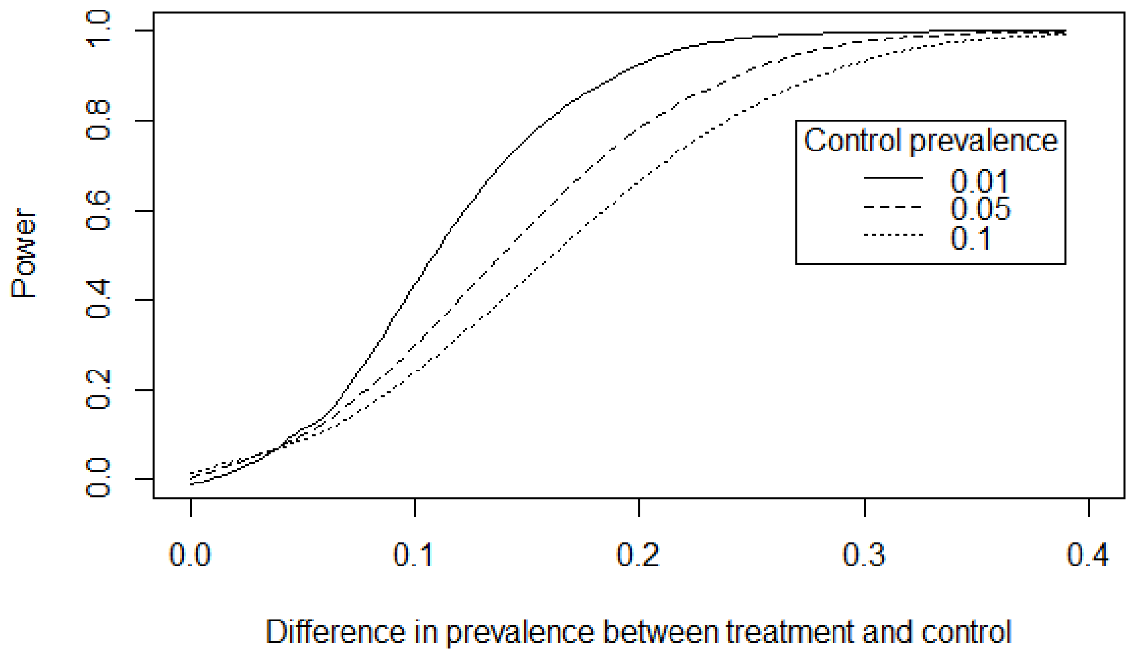


Figure 3.10. Power of Fisher's exact test to detect a difference between TREATMENT and CONTROL groups for the full dataset of 40 CONTROL and 67 TREATMENT fish. Curves were produced by 1000 simulations at each assumed CONTROL and TREATMENT prevalence.

3.3.4.2 Change in pectoral fin score

Out of 94 fish which were scored for pectoral fin condition, there were four instances of increased score (decreased surface area or increased splitting) to either the right or left fin, but this was not associated with turbine passage (Fisher's exact tests: left side p = 1, *odds ratio* = ∞, only a single TREATMENT fish with increased score; right side p = 1, *odds ratio* = 1.14, 1 CONTROL and two TREATMENT fish with increased score).

3.3.5 Theoretical strike probabilities

Based on the mean turbine entrance velocities calculated from turbine rotational speed (Section 3.3.1), strike probability for the mean fish length of 182 mm would be 0.26 at both the FAST and the SLOW speed. The identical estimates for both speeds arises from the fixed relationship between rotational speed and water velocity (Equation 3.3). This result assumes that fish drift passively at the mean water velocity, oriented perpendicular to the plane described by the leading edges of the turbine blades. This result is presented for comparison with the actual damage rates observed. These hatchery origin smolts are much larger than wild Atlantic salmon smolts in Scotland generally. Wild Atlantic salmon smolts captured during the study in Chapter 2 had a mean fork length of 116 mm (range = 90-165mm). Strike probability for these smaller fish would be 0.17 (range = 0.13-0.24).

3.3.6 Serum chemistry

3.3.6.1 AST, CK and LDH

Quality controls for all analytes were < 2 *sd* from the mean on the day of the analyses. There was a significant difference between the UNHANDLED, CONTROL and TREATMENT groups for AST only (Kruskal-Wallis test, $H(3) = 8.56$, $p = 0.0138$) (Table 3.6 and Figure 3.11). There were no differences between TREATMENT and CONTROL groups for any of these analytes (Mann-Whitney U-test, $p > 0.1$) (Table 3.7 and Figure 3.11), but the small sample sizes should be noted, especially for TREATMENT samples, which are very low.

Table 3.6. Results of Kruskal-Wallis tests for each analyte: C, T and UHC indicate the numbers in each of the CONTROL, TREATMENT, and UNHANDLED groups.

	C	T	UHC	*H*	*p*
AST	10	4	10	8.56	0.0138
CK	10	4	10	4.783	0.0915
LDH	11	4	10	1.239	0.538

Table 3.7. Results of Mann-Whitney U-tests: C and T indicate the numbers in the CONTROL and TREATMENT groups.

	C	T	*W*	*p*
AST	10	4	26	0.454
CK	10	4	28	0.3037
LDH	11	4	25	0.54

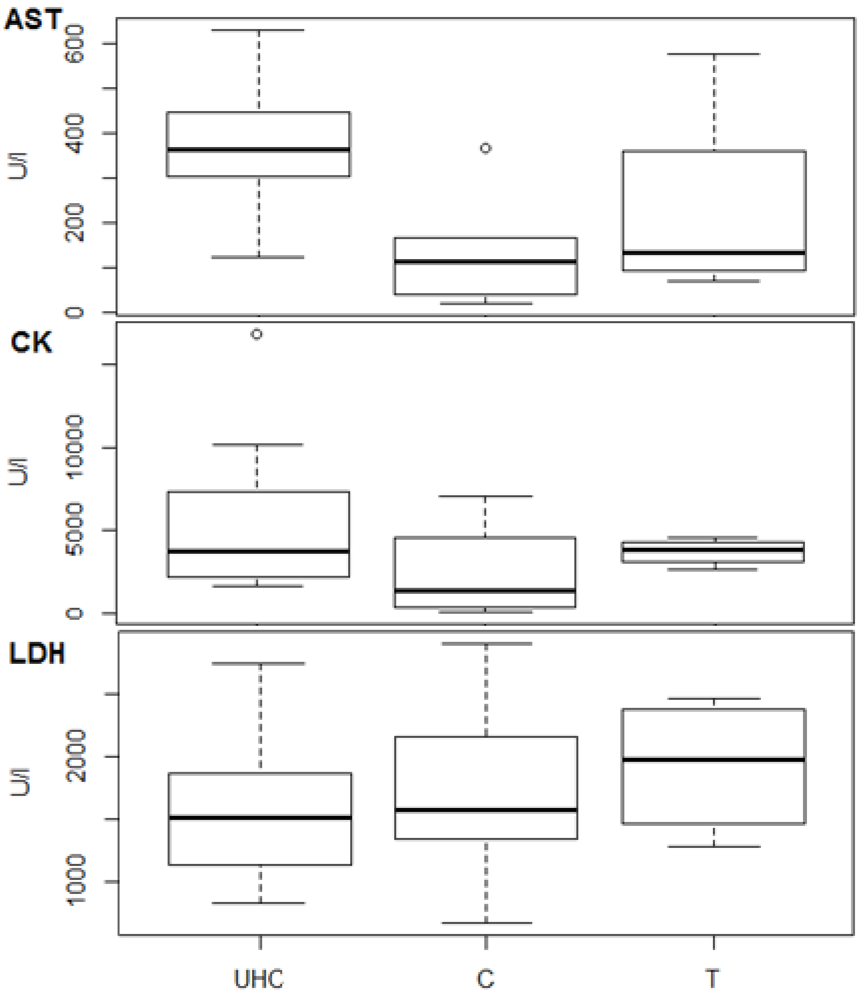


Figure 3.11. AST, CK and LDH activities in the experimental groups, measured in activity units per litre. UHC: UNHANDLED control fish, sampled at the hatchery prior to the trials, C: CONTROL fish released downstream of the turbine and recaptured, T: treatment fish released into the turbine intake and recaptured in the outflow basin having passed through the turbine.

Lag and average temperature were significant in the regression for AST (Table 3.8, Figure 3.12 and Figure 3.13). Removing either temperature or lag from the model reduced the significance of the remaining term to $p > 0.05$, resulting in further selection of an intercept only model. In the final regression model for CK, both treatment and lag were significant (Table 3.8 and Figure 3.14). TREATMENT CK was 4.58 times higher than CONTROL. The initial aggregation of TREATMENT recaptures had elevated levels of CK relative to CONTROL recaptures (Figure 3.14). The FAST group LDH activities were on average 1.4 times higher than for the slow group ($p<0.01$). Average temperature was temporally correlated to speed category (Pearson's correlation coefficient=0.88, $p<0.001$). Repeating model selection without speed category resulted in a final model with only average temperature retained (GLM, estimate = 1576.7, se = 404.8 $p<0.01$).

Table 3.8. Results of generalised linear model regressions for the blood chemistry analytes AST, CK and LDH. N(C): total (and CONTROL) sample size.

Analyte	AST			CK			LDH		
method	log GLM			log GLM			GLM		
N (C)	14 (10)			14 (10)			15 (11)		
	Est	se	p	Est	se	p	Est	se	p
intercept	-11.24	7.088	-	6.474	0.406	-	1428.1	133.0	
TREATMENT	-	-	-	1.521	0.659	0.04	-	-	-
SLOW	-	-	-	-	-	-	996.2	257.6	0.002
lag	1.348	0.530	0.027	1.761	0.646	0.02	-	-	-
Tave	1.885	0.847	0.048	-	-	-	-	-	-

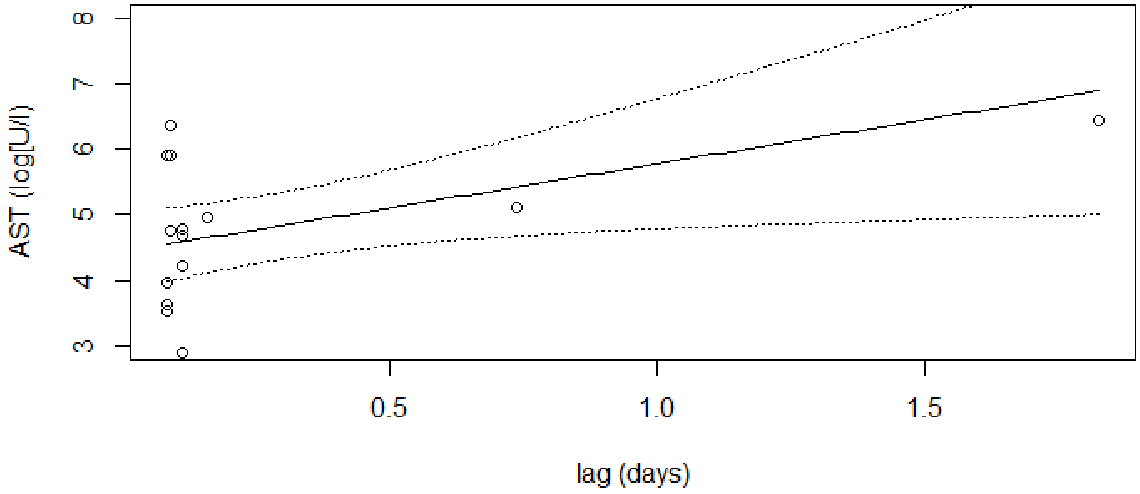


Figure 3.12. Data (points) fitted regression line (solid line) and 95% confidence intervals (dotted lines) for the significant effect of lag on log(AST), with temperature set at the average value.

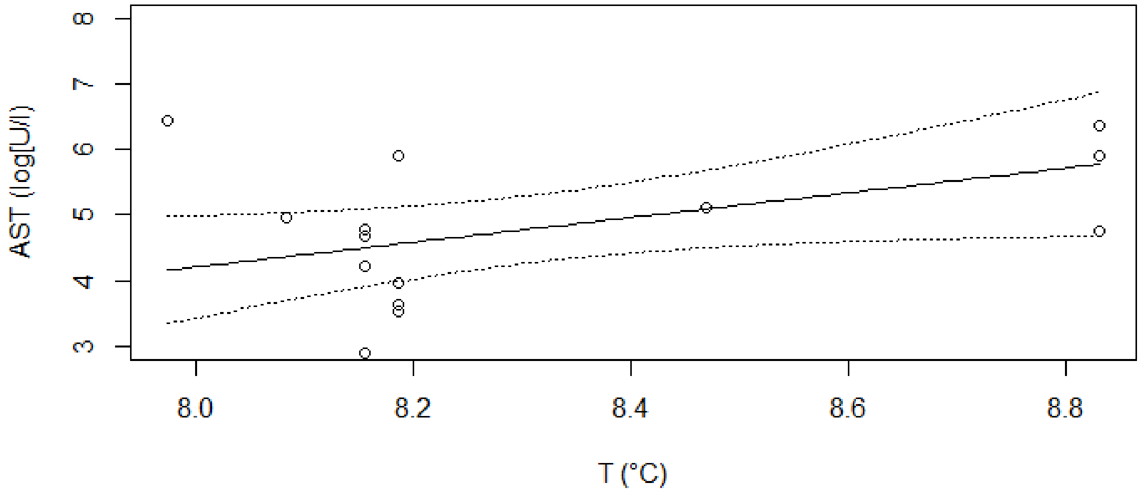


Figure 3.13. Data (points) fitted regression line (solid line) and 95% confidence intervals (dotted lines) for the significant effect of average temperature on log(AST), with lag set at the average value.

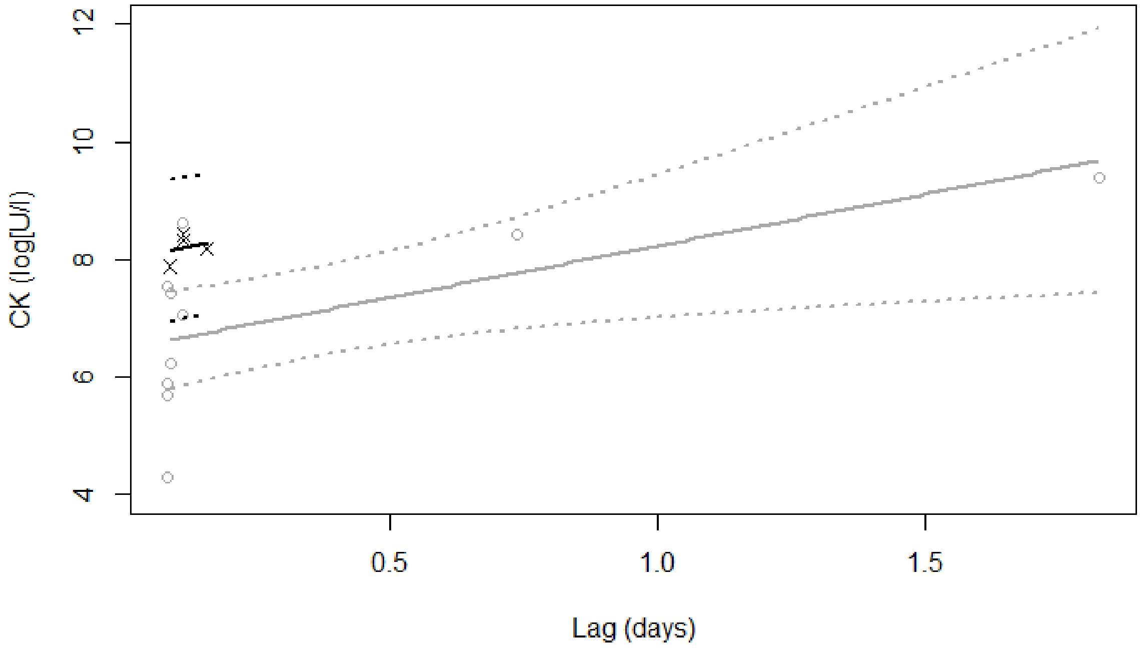


Figure 3.14. The significant relation of lag and treatment with CK activity. Fitted regression line (solid lines) and 95% confidence intervals (dotted lines) are given for the CONTROL (grey lines and grey points), and TREATMENT (black lines and black crosses) groups.

3.3.6.2 ELISA for enolase

Average intra-assay %CV was 12.46% (n=96). This is the average percentage coefficient of variance of all the pairs of wells with duplicate samples, for all assay plates. Inter assay %CV was 12.25% for the low standard and 61.65% for the high standard (N=7). This is the percentage coefficient of variance across plates, of the mean values of each of the duplicate pairs of standards on each plate. Examining the high standard values for each plate revealed an average value for one plate of 19.50 AU, which was much higher than the overall average value (all plates combined) of 8.15. Without this plate the intra-assay %CV for the high standard reduced to 13.7%. The plate was retained in the statistical analysis, with the high inter-assay %CV accounted for by including plate as a random effect in the GLMMs.

The high inter-assay %CVs may have been due to degradation through storage of the high standard used, which had been stored for approximately three years. In order to establish reliability of these results, a subsample (n=96) was re-analysed for comparison, using one of the collected samples (sample 164T) as the high standard. This time the inter-assay %CV for the high standard was 1.378%. The average intra-assay %CV was 19.50%. Limit of detection was determined as 5.826 AU.

Overall, TREATMENT enolase levels were marginally non-significantly higher than CONTROL (Mann-Whitney U-test, w=1539, p = 0.053), and there was no difference between TREATMENT, CONTROL and UNHANDLED groups (Kruskal-Wallis test, $H(2)$=5.88, p > 0.05) (Figure 3.15). The two logistic regressions of enolase detection above the minimum detected value (1.58 AU) and the calculated limit of detection (5.826 AU) resulted in no significant terms. The linear regression on non-zero data resulted in significant terms for average temperature, condition factor and method of capture (Table 3.9, Figure 3.16 [condition factor and method of capture] and Figure 3.17 [temperature and method of capture]). Of the fish used in this regression, only two were found in the recapture box, an additional two were caught by crowding into the box, and the rest (n=30) were corralled using a seine net. The two fish that were captured by crowding had enolase levels 1.23 times higher than those corralled using the seine net and exceeding the 95% confidence interval for the seine-net. The two captured in the box were on average 14.15 times higher than the seine-net fish, one had the highest enolase level, but the other was within 2 *sd* of the regression line for seine-netted fish.

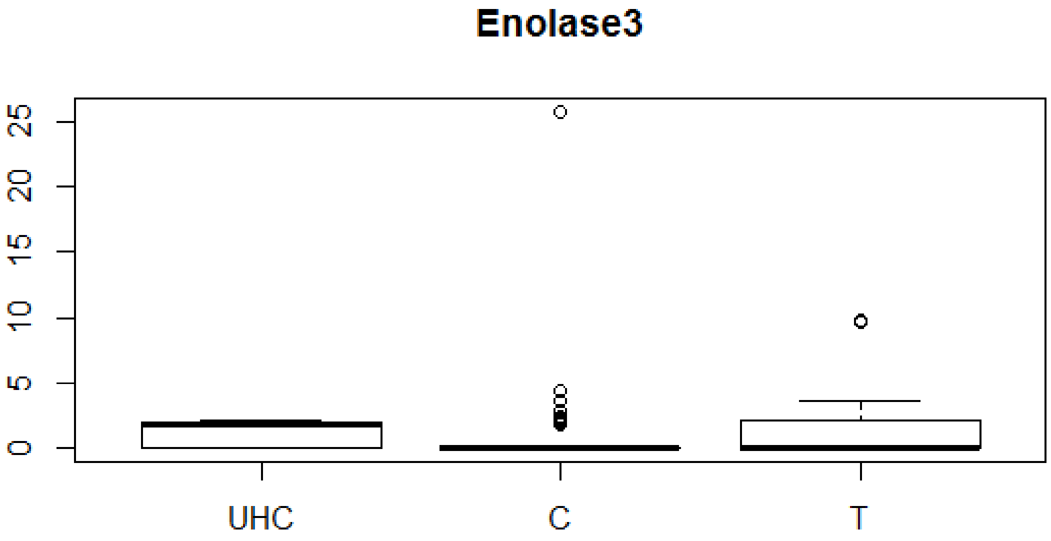


Figure 3.15. Enolase activities in the experimental groups. UHC: UNHANDLED control fish, C: CONTROL fish released downstream of the turbine and recaptured, T: treatment fish released into the turbine intake and recaptured in the outflow basin having passed through the turbine.

Detailed visual comparison of the photos of these fish from before and after the trials showed no new scale loss, although there were distinctive marks present on the fish before the trials.

3.3.7 Correlation between scale loss and blood chemistry responses

There was no correlation between AST, CK or LDH activities and the scale loss metrics (average score and change in average score, or maximum score of either side and change in maximum score), as tested by addition of these pairs of metrics to the final GLM for each analyte ($p > 0.05$, all tests).

Table 3.9. Results of mixed effects models for enolase. Presented are the sample size (N) estimated effect (estimate) and standard error (se) for each covariate retained in the models after stepwise backwards deletion of terms not significant in likelihood ratio test comparisons between models with and without each term. Three regressions were carried out: logistic regressions with the data dichotomised around two chosen thresholds, and a linear regression with the response log-transformed. Fulton's condition factor (100 x fish mass (g)/ [fork length (mm)^3]) is scaled.

Type	Logistic regression		Ordinary regression		Logistic regression	
Threshold for dichotomising data	1.58		-		5.83	
Response transformation	logit		log		logit	
N	79		30		79	
	estimate	se	estimate	se	estimate	se
Fixed effects						
Constant	-0.85	1.81	-3.82	0.93	0.04	0.02
Average temperature	-	-	0.82	0.10	-	-
Condition factor(scaled)	-	-	-0.13	0.03	-	-
Method of capture: crowded	-	-	-2.44	0.28	-	-
Method of capture: seine	-	-	-2.65	0.21	-	-
Random effects						
plate	-	4.52	-	0.61	-	0.03
Batch	-	-	-	1.01	-	0.19

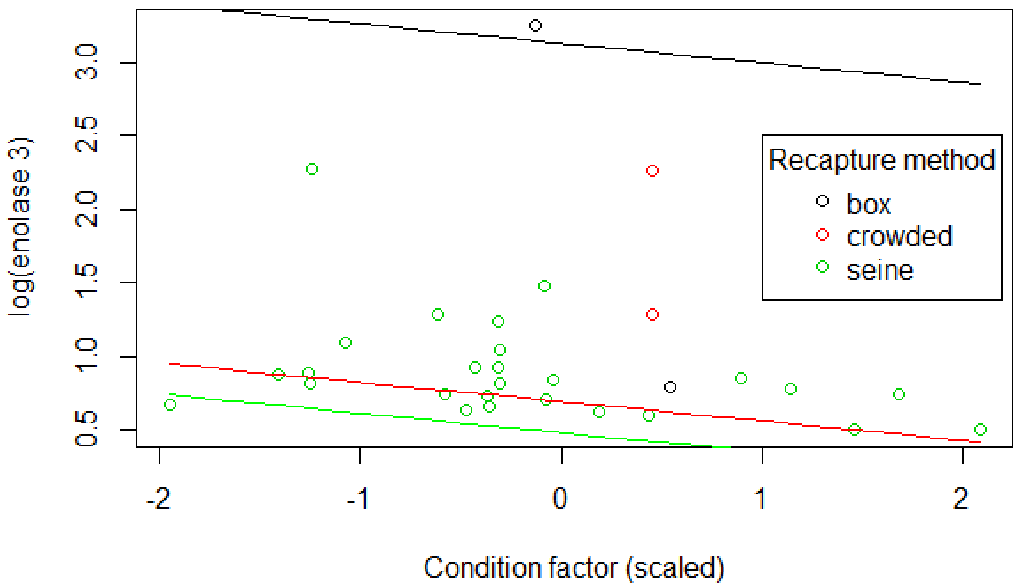


Figure 3.16. Data and fitted regression lines for the significant effect of condition factor on log-transformed enolase. Fulton's condition factor (100 x fish mass (g)/ [fork length (cm)^3) is scaled. Regression lines use the mean value for temperature.

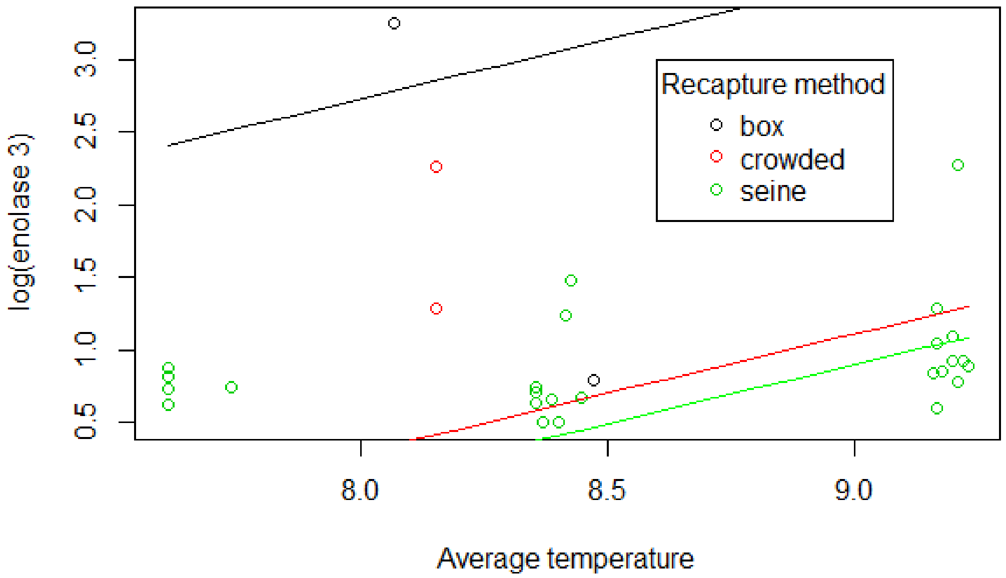


Figure 3.17. Data and fitted regression lines for the significant effect of average temperature on log-transformed enolase activity (AU). Regression lines use the mean value for condition factor.

3.4 Discussion

In this study, no severe injuries such as haemorrhage or haematoma were observed, thus the focus of analysis has been on unbiased testing for changes to scale coverage, and investigation of subtle, invisible damage using blood chemistry correlates.

3.4.1 Change to visible condition resulting from the passage trials

No external injuries other than missing scales, reduced fin area and fin splits were observed in experimental fish that were either passed through the turbine, or released and recaptured below it as a control. Incidences of change to fin condition were negligible and not significant between TREATMENT and CONTROL groups. The comparisons of scale loss score-change between TREATMENT and CONTROL groups revealed no significant difference in the proportion of fish with increased scale-loss associated with turbine passage, when several levels of severity of new scale loss (as defined in the methods, section 3.2.7) were considered. The data from this study provide an estimate of 1.49% prevalence of 5-9% new scale-loss, and a further 0.97% with 10-30% scale-loss, beyond the estimated prevalence of new scale-loss to CONTROL fish.

The lack of visible injury or significant prevalence of new scale loss is in general agreement with available evidence supporting the perspective of low risk from ASHTs to fish. Kibel (2007) captured wild Atlantic salmon smolts that had naturally migrated through an ASHT, and reported that 4.4% of 249 smolts suffered scale loss of less than 10%, and 3% of those were deemed to have been damaged by the recapture net. In the initial part of their study they did identify one severe (likely to cause mortality) injury to a smolt, which they associated with a hazardous pinch point between the trough and the rounded end the turbine blade (pictured in their report), and this led to the recommendation that this be modified. Small details such as this highlight the need for careful evaluation of these turbines to prevent oversights from causing significant damage to migrating fish. Bracken & Lucas (2013) carried out a release-recapture study of *Lampetra* sp. ammocoetes, river lamprey transformers and an adult brook lamprey, and found swimming impairment in one transformer (1.5%

of all recaptured lampreys), which they attributed to turbine passage. Spah (2001) found that 4.4% of fish suffered limited scale loss, likely caused by contact with the unprotected leading edge of the blades. Merckx and Vriese (2007) found no damage to a range of fish species. One study has found greater rates of damage to a variety of fish species (Schmalz, 2010). In that study the edges of the blades were found to have been damaged, potentially by gravel, which could have increased the rate of damaging impacts on fish.

In this study, frequency of change in scale-loss was not significantly affected by turbine speed, but for condition α at the SLOW speed the estimates for TREATMENT and CONTROL were notably lower, and the difference between these estimates was greater (Figure 3.11). A possible explanation is that low severity scale-loss from contact with the recapture system was more prevalent at the higher water velocities during the FAST trials, when velocity in the outflow exceeded 1 ms^{-1} in the centre of the outflow basin, as compared to approximately 0.5 ms^{-1} at the SLOW speed. Based on average fork length (182 mm), sustained swimming speeds for these fish is expected to be around 0.8 ms^{-1} (Booth, 1998) and it is conceivable that fish entering the outflow basin would be more likely to come into contact with - and sustain damage from - the river bed and recapture system at the FAST speed. Whilst this is a possible explanation, it should be noted that the influence of the recapture system was minimized in the design by placing the barrier fence as far as possible from the flow exiting the turbine. Score-change conditions α and γ were correlated with the lag between release and recapture, which suggests a damage effect from the recapture structure which is dependent on exposure time. Again these results are supported by existing reports: in their field experiments, Kibel *et al.* (2007) found no differences in incidence of scale loss between three turbine speeds (20-23, 25-26 and 29-31 RPM), when using hatchery-origin brown trout. They did, however, find that naturally migrating Atlantic salmon smolts sustained greater levels of scale loss from the recapture net when it was checked and emptied hourly than at 15 minute intervals, and this was surmised to be because of increased contact with the net.

Score-change condition α was related to the initial scale-loss score (averaged over the sides of the fish), with the odds of a score-change decreasing by a factor of 0.07 for each successive scoring category (i.e. 1-4, as described in Section 3.2.6.3). This may be attributable to the greater likelihood of identifying more subtle changes to scale coverage at the lower scoring categories, which had narrower ranges of scale loss area.

The simulation-based power analysis (Figure 3.10) demonstrates that reliable detection of a treatment effect requires a prevalence of 20% greater in the treatment group than the CONTROL group, given the expected CONTROL prevalence of approximately 5%. Thus although the data do not support the view that turbine passage results in scale loss, it does not rule out the possibility of turbine-induced scale-loss at a low prevalence. Injured fish with extensive scale-loss may have a reduced osmoregulatory ability (Zydlewski *et al.*, 2010) and decreased performance and survival during smolt migration. Viewing the estimate provided by the data as a worst-case, 1% of the smolts in this study may have suffered over 20% scale loss associated with turbine passage. These would be within the range of maximum tolerated descaling for Atlantic salmon smolts of 20-30% (Kostecki *et al.*, 1987). The converse view is that this 1% may have had as little as 10% scale loss (the scoring category covered 10-30% new scale loss), and it may not have been caused by the turbine, but by other elements of the trials.

Although the results discussed so far demonstrate no significant detectable proportion of fish affected by new scale loss, and this is consistent with previous studies, several authors have discussed the potential for subtle turbine-induced effects to lead to indirect (Travade *et al.*, 2010; Cada 2003; Colotelo *et al.*, 2009; Hasler *et al.*, 2009; Schweizer *et al.*, 2012) or delayed (Cooke *et al.*, 2011; Budy *et al.*, 2002; Thorstad *et al.*, 2012) mortality. Indeed delayed mortality has been demonstrated for large-scale hydropower systems (Ferguson *et al.*, 2006). There may also be an accumulation of such effects on the onward performance and survival of Atlantic salmon smolts. In order to attempt to address these concerns in this study, a novel approach using blood chemistry markers for tissue damage was used, and the outcomes of those efforts are discussed below.

3.4.2 Serum chemistry

The measurement of intracellular enzymes in the serum for identifying subtle trauma to fish from hydropower turbine passage has been suggested (Hasler et al., 2009) but no prior application of this method in this context have been found. However the methodology is supported by examples in the literature which report positive signals associated with angling stress (Butcher et al., 2011; 1999; Killen et al., 2003; Morrissey et al., 2005; Rapp et al., 2012; Wells et al., 1986), pollution induced tissue damage (Escher et al., 1999) and harvesting methods in aquaculture (Dobšíková *et al.*, 2006; Dobšíková & Svobodova, 2009; Grizzle *et al.*, 1992; Grizzle & Lovshin, 1994,1996). Enolase in particular has never before been used as an indicator of physical trauma. Hence there is a paucity of comparable studies for contextual comparison of these results. Even for routine diagnostic purposes in aquaculture, pre-analytical and statistical methods have not yet been standardized (Braceland *et al.*, 2016). The serum chemistry results are discussed summarily below, followed by a more in depth exploration in the following paragraphs, structured by statistical method. Whilst it is recognized that the power of these results is limited by low sample numbers, a detailed interpretation is presented in the interests of perhaps informing future direction in the use of these methods.

In summary, CK activities were significantly higher for the TREATMENT group than for the CONTROL group, but this effect was not seen for any of the other analytes, and it is uncertain whether this trend is actually due to a differential effect of the treatment on CK activities, or whether it is attributable to chance variation between individuals. Both AST and CK had significant positive trends with release to recapture lag, and AST was also correlated with average temperature. It is expected that any effect of physical trauma on blood chemistry would vary over time as proteins from damaged tissue distribute into the vascular system, and are eventually re-assimilated. These processes could well be temperature dependent, and the result for AST supports that view. Handling effects on blood chemistry have been shown to be both time and temperature dependent. Grizzle *et al.* 1992 found that AST activities in channel catfish (*Ictalurus punctatus* Rafinesque) exposed to different harvesting methods began to show effects at 2 hours after harvesting, but that at cooler temperatures (10.5^{O}C - 17.5^{O}C), the peak occurred around a day, with levels declining more slowly (significant effects extending to four days) than at warmer temperatures (27 ^{O}C - 28 ^{O}C) where AST peaked at 3 hours and generally declined by two days. Yousaf and Powell 2012 point out that CK and LDH responses peak at 10-12 days in humans. There are other possible explanations such as differential catchability or propensity for damage with lag, which is itself also related to average temperature, since temperatures fluctuate daily, and average temperature stabilizes with increasing lag. The relation of LDH to speed category is not seen in the other analytes, and it is proposed that this effect is potentially due to the effects of temperature and lag, which were distinctly different between the trials for the two speed categories. Here speed category was the only significant predictor, with no effect of treatment, which indicates that this is not a turbine effect. The regressions for enolase show effects for method of capture, average temperature and condition factor. The sparsity of samples for box-captured and crowded fish, and lack of change to visual condition render the first effect inconclusive. The effects of condition factor and temperature, although significant statistically, are too slight to be considered biologically significant (Figures 3.16 and 3.17).

Non-parametric tests

Non-parametric statistics showed no significant differences between TREATMENT and CONTROL groups for any of the blood analytes, but for AST there was a difference with the UNHANDLED group. This may reflect a temporal trend, or an effect of transport, handling or these factors acting in combination, but differentially on the UNHANDLED group, which was sampled at the hatchery before the trials. Interestingly, it is this group that had the higher AST levels. Enolase activities in the TREATMENT group were marginally but not significantly greater than the CONTROL group, but the UNHANDLED levels were the highest, hence there is no strong evidence for a consistent treatment effect on enolase.

Regression models

The four analyte responses were each modelled against both the experimental treatment manipulations and a number of covariates which were outwith experimental control. The only result of direct relevance to a turbine passage effect was the significant correlation of CK with treatment.

In the final regression model for CK, both treatment and lag were significant. Although there were only two fish sampled after the initial aggregation of lags, the correlation suggests increasing CK levels with lag (Figure 3.14), and it is this correlation which leads to significantly lower response in the CONTROL group where none was seen for the non-parametric test. Intriguing as it is, this trend is based on very few data (Figure 3.14). It may be that there is a time-dependent response as enzymes from damaged tissue distribute into the circulatory system, although lag was not important for LDH or enolase. There are alternative explanations: fish recaptured after a longer lag may have been more likely to be damaged by the recapture system, or fish with elevated CK activities may have evaded capture for longer because of altered behaviour. CK is associated with skeletal (and also cardiac) muscle (Yousaf & Powell, 2012), where the effects of blade strike might be expected, but this effect is not corroborated by the enolase response, which has been shown to have high specificity for white muscle (Braceland *et al.*, 2014), and which used a much higher sample size. It may be that the chronic response of enolase to disease induced tissue lysis which Braceland *et al.* (2014) demonstrated does not translate to the circumstance of acute physical or physiological trauma.

The final model for AST revealed a positive influence of both lag and average temperature on AST, and this could reflect a time-dependent, temperature mediated response after the pre-trial handling. The explanations offered for the effect of lag on CK above also apply here. However it should be remembered that at the sub-day timescale of the majority of recorded lags (mean=0.271 days, *sd*=0.477), average temperature is related to lag within the same release batch – that is, average temperature between release and recapture is defined by this time period, and becomes more stable as lag increases beyond diel oscillations. Removing either temperature or lag from the model reduced the significance of the remaining term to $p>0.05$, resulting in further selection of an intercept only model, thus there is dependence between these two terms, and it is not possible to separate their effects.

LDH activity was significantly related to speed category but not to experimental treatment group, nor any of the other covariates. One possible explanation for this is that speed category was correlated with average temperature, and although this was not found to be significant during model selection, it may have contributed to the overall effect of turbine speed. The fish sampled at the SLOW speed experienced higher average temperatures between release and recapture, as well has having greater lags, so the sampling period may have captured LDH levels at a higher point during a hypothesized response. Removing the speed category and repeating model selection resulted in a final model with only temperature being significant, which helps to validate this argument. Therefore it must be concluded that the effects of speed category and temperature cannot be separated for these samples. There may also have been differential swimming behaviour at the slower speed, possibly affecting the rate of distribution of this enzyme after any tissue damage resulting from handling or the trials. The opposite direction of effect on scale-loss score-change with speed category indicates that the LDH response is either not caused by the same stimulus as caused the scale loss (but rather natural variability or some unknown stimulus), or there is some mechanism causing the effects to be in opposite directions.

For enolase, several effects were found to be important for the regression on the non-zero data. Method of capture was a significant predictor, with those fish captured naturally from the box associated with elevated enolase levels. It may be that these fish were more likely to drift into and remain in the recapture box, or were less able to evade crowding, but the numbers are too low for this to be anything other than conjecture (it should be noted that the samples for AST, CK and LDH all originated from fish which naturally entered or were crowded into the box, and thus this result does not contradict the offered explanations for the lag effect on those analytes). The enolase activity estimates for crowded and seine-coralled fish at average temperature and condition factor are below the limit of detection. There does appear to be a positive effect of average temperature on enolase levels and variability, and this may reflect a temperature dependent activity or response. In any case, the effect, although significant is very slight - the predicted increase in enolase levels for the seine-coralled fish remaining below the limit of detection for the assay for the range of average temperatures encountered in the study period. The negative effect of condition factor on enolase levels, although significant, is also very slight, with predicted values below the limit of detection.

The simulation based power analyses (Appendix A3.6) show the large required prevalence and effect sizes needed for reliable detection of an effect on the blood analytes, given the sample sizes attained and inter-individual variability of the analyte activities. The positive effect of treatment on CK reflects an apparently large effect size, however this result should be applied cautiously and further corroboration with larger sample sizes is recommended. While larger samples were available here, they were analysed much later (June 2015) than those on 15 April 2014, and by a different laboratory, and this generated divergent results, with those from June 2015 regarded as being most inconsistent, precluding combination (Appendix A3.4).

3.4.3 Potential mechanisms for damage

The expected mechanisms that could result in new scale loss due to turbine passage are strike by the leading edge, abrasion, or grinding between stationary and moving turbine parts (Potter *et al.*, 2012). Additionally, scale loss can result from abrasion against the holding environment and during capture and handling. Changes to the serum analytes, where not corroborated by visible damage, would likely be caused by impact from the leading edge or deformation by squeezing or bending between stationary and moving parts. The results are discussed in relation to these risks below.

Grinding

In trials using euthanized Atlantic salmon smolts (Appendix A3.1), distinctive patterns of scale loss - consistent with grinding between the outer edge of the turbine blade and the turbine housing trough - were observed in a proportion of the TREATMENT fish only. The lack of any similar severe symptoms in live fish goes some way to providing evidence that they are not exposed to this injury mechanism, or that if they are, the effects are neither severe nor easily identified.

Strike

The theoretical strike probability for a passive smolt of mean length for this study, aligned perpendicular to the flow, but otherwise exhibiting no behavioural responsiveness, in this system is 0.26 (Section 3.3.3). The identical strike probability for both speeds used is a direct result of the assumption that water velocity has a fixed relation to turbine rotational speed. However this probability does not account for any behavioural response or the varying likelihood of a detectable effect of strike which might be expected at varying turbine blade velocities and position along the fish. If fish have a different velocity to the water at the entrance to the turbine, strike rate will be altered (Vowles *et al.*, 2014b). Moreover, if fish are able to react at the timescale of individual blade sweeps this will further influence strike rate.

If the observed but statistically insignificant 1.46% overall prevalence of new scale loss to TREATMENT fish was caused by strike, this is substantially lower than the theoretical strike rate (26%). It is ordinarily expected that observed strike rates should be lower than theoretical, due to the expectation that a portion of strikes – if they occur - will not result in observable or severe effect (Turnpenny, 2000). Although underwater cameras were installed at the entrance to the turbine to observe the behaviour of fish approaching the turbine, no instances of contact between fish and the leading edge of the turbine were observed, either due to this not happening, or reduced clarity of images under low light conditions in the intake basin. Kibel (2007) did observe 4% of Atlantic salmon smolts being touched by the leading edge using this method, and Kibel (2011) derived a correction relationship for strike probability, based on a linear regression between predicted probability and the observe incidences of contact at different speeds (Equation 3.5). Applying this correction to the theoretical strike rate results in a value of 3.27% for the mean fish length (182 mm) used in these trials. Whilst this relationship cannot be checked under the present study, it does correspond more closely to the estimate of prevalence of new scale loss.

$$p_{actual} = -0.0346 + 0.2592 p_{predicted} \qquad 0 \leq p \leq 1$$

Equation 3.5

where

p_{actual} = corrected strike probability, and

$p_{predicted}$ = uncorrected strike probability.

Since average velocity is assumed to be fixed by RPM, the probability of strike is also fixed for a given fish length. Under this assumption, any speed-related effect of turbine passage must be caused by an increased severity of strike improving the detectability of response, or from another source not accounted for by the strike model, for example fish behaviour or other mechanisms of damage. The scale-loss results indicate a greater change in minor scale loss (condition α) between TREATMENT and CONTROL smolts at the SLOW speed.

It might be expected that strike by the turbine blade leading edge would induce distinct marking injuries such as linear haematoma and scale loss. By this argument, the irregular scale loss patterns observed could rather be a result of abrasion within the turbine (that is, if they arose from turbine passage). If blade velocity is too low to cause such visible injury, the blood chemistry responses may be a more reliable indicator of strike. The elevated CK levels seen for the turbine-passed TREATMENT fish could have been a result of this. However, all of the elevated samples were from the SLOW trials. The leading edge of the turbine would have a maximum tangential velocity at 8RPM of 1.21 ms^{-1}, or, assuming the worst case, that the fish were impacted at the maximum velocity attained during the trials of 14 RPM, 2.13 ms^{-1} (circumference x rotational speed = π x 2.9 m x 14 RPM / 60 s). Such low speed impacts with a rounded rubber edge seem unlikely to illicit a cell rupture response, but this is an area for further investigation. Potentially it is not the impact, but the deformation of a fish from a sideways push which may be damaging. The impact pressure experienced by the fish would be a function of the blade velocity relative to the fish, and the thickness of the blade (Amaral *et al.*, 2015), but also of the fish's inertia and frictional resistance from the water surrounding the fish to sideways acceleration. The resulting deformation would be greater than in a less resistive medium (air for example). Recommended maximum blade velocities in the literature are 4 ms^{-1} for Francis turbines (Monten, 1985), 7 ms^{-1} for Kaplan turbines (Turnpenny, 2000), and 9 ms^{-1} for ASHTs (Kibel, 2009) with a hard rubber edge protector, this last value for fish up to 1 kg in weight.

Several recent laboratory and field studies have examined potential strike by novel hydrokinetic turbines to fish. These investigations have been stimulated by a growing interest in such turbines for use in river and tidal currents. Both axial flow turbines (NAI 2009; Hammar *et al.*, 2013; Amaral *et al.*, 2015) and cross flow turbines (EPRI 2011a; Castro-Santos & Haro, 2013; Viehman *et al.*, 2015) have been investigated, to assess approach and passage behaviour, and quantify mortality and injury probabilities. For axial flow designs, which are comparable to the leading end of an ASHT, total survival probabilities (given entrainment) have been reported as ranging from 0.91 to 1 for a range of sizes and species of fish (Amaral *et al.*, 2015). The relevant turbine characteristics for strike were comparable to those for ASHTs: numbers of blades ranged from three to seven; blade leading edge thickness was 15.2 mm and 127 mm where reported, diameters were 1.5-3.7 m and blade tip strike speeds were 1.9-7 ms^{-1} (Amaral *et al.*, 2015). The only tests with salmonid smolts of any species have been with Atlantic salmon smolts, using a vertical axis cross-flow turbine (Castro-Santos & Haro, 2013) in a flume. Survival probability was 0.98, but was not significantly different to CONTROL fish not exposed to the turbine.

3.4.4 Avoidance behaviour

The extended periods of avoidance behaviour by introduced hatchery Atlantic salmon smolts were not seen for the bulk of naturally passing wild PIT tagged Atlantic salmon and sea trout smolts (Chapter 2), which mostly passed the length of the turbine channel at night, in under 30 minutes. However a few of the wild smolts in Chapter 2 did stay in the channel for longer periods, and this was associated with daytime presence. Additionally, wild smolts were seen holding station in front of the trash rack during the day.

Introducing the hatchery-origin smolts into the alien environment of the turbine intake from holding containers may have induced refuge seeking behaviour which was seen to a lesser degree in naturally moving wild smolts, that is, differences in rearing environment and recent experience may have caused differential behaviours. Visual cues appear to play a role in the avoidance of other behavioural obstacles, such as accelerating flow (Vowles *et al.*, 2014a). Thus introduced smolts may have been stimulated to avoid turbine entry until visual orientation was lost at the onset of darkness, increasing the likelihood of drifting into the turbine. Another explanation is that smolts have a natural tendency to hold station in the day, and move downstream in the hours of darkness (Thorpe & Morgan, 1978, Riley, 2012; 2014). In any case, with regard to the tendency for nocturnal turbine passage, the hatchery fish behaved equivalently to wild migrating smolts.

Several studies have incorporated the evaluation of behaviour of fish proximal to novel turbine types (EPRI 2011b; Castro Santos & Haro, 2013; Hammar *et al.*, 2013; Vowles *et al.*, 2014b, Amaral *et al.*, 2015; Viehman *et al.,* 2015). These have identified avoidance and evasion behaviours for a range of fish taxa that were related to turbine operational parameters and environmental conditions. In general for these studies, there has been ubiquitous and efficient avoidance of entrainment, but this varied among species. Water velocity on approach and Illumination (light or dark) appear to be the primary influencing factors affecting degree of avoidance. The only study which tested Atlantic salmon smolts (Castro Santos & Haro, 2013) observed a non-significant, slightly slower ground speed of smolts approaching a vertical axis turbine in an artificially illuminated flume compared to a control condition with no turbine in place. In this case, the proportion of fish passing through the blade swept area was significantly higher than the area to either side of the turbine, but this was attributed to avoidance of the flume walls. It would appear, from daytime introductions of Atlantic salmon smolts in the present study, that the ASHT presented a greater behavioural barrier under lit conditions than the vertical axis turbine used in that study. Further investigation of lighting conditions and form of visual cues may explain such contrasting behaviour.

Fish reactions to turbines and associated stimuli can be important in determining injury rates (Vowles *et al.*, 2014b) and in influencing connectivity for fish moving at novel turbines. The characterisation and relation of these behaviours to turbine design and operating parameters is likely to be a productive means of informing turbine deployments which are sympathetic to fish moving past them.

3.4.5 Critique of methods

In this study a pragmatic approach was taken to identify potential hazards to smolts from turbine passage, and quantify the severity and prevalence of the resulting effects. The difficulty in obtaining large numbers of wild Atlantic salmon smolts meant that the study was restricted in the main to hatchery-origin Atlantic salmon smolts (though some observations on potential turbine impacts on wild fish recorded incidentally in this study are given in Appendix A3.2). The study design was restricted to a single turbine, although two turbine speeds were used.

Considerable difficulties were encountered in controlling the lag between treatment exposure and sampling, due to practical constraints at the site and limited resources. Ideally, the fish would have passed through the turbine immediately after release. These, and the control releases would be recaptured and sampled at a pre-determined and consistent lag after exposure. This may be conceivably achieved by engineering bespoke introduction and recapture systems. These would need to ensure passage into the turbine after release, and the recovery of fish from the entire downstream area. For example, the presence of refuge areas above the turbine could be removed by contouring the intake basin as a duct with the diameter of the turbine. Below the turbine, a recapture basin lined with a lifting cage could be employed. This was not achievable in the present study due to time and resource constraints. Potentially confounding factors have been included in the data analysis as far as is possible.

The interpretation and wider application of the outcomes should be made with reference to these restrictions. The outcomes are limited by the resolution of the responses measured and tested, the numbers of fish used, and also prior knowledge of expected response in the case of the blood chemistry parameters. Observations were restricted to external signs of trauma, or elevated blood chemistry markers for damage resulting from contact with turbine infrastructure, with the expected mechanisms of strike by the leading edge of the turbine, or abrasion or grinding between stationary and moving turbine parts.

3.4.5.1 Scale-loss methods

The categorization of scale loss and definition of score-change conditions for statistical testing carry an inherent cost in terms of sensitivity and resolution. However, given the quality of images used and the difficulty of more sensitive methods (see below), it is felt that this approach is justified in this case. Alternatives include the detailed measurement of scale loss on fish retained frozen (Kostecki *et al.*, 1987), but this would require the same detailed information on scale-coverage prior to the trials. This was not feasible given the improbability of acquiring completely unmarked smolts from hatchery sources. Otherwise this method would be limited to the bulk comparison of scale coverage between treatment and control groups after exposure without prior assessment. This may be a useful approach with large sample sizes, but is costly. Another approach would be the development of standardized photographic and image analysis methodologies, as have been used in other fields (for example, the quantification of retinal area (Croft *et al.*, 2014). Further testing and validation would be required before this approach would yield high-resolution results. There is also a lack of information on the consequences of mild to moderate scale-loss on the onward performance and survival of smolts.

3.4.5.2 Blood chemistry methods

Two key pieces of information, which are missing for a full understanding of the blood chemistry outcomes of this study are:

- the actual effect (under laboratory conditions) on blood chemistry responses of exposure to the types and severity of damage stimulus expected, given the population and environmental variability encountered; and
- the onward consequences for the fish, given any observed deviations in blood chemistry levels after turbine passage.

The first issue may in principal be resolved by controlled experiments on individual fish, but the scope for this is limited by ethical and licensing constraints. The second issue could also be investigated in the same way with extended holding and observation under laboratory conditions. A priority would be a validation of the serum chemistry responses to physical trauma of both the visually obvious and invisible type. Secondly, the medium- to long-term consequences of such trauma, including mild to moderate scale loss, could be assessed in order to establish the biological significance of these responses.

Gadomski *et al.* (1994) experimentally tested the effects of descaling on physiological stress responses and predation using yearling Chinook salmon. They found that 10% descaling resulted in a significant stress response, but there was no difference in predation by northern pikeminnow (*Ptychocheilus oregonensus* (Richardson)) between control and descaled groups. Kibel (2007) kept juvenile brown trout that had passed through an ASHT, as well as a control group that had not, for 48 hours to assess symptoms of longer term damage such as listing, poor swimming, remaining on the bottom of the holding tank, or infection. They reported that there were no fish "*displaying any behaviour such as listing to one side, lethargy or remaining motionless on the bottom for long periods that might indicate a delayed effect*".

The relevance of such trials to wild migrating Atlantic smolts is complicated by a host of environmental factors which could affect the individual outcome. A possible approach to assessing this at a population level in the wild would be to use paired releases of smolts, down the turbine and alternative route (Skalski, 2002; 2010), with subsequent tracking of survival down-river, through estuary emergence to sea migration and return to freshwater for spawning. The initial part of this approach of monitoring onward in-river migration was incorporated into the study described in Chapter 2. Extensions to this tracking may be accomplished with acoustic tags and receivers placed along the migration route out to sea (as for example Hubley *et al.*, 2008; Dempson *et al.*, 2011; Gauld *et al.*, 2013), and current efforts to establish post smolt migratory behaviour for smolts exiting the River Conon, Scotland (M. Newton, 2016, pers. comm)). In order to assess possible turbine effects on the rate of fish ultimately returning to spawn, the only suitable technology at present would be passive integrated transponder tags, for their unlimited longevity and unique identification *in situ*. This approach depends on the existence of suitable locations in the river for PIT antennas to detect all returning tagged fish, and requires very large sample sizes to detect any potential effect, given the low rate of return of adult Atlantic salmon.

The simulation-based power analyses demonstrate the difficulty of detecting low prevalence, or low effect-size effects under the assumed model. Thus whilst the results of this study provide evidence of no high prevalence or extremely severe effects, there remains the possibility of more subtle effects to a small proportion of fish. In order to address this issue with the described methods, sample sizes should be increased to attain the desired power to detect these effects.

3.5 Conclusions and recommendations

The results as a whole support the perspective of low risk of injury to Atlantic salmon smolts ASHT passage. However this conclusion should be applied with reference to the site specificity of this study, the origin and size of fish used, the limitations of sensitivity of measures of damage, and the statistical power of to detect a given effect size and prevalence. Under the conditions of this study, there was no evidence of significant external injury to Atlantic salmon smolts. The prevalence of new scale-loss was not significantly associated with turbine passage when compared with control groups. Although not statistically significant, the data do provide an estimate of 2.46% of turbine-passed smolts as having 5-30% new scale-loss, over and above the 5% prevalence of new scale-loss observed in the control group. The absence of injury allows no conclusions to be drawn with respect to potential causes of turbine induced damage However evidence from trials with euthanized fish (Appendix A3.1) showing a possible grinding effect between the turbine blade and the housing trough suggests that this mechanism does not occur with live fish.

The serum chemistry correlates for tissue damage showed no differences between TREATMENT and CONTROL groups, with the exception of CK, which had a 4.58-fold elevation in turbine-passed smolts over control smolts. This result should be treated with caution, since it was derived with a small sample size, and it is not corroborated by the other serum markers. Further research is required to fully validate the use of serum chemistry markers for the types of trauma which might be expected from turbine passage, and to establish the biological significance of any detectable deviations in the activities of these markers, and the trauma causing them. Although the there is no detectable proportion of fish with elevations in the responses measured (with the exception of CK), and this is consistent with previous studies, the potential long-term significance of a low but undetectable prevalence of the worst case outcome of these responses at a population level remains a question to be resolved.

Appendix A3.1: A comparison of live and euthanized Atlantic salmon (*Salmo salar* L.) smolts as models for assessing scale loss from passage through an Archimedean screw turbine

Introduction

Incentives to increase renewable energy production have resulted in several innovative turbine technologies designed to exploit previously marginally economical very low head hydropower potential. The most widely applied of these has been the Archimedean screw turbine (Kibel, 2007) which may be retro-fitted to existing low-head historic barriers. There is a need to assess the potential impacts of such novel technologies on aquatic biota. Of particular concern are the risks posed to fish species which rely on longitudinal connectivity for migration between freshwater and marine habitats. Passage through conventional hydropower turbine infrastructure can result in direct or delayed mortality resulting from mechanical damage, rapid changes in water velocity and pressure and high shear stresses (Coutant & Whitney, 2000; Turnpenny *et al.*, 2000, Larinier & Travade, 2002; Brown *et al.*, 2008). For the most part, new technologies aimed at low-head applications lack these characteristics. Nevertheless several mechanisms for damage to fish have been identified, namely: impact by the leading edges of the turbine, grinding between moving and stationary turbine parts, or abrasion. Recent evidence (Vowles *et al.*, 2014) suggests that where low water velocities and rotational speeds are utilized, fish behaviour may become relatively more important as a determinant for potential injury or mortality, when compared with traditional high velocity situations.

In this study, euthanized salmon smolts were used to assess the potential for damage to passively drifting fish by an Archimedean screw turbine. The results are compared with those from tests with live, actively swimming fish in order to evaluate the utility of such passively drifting models for the assessment of turbine damage.

Methods

Tests with euthanized hatchery-origin fish were carried out on 20 April 2014, and tests with wild euthanized fish were carried out on 18 April 2014. Smolts were euthanized by overdose with benzocaine, followed by pithing. The experiments were executed at a turbine speed corresponding to operation near to maximum capacity (FAST, 26RPM). Two experimental groups were used to assess change in condition of fish from passage through the turbine: a turbine treatment group was released directly above the turbine and recaptured below (TREATMENT) and a recapture control group was released directly below the turbine and recaptured as a control for possible change to fish condition resulting from recapture (CONTROL). Ten each of TREATMENT and CONTROL hatchery origin fish were released simultaneously and collected below the turbine. CONTROL fish were distinguishable from TREATMENT fish by clipping of the adipose fin. The site, release, and recapture procedures are as described in section 3.2. In order to assess potentially different effects on the considerably smaller, leaner wild smolts, tests were carried out with ten each of euthanized TREATMENT, live TREATMENT and live CONTROL FISH wild fish.

The procedures for the live hatchery smolt tests were as described in section 3.2. Only the results from the tests with live fish at the FAST turbine speed are presented for comparison with the results from tests with euthanized fish, which were carried out at the same speed. In addition to the procedures described in section 3.2, 10 each of wild origin TREATMENT and CONTROL smolts were tested.

Results

A substantial portion (35.7%) of the dead TREATMENT fish exhibited a distinctive pattern of scale loss not seen in the live fish, nor in the dead CONTROL fish (Figure A3.1.1). This pattern was also seen in fish excluded from scale loss scoring due to *Saprolegnia* infection (47.3% of a total of 19). There were no significant differences in numbers of fish in any of the three scale loss conditions between TREATMENT and CONTROL groups, neither for the live nor the euthanized fish (Fisher’s exact test, $P>0.1$) (Table A3.1.1). There was a significant difference between live and euthanized groups in the proportion of TREATMENT fish with scale loss condition γ (Fisher's exact test, $p<0.05$). Of the 20 live wild fish, three CONTROL and four TREATMENT fish were recaptured, and none showed any of the three score-change conditions. Of the 10 euthanized wild fish passed through the turbine, seven were recaptured, two exhibited score-change condition 1 (any.change), and one appeared to have been squeezed (Figure A3.1.4).

Table A3.1.1. Results of Fisher's exact tests comparing numbers of fish with each of the three score change conditions between TREATMENT and CONTROL groups.

Hatchery fish with sapro score=0							
		Any positive change		summed side changes>1		maximum change to either side>1	
Group	N	odds	p	odds	p	odds	p
LIVE FAST Treatment-Control	89	0.84	0.82	0.37	0.13	1.02	1
Dead Treatment-Control	21	2.3	0.4	2.31	0.62	2.31	0.62

Table A3.1.2. Results of Fisher's exact tests comparing numbers of fish with each of the three score change conditions between LIVE and DEAD groups.

Hatchery fish with sapro score=0							
		Any positive change			summed side changes>1	maximum change to either side>1	
Group	N(D)	odds	p	odds	p	odds	p
FAST TREATMENT LIVE:DEAD	73(14)	2.42	0.233	2.92	0.2	5.32	0.04
FAST CONTROL LIVE:DEAD	37(7)	0.86	1	2.27	0.48	0.47	0.66

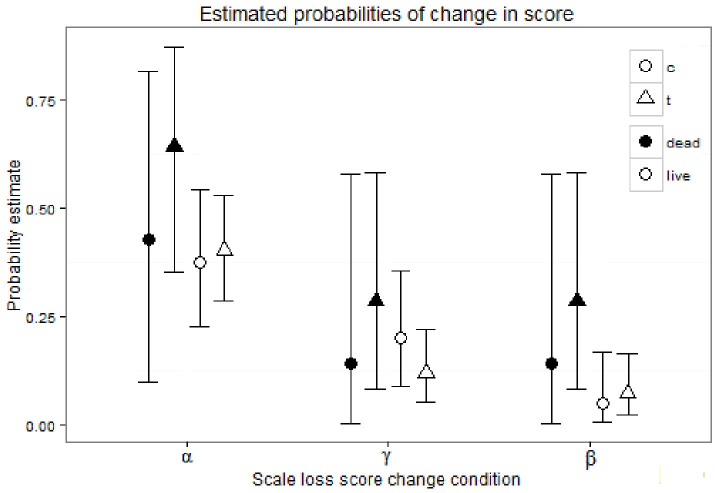


Figure A3.1.1. Estimated probabilities, based on the data collected of changes in scale-loss scoring category, at the three conditions for CONTROL and TREATMENT groups for the dead and live fish trials. Solid symbols represent the estimates for the dead fish. Hollow symbols represent the estimates for the live fish. Circles represent CONTROL fish and triangles represent TREATMENT fish. 95% confidence intervals for the estimates are are derived from a binomial distribution and shown with black lines. Score change conditions are: α = any positive change in scale loss score; γ = any positive change greater than 1, when the scores for each side of the fish are summed; β = any positive change greater than 1 for either side of the fish.

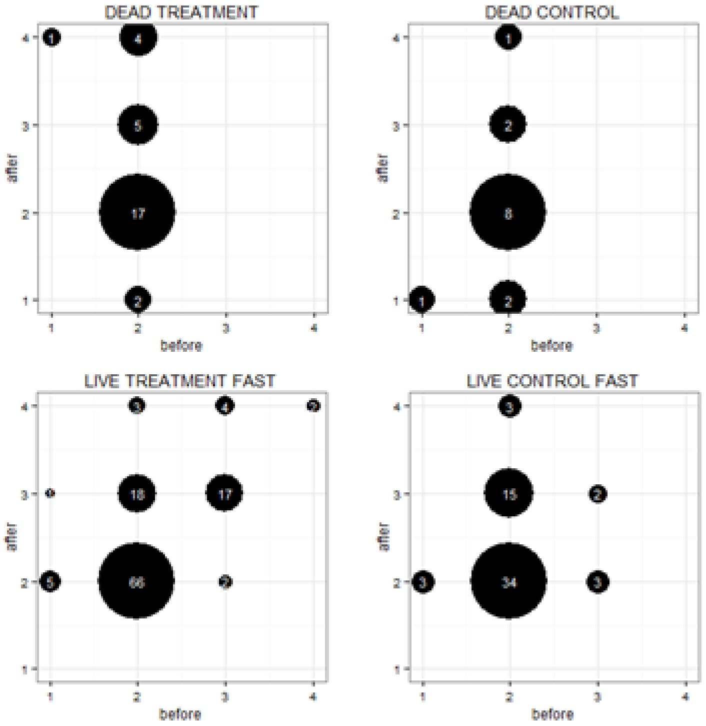


Figure A3.1.2. Scores assigned to each side of each fish before and after the trials. Each side of the fish is counted separately in order to illustrate changes in score without summarizing scores over both sides. This avoids any masking of effects to just one side of a fish, but effectively doubles the apparent sample size for these plots. Column 1: trials with dead fish; column 2: trials with live fish at both turbine speeds combined; column 3 trials with live fish at FAST turbine speed; column 4: trials with live fish at SLOW turbine speed. Row 1: turbine TREATMENT fish; row 2: recapture CONTROL fish. The area of the points in each panel is proportional to the number of sides with the assigned scores. Points on the diagonal (score before = score after) indicate no change in score. Points above the diagonal indicate an increase in score resulting from the trial. Points below the diagonal indicate a decrease in score (improvement in fish condition) and are a result of scoring errors).

Figure A3.1.3. Example photograph of one of the euthanized TREATMENT Atlantic salmon smolts exhibiting the distinctive scale loss pattern after turbine passage.

Figure A3.1.4. Dorsal view of the euthanized wild TREATMENT smolt showing possible effect of turbine passage.

Discussion

Although there were no significant differences between TREATMENTs and CONTROLs in the numbers of fish with any of the new scale loss conditions, the distinctive patterning observed in 37.5% of the dead TREATMENT fish reflects a real effect from the turbine. This pattern of descaling is consistent with that expected from abrasion by the outer edge of the turbine blade, if a fish was to be held against the gap between the trough and the turbine blade. It is proposed that the euthanized fish, being unable to maintain a position in the water away from moving parts, were drawn towards this gap by water flowing from upper to lower turbine compartments under the differential head.

This pattern of damage was not observed in any of the live fish, supporting the idea that live fish were avoiding contact with these hazard areas by swimming. The significant difference in substantial new scale loss to one side (condition 3) between live and dead TREATMENT fish supports the practical conclusion that passively drifting euthanized fish are not appropriate models for assessing scale loss. Whilst the lack of any significant proportion of live fish with new scale loss is reassuring, the grinding/slicing effect observed on euthanized fish identifies some important potential risks which merit further investigation: the effects on passively drifting fish raises concerns about potential effects on fish with reduced swimming/reaction ability due to infection or low temperatures. Another concern may be that smaller fish with weaker swimming ability and smaller size may be at more risk of being drawn into the hazardous area. A limited number of tests with euthanized wild smolts did not show the same scale loss pattern, but in one out of six recaptured fish the fish had been squeezed in a manner consistent with the proposed mechanism. Tests with live wild smolts did not show scale loss score changes at any of the three conditions.

Vowles *et al.* (2014) found an increased strike probability with live salmonids as compared to passively drifting euthanized fish when encountering a waterwheel type hydrostatic energy converter. By contrast, in this study the effect of grinding between the turbine blade and the trough was only observed in euthanized salmonids. These opposing directions of effect between passive and active models highlight the importance of considering each of the potential mechanisms for damage from turbine passage, and identifying the differential effects of these on fish of differing size, morphology and swimming behaviour in order to arrive at a sensible compromise on design and operational constraints to protect the fish species present. These considerations are more widely applicable to emerging novel turbine technologies, both in rivers and those utilizing tidal currents.

Appendix A3.2: Results from wild salmon smolts and brown trout - experimentally passed fish and fish naturally passing or resident in the vicinity of the turbine.

Experimentally passed wild salmon smolts

Twenty wild smolts were used in the turbine passage experiments. Ten each of TREATMENT and CONTROL fish were processed and released as described in the protocol for the hatchery-origin fish. Of these, seven were recaptured: three CONTROL and four TREATMENT (mean length ± sd [range] = 131 ± 5.88 [121-136] mm). None of these recaptured fish exhibited any of the three score-change conditions described in section 3.2. Table A3.2.1 gives the blood chemistry results for these seven fish.

Table A3.2.1. Blood chemistry results for wild fish used for experimental turbine passage trials. Units are activity units per litre (AST,CK and LDH), and arbitrary units (enolase)

T/C	AST	CK	LDH	enolase
C	30	279	2817	0
C	NA	NA	NA	0
C	NA	446	1964	0
T	246	87	1647	2.05
T	9	9	1110	0
T	NA	NA	NA	NA
T	372	904	2876	0

Experimentally passed wild brown trout

Ten wild brown trout smolts were processed and released as described in the protocol for the hatchery-origin salmon smolts. Of these, three were recaptured: two CONTROL and one TREATMENT. None of these recaptured fish exhibited any of the three score-change conditions.

Wild fish captured below the turbine during the passage trials, but which were not experimentally introduced during the turbine passage experiments

Eighty-seven wild salmon smolts, three salmon parr, 51 brown trout, six stickleback (*Gasterosteus aculeatus* L.), three stone loach (*Noemacheilus barbatulus* L.) and two minnow (*Phoxinus phoxinus* L.) were captured below the turbine during the period that the turbine was screened off from the rest of the river, both upstream and downstream.

Although the photographs of these fish have not been processed systematically as were those of the hatchery-origin smolts, no obvious signs of injury were observed, with two exceptions: a minnow which was missing the caudal fin, and a brown trout (fork length 264 mm, weight 217 g) with a tear to the skin and abrasions. A serum sample from this fish was analysed, and resulted in the following enzyme activities: AST, 6 U/l; CK, 6 U/l, LDH, 355U/l, enolase not detected. These injuries cannot be attributed to turbine passage, since passage was not confirmed (the fish may have been residing in the outflow), and condition prior to passage (if it occurred) was unknown.

Wild salmon smolts captured below the turbine after removal of the upstream screen

During the passage experiments, there was an accumulation of wild migrating smolts in the intake channel above the turbine, which was blocked by a fine screen for the purpose of preventing experimental fish from escaping upstream. On 24 April 2014, this screen was removed, and the following morning those wild fish which had passed through the turbine into the recapture area were captured and transferred to a holding tank. Sixty-four of these wild salmon smolts were processed and photographed, and none of these exhibited notable injuries other than scale loss. There was no control group for comparison of scale loss for these fish.

Appendix A3.3: Results of analyses carried out on the full dataset, including fish with visible *Saprolegnia* infection

In this appendix, the full set of scale-loss and blood chemistry results, including fish with visible sign of *Saprolegnia* infection are presented. These results are presented both for comparison with the uninfected fish, and to explore any relation between the measured responses and the presence and degree of *Saprolegnia* infection. The comparison of scale loss between live and euthanized fish is made for this full set, and both the initial results of samples processed for AST, LDH and CK during the experiments, and the later set (discarded due to uncertainty of reliability, see appendix A3.4), are shown.

Fish were scored from 0 to 5 for *Saprolegnia* infection using a reference sheet and the photos taken during the trials. A score of 0 denoted no visible infection, whilst scores from 1 to 4 represented increasing levels of *Saprolegnia* coverage on the fins and skin of the fish, according to the following grading system, and as shown in figure A3.3.1.

> **Grade 1**: small spots on leading edges of pectoral, dorsal or pelvic fins;
> **Grade 2**: clearly visible coverage of at least one fin, occasionally with encroachment onto nearby skin;
> **Grade 3**: at least one fin more than 50% covered and encroachment onto skin; and
> **Grade 4**: complete coverage of at least one fin or more than 50% coverage of the tail fin, with encroachment onto comparably large areas of nearby skin.

The experimental protocol and statistical analyses were as described in section 3.2, with the addition of *Saprolegnia* score as a covariate in regression analyses. Association between TREATMENT group and *Saprolegnia* presence was tested using a Chi-squared test.

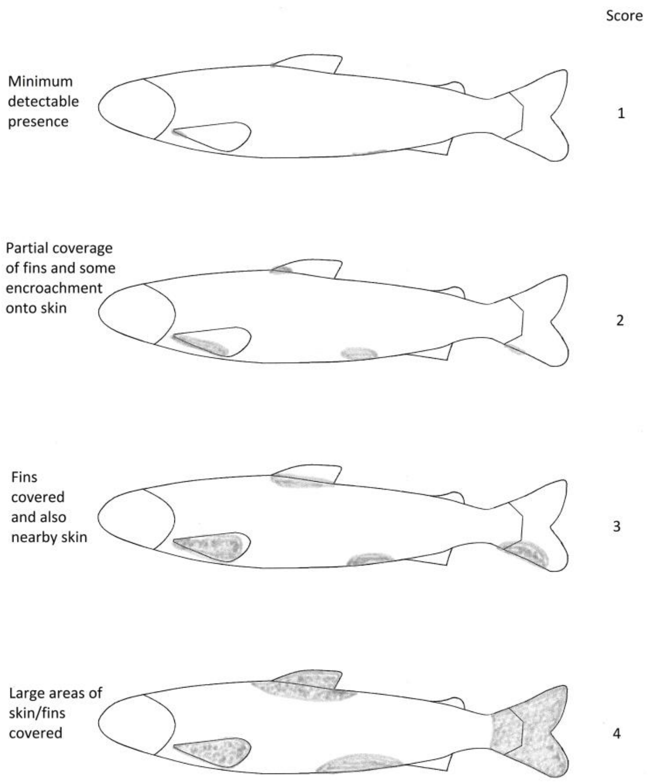


Figure A3.3.1. Scoring categories for *Saprolegnia* infection.

Saprolegnia infection

44.8% of recaptured fish had visible *Saprolegnia* infection. Figure A3.3.2 shows the proportion of TREATMENT and CONTROL groups in each *Saprolegnia* scoring category, for the recaptured fish. A chi-squared test showed no association between scoring category and TREATMENT (χ^2 = 1.60, df = 4, p = 0.81) for TREATMENT and CONTROL groups.

Scale loss

There were no significant differences in prevalence of any of the score-change conditions between TREATMENT and CONTROL groups, for the live or euthanized fish (Fisher's exact tests, *P>0.05*, Table A3.3.1), although there was a close to significant difference for the euthanized fish (*P=0.06*). Figures A3.3.3 and A3.3.4

graphically illustrate the difference in prevalence of the score change conditions between live and euthanized fish, and between the two turbine speeds.

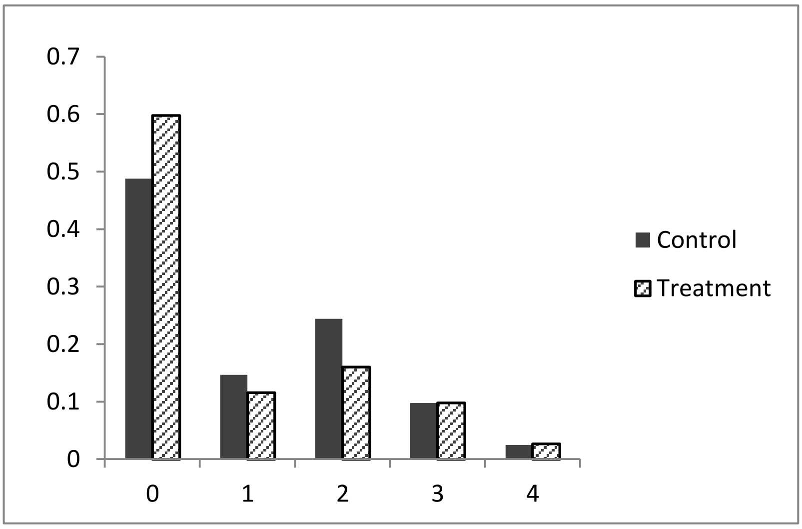


Figure A3.3.2. Proportions of TREATMENT and CONTROL samples assigned to the five *Saprolegnia* scoring categories.

Table A3.3.1. Results of Fisher’s exact tests for differences in frequencies of fish with changes in score between TREATMENT and CONTROL groups, for all the live fish, and the live fish subsetted by turbine speed category, and for the dead fish, which were trialled only at the FAST turbine speed.

All hatchery fish							
		Any positive change		summed side changes>1		maximum change to either side>1	
	N	odds	p	odds	p	odds	p
Live Treatment-Control	192	0.81	0.53	0.69	0.60	2.20	0.47
Live FAST Treatment-Control	145	0.92	0.86	0.55	0.28	1.69	0.70
Live SLOW Treatment-Control	44	0.43	0.30	inf	1.00	inf	1.00
Dead Treatment-Control	29	4.75	0.06	6.18	0.11	6.18	0.11

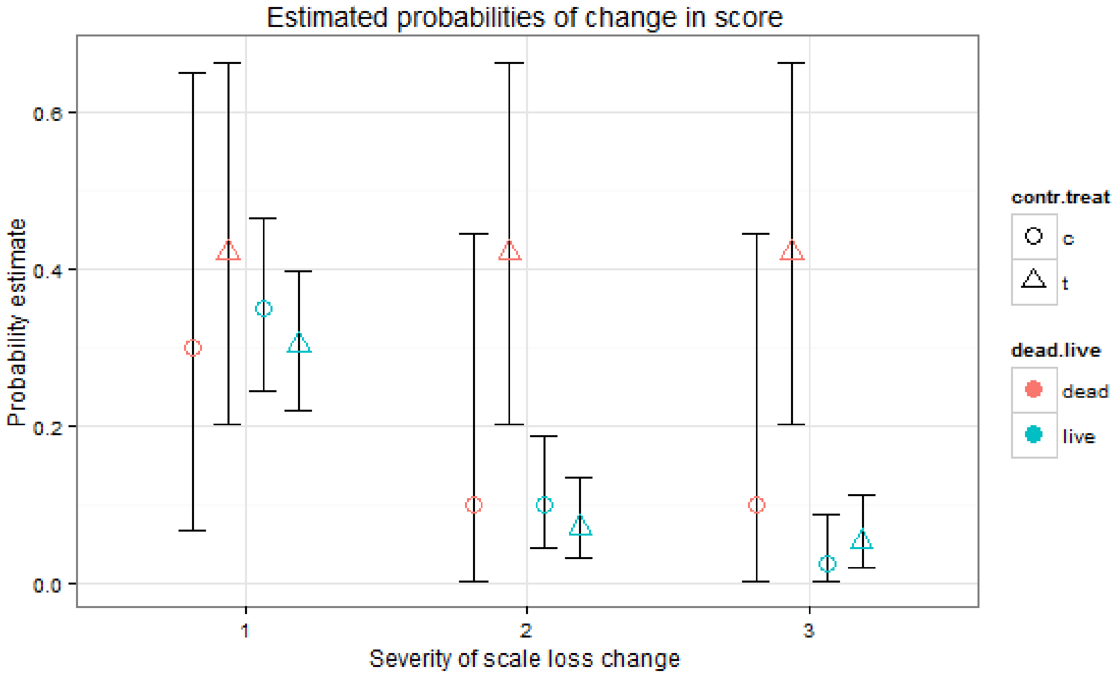


Figure A3.3.3. Estimated probabilities, based on the data collected, of changes in scale-loss scoring category, at three severities for CONTROL and TREATMENT groups for the dead and live fish trials. Red symbols represent the estimates for the dead fish. Blue symbols represent the estimates for the live fish. Circles represent CONTROL fish and triangles represent TREATMENT fish. 95% confidence intervals for the estimates are shown with black lines. Severity is given as: 1 = any positive change in scale loss score; 2 = any positive change greater than 1, when the scores for each side of the fish are summed; 3 = any positive change greater than 1 for the side of the fish with the maximum change.

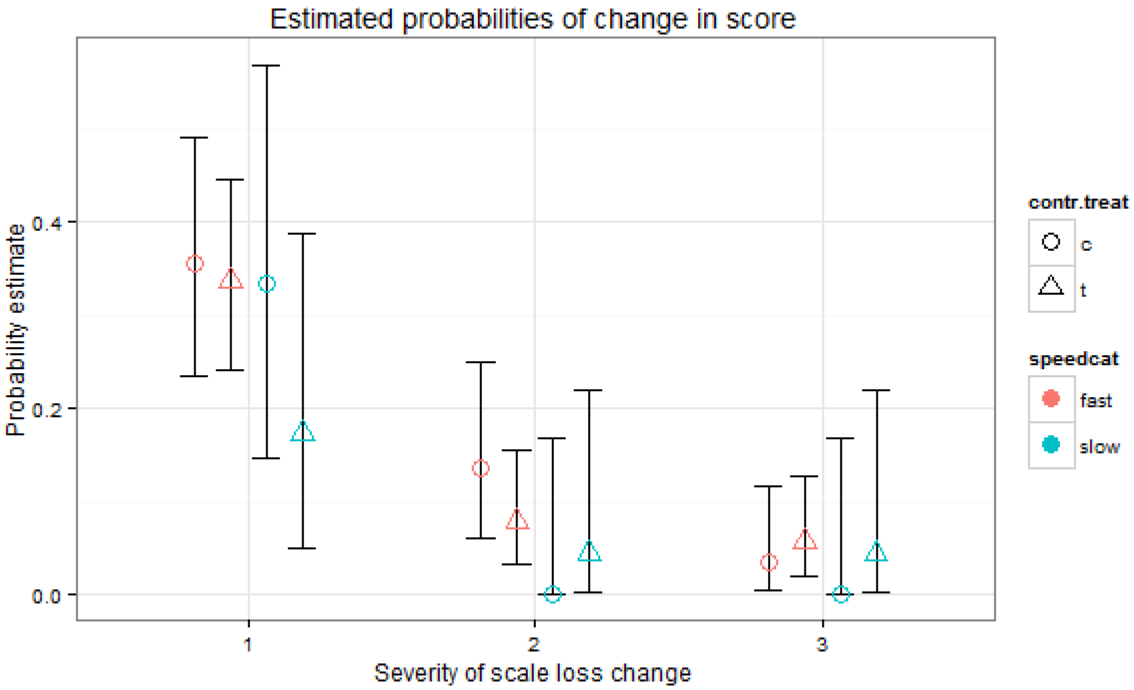


Figure A3.3.4. Estimated probabilities, based on the data collected of changes in scale-loss scoring category, at three severities for CONTROL and TREATMENT groups for the live fish trials. Red symbols represent the estimates for the FAST turbine speed trials. Blue symbols represent the estimates for the SLOW turbine speed trials. Circles represent CONTROL fish and triangles represent TREATMENT fish. 95% confidence intervals for the estimates are shown with black lines. Severity is given as: 1 = any positive change in scale loss score; 2 = any positive change greater than 1, when the scores for each side of the fish are summed; 3 = any positive change greater than 1 for the side of the fish with the maximum change.

Blood chemistry

AST, CK and LDH

a) Initial analyses

Of the covariates tested, only condition factor was significantly related to CK activity, and speed category to LDH activity ($P<0.05$, table A3.3.2). Whilst other covariates variously contributed to model fit for the three analytes, they were not significantly correlated with analyte activities.

Table A3.3.2. Regression results for the intitial analyses of AST, CK and LDH, including saprolegnia infected fish.

analyte	AST			CK			LDH		
method	log GLMM			log GLMM			GLMM		
N (C)	22 (11)			22 (11)			22 (11)		
	Est	se	p	Est	se	p	Est	se	p
intercept	4.61	0.29	<0.001	7.56	0.41	<0.001	1205.3	169.3	<0.001
TREATMENT	-	-	-	0.9	0.55	0.12	412	232.8	0.095
SLOW	0.73	0.37	0.064	-	-	-	1206.8	235	<0.001
lag	0.68	0.36	0.08				-	-	-
Tave	0.35	0.17	0.05	-	-	-	-	-	-
condfac	-	-	-	0.65	0.26	0.02	-208.6	101.2	0.055

b) Second analysis batch

Tables A3.3.3, A3.3.4 and A3.3.5 set out the regression results for AST, CK and LDH including *Saprolegnia* infected fish, with comparison to the *Saprolegnia* excluded set of results. Inclusion of *Saprolegnia* infected fish resulted in new correlations between AST and speed category, condition factor and length; between CK and lag and speed category; and between LDH and average temperature. Meanwhile correlations between CK and temperature deviance and condition factor were removed, as was correlation between LDH and length. AST was not correlated with *Saprolegnia* score, whilst both CK and LDH were negatively correlated with *Saprolegnia* score.

Table A3.3.3. Comparison of AST regression results for the full set and *Saprolegnia* excluded set of recaptured fish. LR= Logistic regression, OR= ordinary regression, df= degrees of freedom, LD= lower limit of detection, est=Estimate and se=standard error.

	with *Saprolegnia*						without *Saprolegnia*					
	LR		OR		LR		LR		OR		LR	
data subset	full		non-zero		full		full		sapro.score=0 & non-zero		sapro.score=0	
Dichotomising threshold	3 (LD)		-		10		3 (LD)		-		10	
response transformation	logit		log		logit		logit		log		logit	
df	129		100		127		75		64		75	
	est	se	est	se	est	se	est	se	est	se	est	se
fixed effects												
constant	1.51	0.46	2.54	0.13	-0.44	0.25	2.35	0.83	2.48	0.14	0.04	0.31
Speedcat-SLOW	-	-	-	-	1.15	0.61	-	-	-	-	-	-
Condfac (scaled)	-	-	0.23	0.11	-	-	-	-	-	-	-	-
Length (scaled)	-	-	-0.27	0.10	-0.44	0.21	-	-	-0.28	0.11	-	-
Random effects												
Batch	-	0.41	-	0.24	-	0.38	-	-0.67	-	1.18	-	0.46

TableA3.3.4. Comparison of CK regression results for the full set and *Saprolegnia* excluded set of recaptured fish. LR= Logistic regression, OR= ordinary regression, df= degrees of freedom, LD= lower limit of detection, est=Estimate and se=standard error.

	with *Saprolegnia*						without *Saprolegnia*					
	LR		OR		LR		LR		OR		LR	
data subset	full		non-zero		full		sapro.score=0		sapro.score=0 & non-zero		sapro score=0	
threshold for dichotomising data	6 (LD)		-		47		6 (LD)		-		47	
response transformation	logit		log		logit		logit		log		logit	
df	130		97		96		74		68		76	
	est	se	est	se	est	se	est	se	est	se	est	se
fixed effects												
constant	2.26	0.73	4.44	0.18	-0.008	0.33	-9.97	18.4	4.55	0.21	0.139	0.38
sapro score	-0.98	0.26	-0.49	0.14	-1.29	0.33	-	-	-	-	-	-
Speedcat-SLOW	-	-	-	-	1.69	0.69	-	-	-	-	1.89	1.17
Tave	-	-	-	-	-	-	-	-	-	-	-	-
delT	-	-	-	-	-	-	14.14	6.70	-	-	-	-
Condfac (scaled)	-	-	-	-	-	-	-9.79	3.25	-	-	-	-
Length (scaled)	-	-	-	-	-	-	-	-	-	-	-	-
relrecaplag	0.51	0.19	-	-	-	-	10.92	8.25	-	-	-	-
Random effects												
plate	-	-	-	-	-	-	-	-	-	-	-	-
Batch	-	1.678	-	0.098	-	0.26	-	161.5	-	0.27	-	0.44

Table A3.3.5. Comparison of LDH regression results for the full set and *Saprolegnia* excluded set of recaptured fish. LR= Logistic regression, OR= ordinary regression, df= degrees of freedom, LD= lower limit of detection, est=Estimate and se=standard error

	with *Saprolegnia*						without *Saprolegnia*			
	LR		OR		LR		OR		LR	
data subset	full		non-zero		full		sapro score=0 & non-zero		sapro score=0	
threshold for dichotomising data	21 (LD)		-		1048		-		1048	
response transformation	logit		none		logit		none		logit	
df	131		112		128		73		76	
	est	se	est	se	est	se	est	se	est	se
fixed effects										
constant	3.73	0.87	1632.2	127.2	11.18	4.53	2208.15	334.4	3.51	1.31
sapro score	-1.23	0.305	-	-	-0.82	0.22	-	-	-	-
Speedcat-SLOW	-	-	-	-	3.30	1.32	-	-	-	-
Tave	-	-	-	-	-1.01	0.52	-	-	-	-
delT	-	-	-	-	-0.38	0.24	-195.98	95.83	0.53	0.28
Condfac (scaled)	-	-	-	-	-	-	-	-	-	-
Length (scaled)	-	-	-	-	-	-	-249.06	110.9	-	-
relrecaplag	-	-	-	-	-	-	-	-	-	-
Random effects										
plate	-	-	-	-	-	-	-	-	-	-
Batch	-	1.557	-	315.9	-	0.89	-	126	-	1.00

TableA3.3.6. Comparison of enolase regression results for the full set and *Saprolegnia* excluded set of recaptured fish.

	with *Saprolegnia*						without *Saprolegnia*					
Type	LR		OR		LR		LR		OR		LR	
data subset	full		non-zero		full		sapro.score=0		sapro.score=0 & non-zero		sapro.score=0	
threshold for dichotomising data	1.58 (LD)		-		2		1.58 (l)		-		2	
response transformation	logit		log		logit		logit		log		logit	
N	156		68		156		96		37		96	
	est	se	est	se	est	se	est	se	est	se	est	se
fixed effects												
constant	-0.67	1.9	1.1	0.24	-2.32	1.16	35.20	22.95	0.15	0.45	1.97	1.04
sapro score	1.49	0.3	0.3	0.08	1.20	0.28	-	-	-	-	-	-
Speedcat-SLOW	-	-	-	-	-	-	-8.21	4.43	-	-	-	-
Tave	-	-	-	-	-	-	4.09	2.68	-	-	-	-
delT	-	-	-	-	-	-	-	-	0.33	3.14	-	-
Condfac (scaled)	-	-	-	-	-	-	-	-	-0.20	0.07	-	-
Length (scaled)	-	-	-	-	-	-	-	-			-	-
relrecaplag	-	-	-	-	-	-	-	-			-	-
Random effects												
plate	-	-	-	0.4494	-	2.63	-	7.282	-	0.51	-	2.31
Batch	-	4.41	-	0.4077	-	0.57	-	-	-	0.32	-	-

Enolase

Both the presence and magnitude of detectable enolase levels were significantly positively correlated with *Saprolegnia* score. The incidence of enolase levels greater than 2 arbitrary units was similarly correlated with *Saprolegnia* score The incidence of enolase levels greater than the limit of detection (5.826 AU) was not correlated with any of the covariates (likelihood ratio tests, backward model selection, p>0.1). All other covariates, including the experimental treatment and manipulated turbine speed made no significant contribution to the fit of the models (likelihood ratio tests, backward model selection, p>0.1.

Discussion

The lack of association between *Saprolegnia* presence and treatment group provides some justification for the inclusion of *Saprolegnia* infected fish in the analyses of change to scale loss and blood biochemistry responses, since no bias is indicated. The scale loss results support the conclusion from the *Saprolegnia* excluded results: that there is no significant turbine-induced scale loss, that euthanized fish experience greater levels of scale loss associated with turbine passage, and that both TREATMENT and CONTROL fish experience more scale loss at the faster turbine speed. The significant correlation between enolase and *Saprolegnia* score suggests that this infection is associated with elevated levels of this enzyme in the blood, and increases confidence that this analyte is of use in detecting tissue damage. The negative correlation between *Saprolegnia* score and CK and LDH levels contradicts the assumed mechanism of enzyme release following infection induced cell lysis. The varying importance of regression covariates between the *Saprolegnia* excluded and included datasets shows that the results are sensitive to the inclusion of infected fish, and supports their exclusion, but may also be a result of unbalanced data, and further supports the recommendation that the use of these blood chemistry correlates requires very strict control of covariates in the absence of a full understanding of their effects on the responses.

Appendix A3.4: Comparison of initial and full results for the blood analytes AST, CK and LDH

Introduction

The blood chemistry analytes AST, CK and LDH were processed in two batches: a limited number of initial samples were processed during the period of turbine experiments on 14 April 2014 by the University of Glasgow Veterinary Diagnostics Laboratory [UGVDL], whereas the bulk of the samples were processed in June 2015 by Nationwide Laboratories [NWLab]. A small number of samples which were processed in the first batch were reprocessed with the second batch. This appendix serves to:

- compare the data from the two sets of analyses, discuss possible reasons for observed differences, and hence support the decision to rely primarily on the first, smaller dataset for the main results (Chapter 3), and
- present the results of the analyses of the second dataset in relation to the turbine passage experiments, and discuss any trends or discrepancies between the resulting inferences and those of the first dataset presented in Chapter 3.

Methods

The experimental protocol and sampling procedure were as described in section 3.2. Handling and storage of the samples was identical for the two analysis batches up until transport of the first batch for analysis. At this point the samples were taken from -20°C storage and transported frozen to the UGVDL, after which they were thawed and analysed. These samples were then stored at -20°C until the second analysis, when those samples with adequate remaining volume were re-analysed by NWLab. The rest of the samples remained in -20°C storage until they were transported frozen in June 2014 and stored at -80°C until thawing in February 2015 for enolase assay. These samples underwent three further freeze-thaw cycles, ending with the final analysis for AST, CK and LDH by NWLab.

Quality control procedures for the analyses carried out by UGVDL were as described in section 3.2. NWLab use a similar in-house quality control procedure. In-house quality control samples for AST, CK and LDH were tested to check for accuracy. Two samples were used for each analyte – one at a low concentration, and one at a high concentration. The acceptable range is taken as ± 2 standard deviations from the mean concentration for these quality control samples from previous assays.

Statistical analyses

Mann-Whitney U-tests were used to compare the distributions of the two datasets. This is a conservative method for identifying differences, as only the ranks rather than the absolute values of the data are used. In order to identify correlations of the second dataset with other measured variables, generalised linear mixed model regression was used. The blood chemistry data were bounded by limits of detection as shown in Table A3.4.1. Due to the zero-inflated and left skewed distributions of the AST and CK data, three regressions were carried out for each blood analyte: a logistic regression on the binary data dichotomised around the lower limit of detection, an ordinary regression on the continuous part of the data, and a logistic regression on the data dichotomised around the chosen threshold for each analyte (see page 18).

Because of the potential correlation between *Saprolegnia* score and blood chemistry response, these regressions were carried out excluding samples from fish with visible *Saprolegnia* infection. For the LDH data without *Saprolegnia*, only two of 79 data points were below the limit of detection, therefore only ordinary regression was performed on these data. All ordinary regressions other than for LDH were carried out on the log-transformed response. Ordinary regressions on the LDH data were carried out on the untransformed response, as these data required no transformation in order to meet the assumption of normality of residuals.

Table A3.4.1. Limits of detection and distribution of the data with reference to these limits. The limits for enolase were from analyses carried out in UGVDL, whereas the limits for AST, CK and LDH are from the later analyses carried out by NWLab.

	Enolase	AST	CK	LDH
Lower limit of detection	1.58 (5.83)	3	6	21
Proportion of sample below lower limit	0.53 (0.90)	0.25	0.25	0.12
Upper limit of detection	140	1236	2730	3000
Proportion of sample above upper limit	0.01	0	0.01	0.09
Total N	223	204	205	206

Results

Test performance characteristics for the second set of analyses (NW Labs) for AST, CK and LDH

In all cases, quality control samples were <2 *sd* from the mean on the day of the analyses, which is an acceptable accuracy for the assays. Table A3.4.2 shows the historic standard deviations for these samples, which are a measure of the precision of the assay.

Table A3.4.2. Means and standard deviations (in brackets) for the in-house quality control samples.

	AST	CK	LDH
Low standard	25 (2)	160 (5)	148 (7)
High standard	178 (5)	445 (14)	715 (20)

Distributions of the two data sets

When comparing the earlier (UGVDL) analyses with the later (NWLab) results, it is apparent that the earlier values are generally higher and more variable for AST and CK (Figure A3.4.1), with no values under the thresholds of detection (as determined from the later analyses). LDH was higher in the earlier analysed samples, but with less variability. A few of the samples were analysed both by UGVDL in April 2014 and again by NWLab in June 2015. Figure A3.4.2 compares those values against each other.

Most of the NWLab results are lower than the earlier UGVDL results, the AST values have dropped by an order of magnitude and appear to be correlated. Only one CK value is above the lower detection threshold on the second analysis, and it is about 150 times smaller than in the first analysis. The LDH results show no convincing correlation, however the difference in values between the first and second analyses are far less extreme than for the other two analytes (less than an order of magnitude).

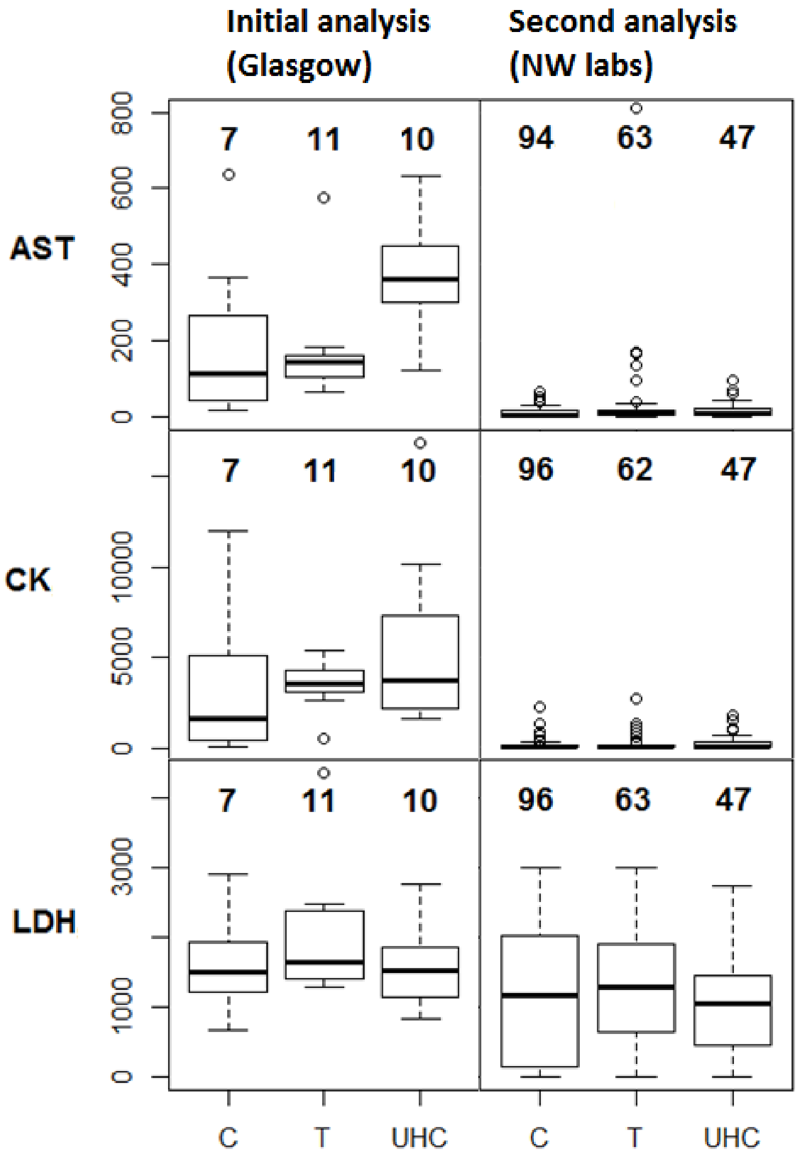


Figure A3.4.1. Comparison of initial results to later bulk analyses. Y-axis units are activity units per litre. X-axis labels are: C - CONTROL, T - TREATMENT and UHC - UNHANDLED CONTROL. Sample sizes for each group are shown within each panel.

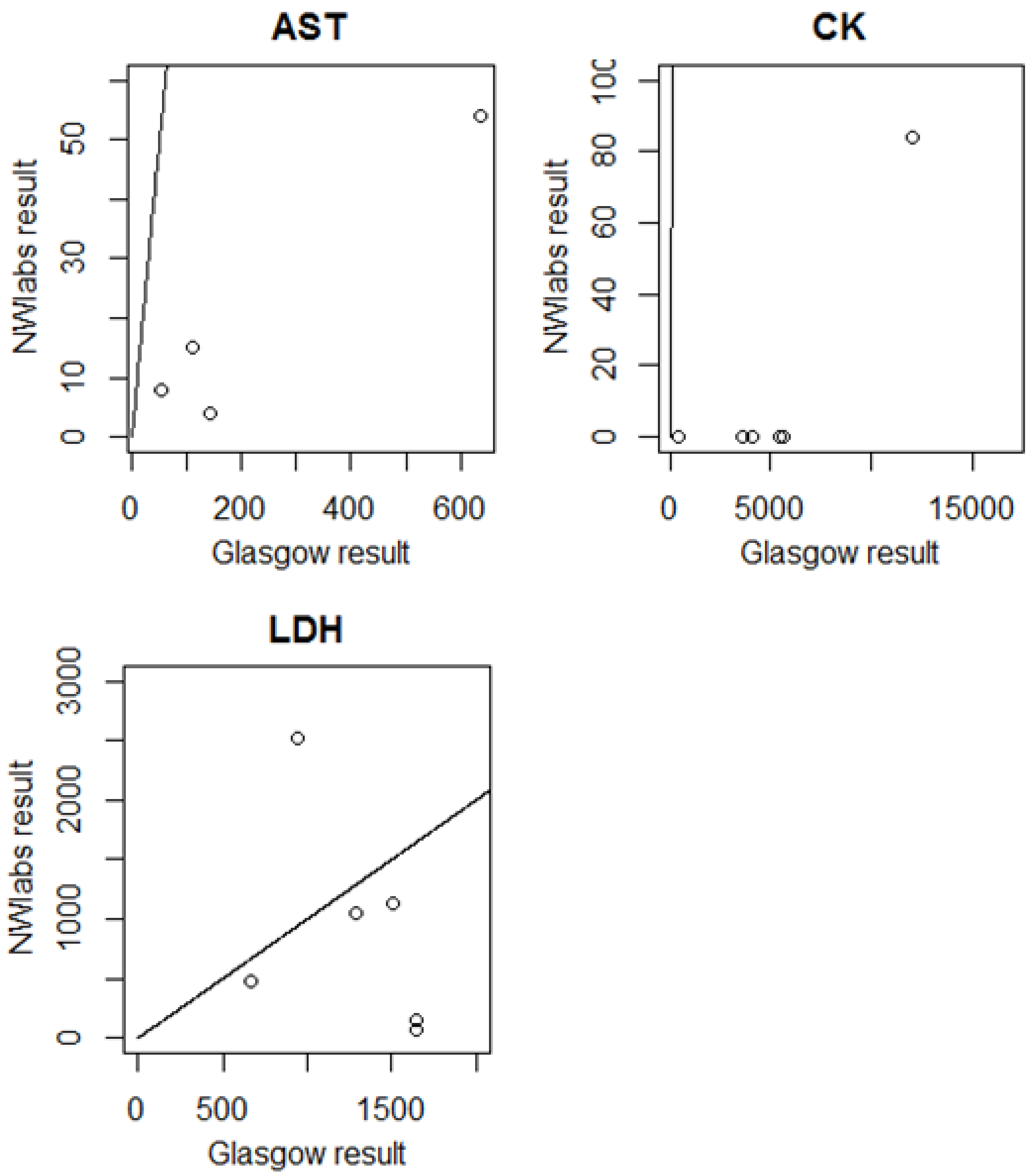


Figure A3.4.2. Comparison of initial results with later results for samples which were analysed on both occasions. The line is y=x. Units are activity units per litre.

Results of analysis of second set of results with regard to turbine trials

Turbine TREATMENT group was not correlated with the detection, magnitude, or detection over the median of the UNHANDLED group for any of the analytes.

The magnitude of AST levels showed a negative correlation with length ($p<0.05$) (Table A3.4.3). Detection of CK was correlated with temperature deviation, condition factor and release-recapture lag ($p<0.05$) (Table A3.4.4). Detection of CK above a threshold of 47 activity units was correlated with speed category ($p<0.05$) (Table A3.4.4). LDH levels were negatively correlated with temperature deviance and length, and detection above 1048 activity units was correlated with temperature deviance.

Table A3.4.3. GLMM results for the second set of AST results from NWLab.

	Logistic regression		Ordinary regression		Logistic regression	
data subset	sapro.score=0		sapro.score=0 & non-zero		sapro.score=0	
threshold for dichotomising data	3 (lower limit of detection)		-		10	
response transformation	logit		log		logit	
df	75		64		75	
	estimate	se	estimate	se	estimate	se
fixed effects						
constant	2.35	0.83	2.48	0.14	0.04	0.31
Length (scaled)	-	-	-0.28	0.11	-	-
Random effects						
Batch	-	-0.67	-	1.08	-	0.46

Table A3.4.4. GLMM results for the second set of CK results from NWLab.

	Logistic regression		Ordinary regression		Logistic regression	
data subset	sapro.score=0		sapro.score=0 & non-zero		sapro score=0	
threshold for dichotomising data	6 (lower limit of detection)		-		47	
response transformation	logit		log		logit	
df	74		68		76	
	estimate	se	estimate	se	estimate	se
fixed effects						
constant	-9.97	18.4	4.55	0.21	0.14	0.38
Speedcat-SLOW	-	-	-	-	1.89	1.17
delT	14.14	6.70	-	-	-	-
Condfac (scaled)	-9.79	3.25	-	-	-	-
relrecaplag	10.92	8.25	-	-	-	-
Random effects						
Batch	-	161.5	-	0.27	-	0.44

Table A3.4.5. GLMM results for the second set of LDH results from NWLab.

	Ordinary regression		Logistic regression	
data subset	sapro score=0 & non-zero		sapro score=0	
threshold for dichotomising data	-		1048	
response transformation	none		logit	
df	73		76	
	estimate	se	estimate	se
fixed effects				
constant	2208.15	334.4	3.51	1.31
delT	-195.98	95.83	0.53	0.28
Length (scaled)	-249.06	110.9	-	-
Random effects				
Batch	-	126	-	1.00

Discussion

Comparison of first and second set of analyses

The marked difference in median and range for AST and CK (Figure A3.4.1) between the first and second set of analyses support the possibility of a lowering of activities. LDH appears to have retained a more similar spread of activities.

The magnitude of the change to the individual sample results for AST and CK (Figure A3.4.2) between the two periods of analysis is concerning because information may have been lost for sample analyte activities which have dropped below the detection thresholds. However, it should be noted that these particular samples were not stored and treated in the same way as the others after the first analysis – in fact these samples were stored at -20^{o}C after the first analysis, whereas the bulk of the samples had been stored at -80 between June 2014 and analysis in June 2015. The comparison of these individual samples for LDH again suggests that this analyte may be more resilient to change.

Possible causes for the changes in activity between the first and second analyses are: an attenuation of enyzyme activity due to denaturing over time, or denaturing due to freeze and thaw. It is likely that the rate of attenuation over time in storage is dependent on storage temperature, and since the samples were transitioned from ambient, to -20°C, to -80°C, the literature pertaining to effects of storage at these temperatures is considered below.

Short term storage at above freezing:

Cuhadar *et al.* (2012) found no effects on AST, CK or LDH after six or 12 hours storage at 4°C. Donnelly *et al.* (1995) found no change in CK stored at room temperature, 4°C or -20°C over 48 hours. Jackson *et al.* (2008) found no stability issues for AST, CK or LDH stored at 4°C up to 36 h, or with freeze thaw cycling for this period. The samples under question were stored on ice for up to 12 hours at most, therefore no stability issues are expected because of short term storage before freezing.

Medium term storage at -20 °C:

Cuhadar *et al.* (2013) reported that AST, CK and LDH were not affected by a storage duration of up to three months at -20°C. Cray *et al.* (2009), found all three analytes to be stable after 30 and 90 days at -20°C. Donnelly *et al.* (1995) found no change in CK stored at room temperature, 4°C or -20oC over 14 days and four months. These samples were stored for less than three months at -20°C before frozen transport to -80°C, therefore no stability issues should have arisen because of medium term storage in a household freezer.

Long term storage at -20 °C or -80 to -20 °C:

Brinc *et al.* (2012) tested the Long-term stability of biochemical markers in pediatric serum specimens stored at −80 °C, and found AST, CK and LDH to be stable over a 10 to 13 month period of storage, relative to baseline measurements before storage, and with one freeze-thaw cycle before analysis. Cray *et al.* (2009), examined the stability of selected analytes in rat serum with long term storage at -20°C and -70°C and found no significant reduction in activity after 360 days at -70°C for any of the three analytes, but larger reductions (7.1% and 59%) for AST and CK after the same time period at –20°C. These samples were stored at -80 for 12 months prior to analysis. These studies indicate no stability issues over that time period.

Freeze-thaw:

Cuhadar *et al.* (2013) found that reduction in CK activity was statistically but not clinically significant after four freeze-thaw cycles, and reduction in LDH was clinically significant after two freeze-thaw cycles. There were no significant changes in AST activity. Paltiel *et al.* (2008) subjected human plasma samples to several freeze thaw cycles after storage at -80 °C. AST activity was determined as stable beyond 20 freeze-thaw cycles, with no significant changes until 30 cycles. Mitchell *et al.* (2005) investigated the effects of repeated freeze thaw on proteins in plasma used for mass-spectrometry. They found the effects to be variable between proteins, with the results suggesting that larger proteins are generally more susceptible to degradation than smaller ones. They concluded that more than two freeze-thaw cycles could begin to compromise data quality by interfering with peak detection, and that freeze-thaw is more problematic than long term storage at -70^{o}C.

These samples underwent two freeze thaw cycles before the analysis of individual enolase activities (first analysis), and three cycles before the reanalysis of a subsample. All the samples had undergone four freeze-thaw cycles before the analysis of the full set for AST, CK and LDH. The literature suggests that AST and CK are stable at four freeze-thaw cycles (Cuhadar *et al.*, 2013), and that AST is stable beyond 20 (Paltiel *et al.*, 2008). Based on this evidence, freeze thaw would not have affected AST and CK, but would have affected LDH. In fact, the data show the opposite: generally much lower values on the second analysis for AST and CK, but less of a change for LDH.

Observed change in activities with reference to literature base on enzyme stability with storage and freeze-thaw

Without a fuller understanding of any potential differential stability of these analytes in the serum of different species, the literature information (which is almost exclusively based on human serum or plasma), can only be an indication. Ideally all samples would be immediately analysed after collection, however this was not practicable given the remote field location and limited resources available. Another strategy would be to analyse aliquots of these samples at strategic points during the samples' history in order to understand how the data may have been affected by pre-analysis handling and storage. The very low sample volumes which could be collected from these juvenile fish and the additional cost of these analyses prohibited this option.

Recent advances

A recent study (Braceland *et al.*, 2016) has identified, discussed and partially explained the current issues with serum biochemistry as a diagnostic tool for Atlantic salmon. They point out that pre-analytical handling can influence analyte concentrations and test the effect of sample type (serum or plasma), time until centrifugation, length of short term post-separation storage at room temperature or 4 ^{o}C and freeze-thaw by using paired samples with and without each of these treatments. Among the range of analytes tested, AST and CK consistently exhibited significant changes in activity with time until serum or plasma separation, with storage at room temperature vs 4^{o}C for 48 and 72 hours after collection, and with 2 and 4 freeze-thaw cycles. Importantly, whilst some of these treatments acted to reduce activities in these analytes (time until separation for AST, short term storage temperature for AST and CK), others increased the activities (time until separation for CK, freeze thaw treatments for both analytes). Interestingly, LDH was in general not sensitive to these treatments, which is in agreement with the lesser difference in activities seen between the UGVDL and NWlab results in this study. They established normal ranges for a suite of analytes, but in general found these to be in agreement with those of Sandnes (1988).

Results from second set of analyses in relation to the turbine passage trials

The lack of any relationship between the turbine treatment groups and these analytes supports the conclusions of the smaller initial sample set (Section 3.3). Correlations between these responses and several of the covariates in certain cases likely reflects the presence of influential data points.

Appendix A3.5: Turbine and river state

Table A3.5.1. Velocity profile taken during the turbine trials. Column headings are: S – distance (m) from edge of channel of sampling vertices; Vx – velocity (m/s) at depth x (m) from surface; Q total discharge (m^3/s^{-1}) for all segments.

Date	10/04/204
Time	19:20
turbine RPM	8
turbine Power	20
total depth	1.94
turbine q	1.2
S	V0.5
1.5	0.224

Table A3.5.2. Velocity profile taken during the turbine trials. Column headings are: S – distance (m) from edge of channel of sampling vertices; Vx – velocity (m/s) at depth x (m) from surface; Q total discharge (m^3/s^{-1}) for all segments.

Date	11/04/204
Time	17:45
turbine RPM	
turbine Power	
total depth	1.7
turbine q	
s	v0.5
1.5	0.608

Table A3.5.3. Velocity profile taken during the turbine trials. Column headings are: S – distance (m) from edge of channel of sampling vertices; Vx – velocity (m/s) at depth x (m) from surface; Vave – average velocity (m/s) over the three depths; Vavemid – average velocity (m/s) at the midpoint between sampling vertices; W – width (m) of segment between sampling vertices; A – area (m2)of segment between sampling vertices; q – calculated discharge (m3/s^{-1}) through segment between sampling vertices; Q total discharge (m3/s^{-1}) for all segments.

Date	18/04/204
Time	20:42
turbine RPM	24
turbine Power	45.2
total depth	1.63

S	V0.2	V0.6	V0.8	Vave	Vavemid	W	A	q
0.59	0.571	0.599	0.578	0.582667	0.291333	0.59	0.9617	0.280175
1.27	0.533	0.584	0.348	0.488333	0.5355	0.68	1.1084	0.593548
1.76	0.583	0.355	0.25	0.396	0.442167	0.49	0.7987	0.353159
2.34	0.514	0.594	0.487	0.531667	0.463833	0.58	0.9454	0.438508
3.03	0.529	0.664	0.439	0.544	0.537833	0.69	1.1247	0.604901
3.63					0.272	0.6	0.978	0.266016
							Q	2.536306

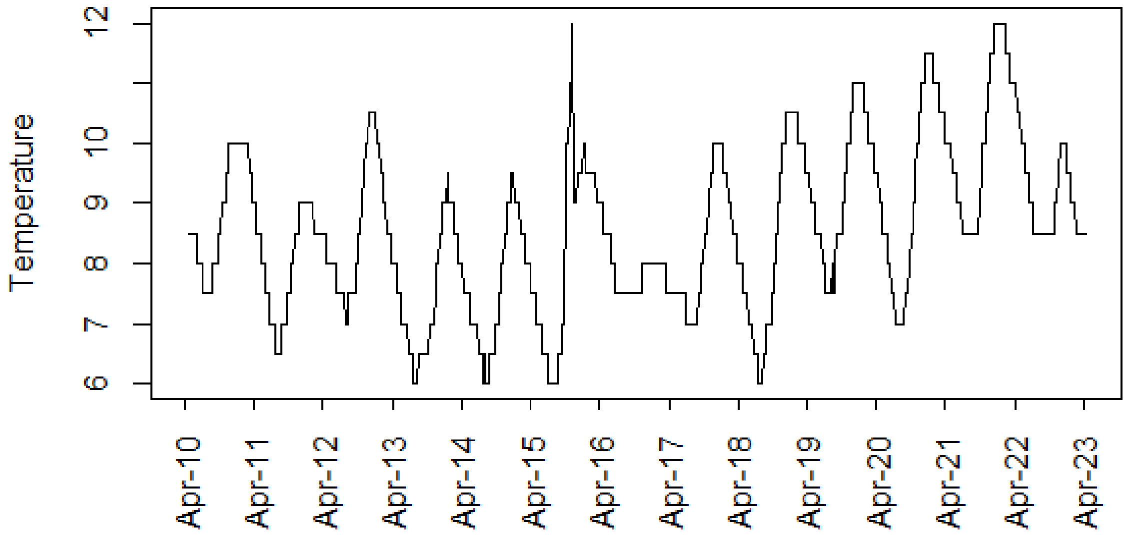


Figure A3.5.1. Logged temperature in the turbine intake channel during the passage trials.

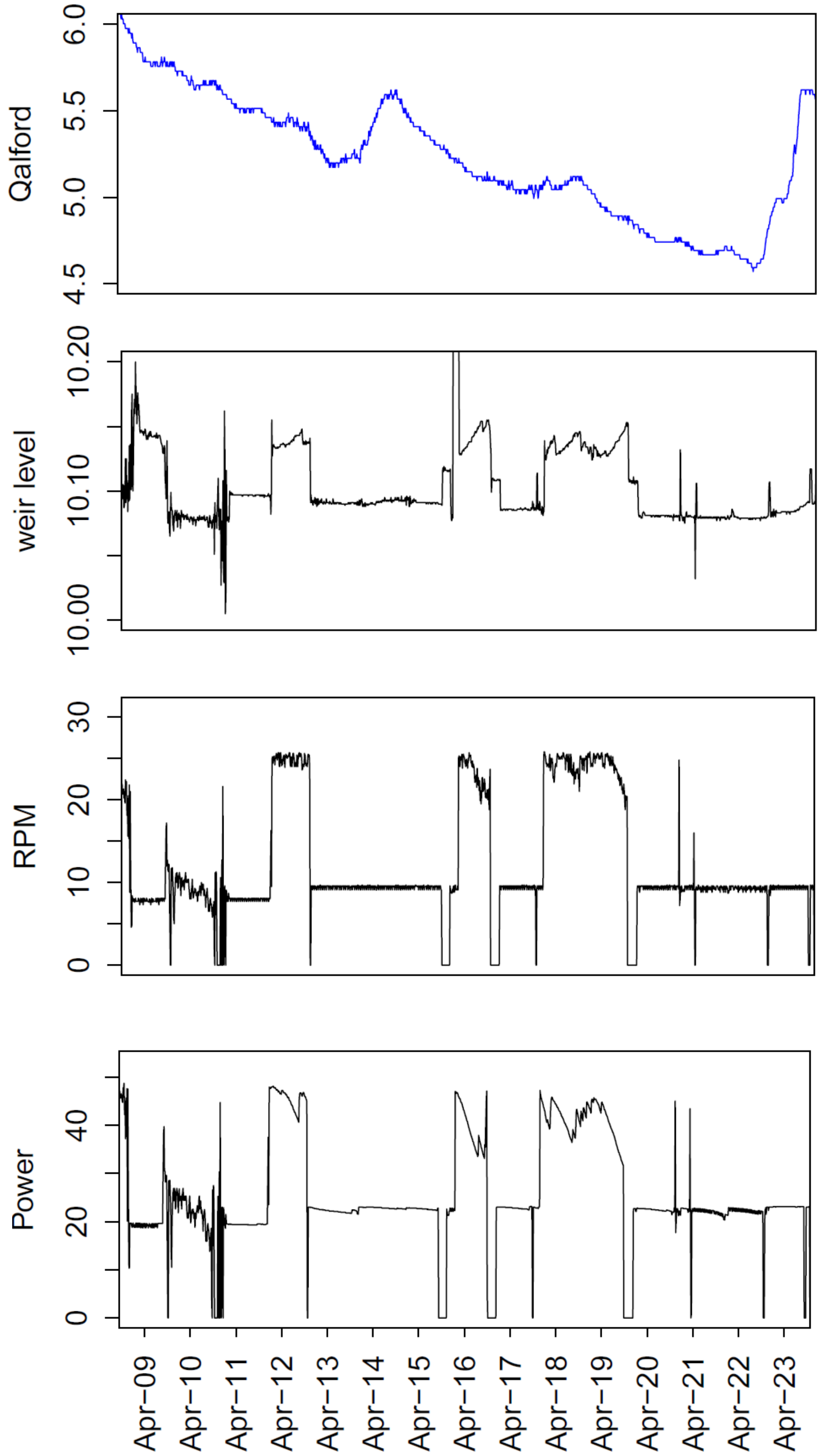


Figure A3.5.2. From top to bottom: discharge, weir level and turbine rotational speed and power output during the passage trials.

Appendix A3.6. Post-hoc power analysis for the blood chemistry analytes.

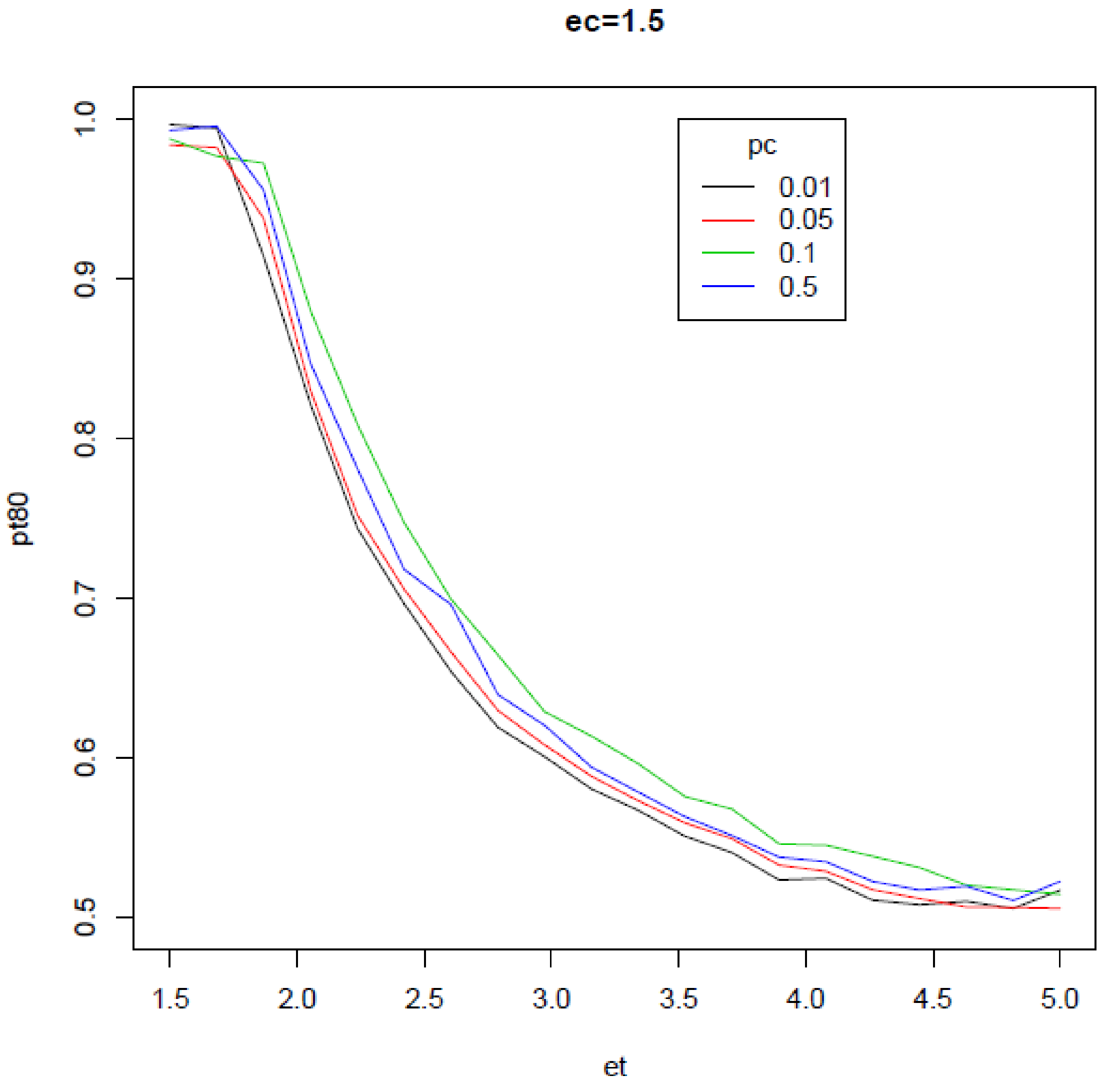


Figure A3.6.1. 80% power contours for detection of a treatment effect. Prevalence is plotted against treatment effect size (a multiplicative factor increase of individual analyte levels), for four prevalences of an effect of 1.5 already present in the control group. The plot gives the treatment prevalence and effect size necessary for detection of an effect in 80% of 1000 simulated datasets. Data were simulated under the assumptions outlined in section 3.2.9.4, for 10 control and four treatment samples.

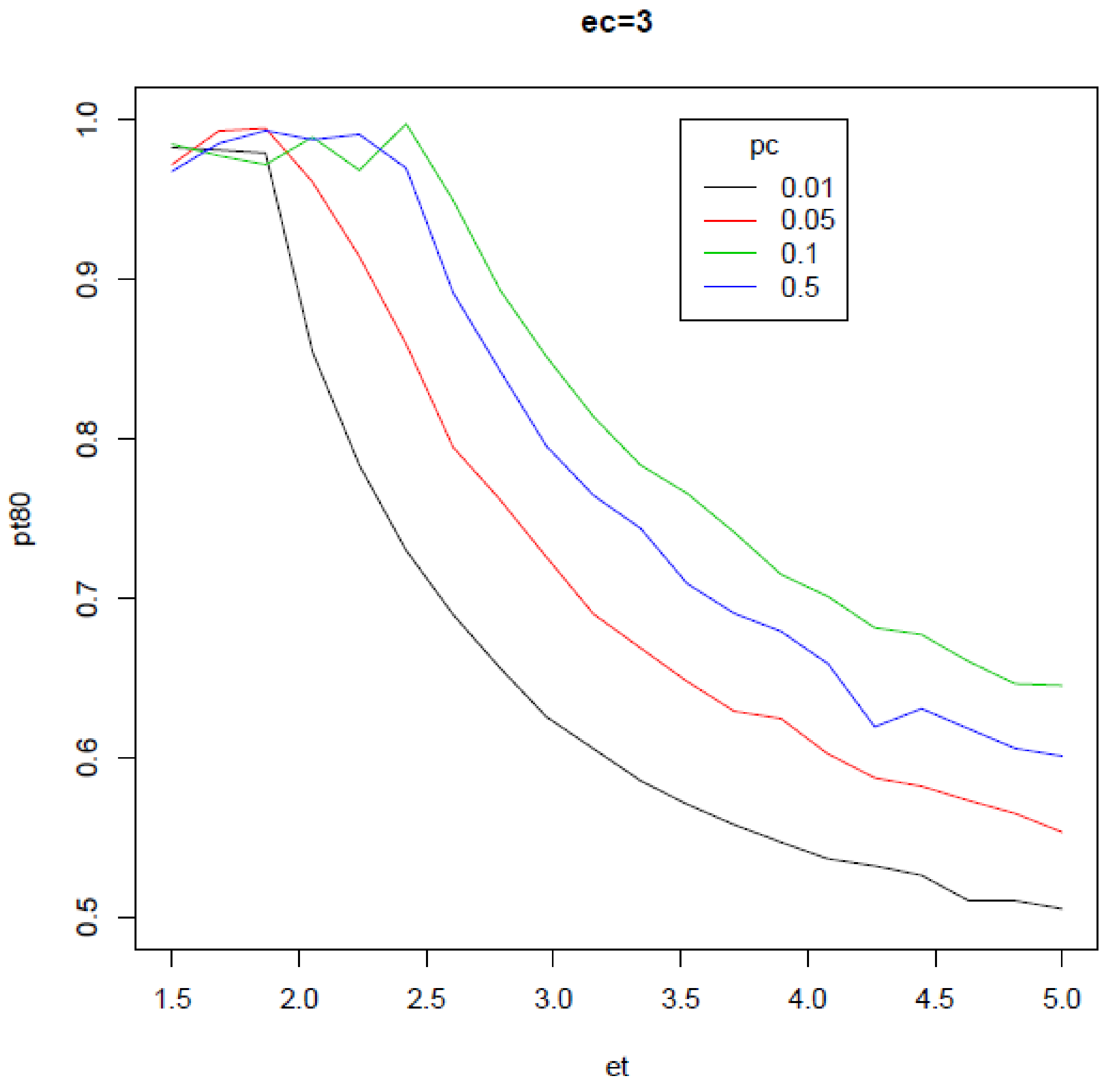


Figure A3.6.2. 80% power contours for detection of a treatment effect. Prevalence is plotted against treatment effect size (a multiplicative factor increase of individual analyte levels), for four prevalences of an effect of three already present in the control group. The plot gives the treatment prevalence and effect size necessary for detection of an effect in 80% of 1000 simulated datasets. Data were simulated under the assumptions outlined in section 3.2.9.4, for 10 control and four treatment samples.

Chapter 4

Upstream passage of adult Atlantic salmon (*Salmo salar* L.) at three low-head Archimedean screw hydropower schemes

4.1 Introduction

The migration of anadromous salmonids, such as Atlantic salmon, from the sea to in-river spawning grounds, is an iconic event of cultural, economic and ecological importance. Human modification of rivers for hydropower generation can hamper or halt these movements. This can be catastrophic for the continuity of migratory populations (e.g. Reale, 2011; Ugedal *et al.*, 2008), because some or all migrants are prevented from completing their lifecycles. For the most part, adult salmonids home to their natal spawning grounds, and thus genetically and phenotypically distinct populations have evolved between and within catchments. By impeding upstream movements with obstacles to migration (Gowans 1999; Gowans *et al.*, 2003), the continuity of these populations is threatened. These consequences are well known for large hydropower installations (Jonsson & Jonsson, 2011), but there is a perception that small, low-head schemes are less damaging, because the magnitude of alterations to river morphology and hydrology are lesser (Abbasi & Abbasi, 2011).

However, such small schemes may pose obstacles to migrants: 1) low head impounding structures (hereafter referred to as weirs) and flow depleted sections of river can halt or delay migration just as can large dams (Powers & Orsborn, 1985; Kemp *et al.*, 2008; Kemp & O'Hanley, 2010; Lucas *et al.*, 2009). ; and 2) fish may be distracted from their course upstream to follow flow coming from the turbine (Arnekleiv & Kraabøl, 1996). The effects of these two potential obstacles are related to the absolute and relative flows going through the diversion and depleted stretch of river, and thus they are inked by the abstraction regime of the hydropower scheme.

This chapter aims to determine characterise the behaviour of upstream migrating adult salmonids at low-head hydropower schemes. Three schemes with differing configurations and operating regimes are compared in order to identify features which may influence passage behaviour. The possible influence of environmental and operating conditions on behaviour is also explored. In the following sections, the potential impacts of such small, low-head schemes on upstream migration are briefly reviewed. The effects on guidance upstream in the face of competing flows from the turbine and the depleted main river (depleted stretch) are addressed first, after which the effects of the physical obstacles posed by the weir are considered. As a final consideration, potential effects on the downstream migration of post-spawned anadromous salmonids are considered.

4.1.1 Attraction to competing flows

Water current is an important cue for the orientation of upstream migrants (Binder *et al.*, 2011). There is an established general view that upstream migrating salmonids approaching a channel divergence will favour the route with higher channel velocity in the absence of other navigational cues (Weaver, 1963; Banks, 1969). This tendency may be explained as an evolutionary trait which enables fish to reliably orient and move upstream, and hence increase eventual spawning opportunities (Williams *et al.,* 2012). Swimming strategies can however vary under different flow conditions, with efficient upstream movement occurring away from the highest velocity when overall current velocity is slow, switching to higher energy cost use of the main discharge where overall velocities are higher (Hinch & Rand, 2000, Standen *et al.*, 2004). Progression upstream is sometimes greater than can be explained by swimming speed alone (Hinch *et al.*, 1996, Hinch & Rand., 1998), which has led to the suggestion that migrants make use of reverse currents and vortices (Hinch & Rand, 2000). Indeed it has been shown under labotatory conditions that fish use turbulence vortices to decrease the energy cost of locomotion (Liao *et al.*, 2003).

Several studies have shown that complex flow patterns and large turbine flows at hydropower tailraces hinder migration (Webb, 1990; Karppinen *et al.*, 2002; Thorstad & Økland 2003). In large hydropower situations, successful bypass passage is often positively related to spillway flow, and negatively related to turbine flow (Thorstad & Økland 2003; Lundqvist, *et al.* 2008) which is consistent with the general opinion that upstream migrants are attracted to areas with the highest water discharge. Williams *et al.* (2012) point out that upstream migrating salmonids tend to seek areas with higher velocity gradients, and also that when encountering high discharge they will move as far as possible into this discharge. For large hydropower situations, because fishway flow is usually very small compared with turbine flow (5-10% in the UK, France and USA (Williams *et al.*, 2012)), these concepts have led to the positioning of fishways as close as possible to both the barrier and the turbine discharge. The fishway entrance is preferentially oriented so that fish move into it with the overall discharge, and the attraction flows at the entrance are often supplemented to provide large enough volume, velocity and turbulence characteristics for effective attraction (Williams *et al., op. cit.*). It has also been recommended that the fish pass and turbine outflows should be close together in certain small hydropower situations, where the flow from the turbine is directly below the weir (Clay, 1995; Larinier, 1998; Armstrong *et al.*, 2010; Robson *et al.*, 2011), as distinct from those situations where the outflow is some distance downstream from the barrier.

For attraction to fish passage facilities, Cowx & Welcomme (1998) emphasize the need for a strong attraction flow, which is either at, or equal to, the main channel velocity. This is supported by the Environment Agency Fish Pass Manual (Armstrong, 2010), which states that this attraction flow should be *"discernible to the fish amongst all the other competing flows and from as far away as possible"*. The manual further recommends that fish passes include an a velocity greater than 1 ms^{-1} at the downstream entrance, and preferably of the order of 2 - 2.4 ms^{-1} for large salmonids, and that where a pass entrance is located competing with the flows from low-head hydro turbines the velocity of the pass jet should be at least twice that of the turbine outflow, and that fish pass discharge should be at least 5-10% of maximum turbine discharge. A search of the literature has revealed no recommendation on maximum velocity from small hydropower turbine outlets, other than in relation to the fish pass requirements. Maximum velocity at two of the study sites used here was set by the SEPA at 0.5 ms^{-1}, and this is common practice in Scotland (A. Duguid, 2016, pers. comm.).

The current recommendations regarding the positioning of turbine outflows and fish passage routes, and their absolute and relative attractant flow volumes and velocities is based on experience at large hydropower situations and the concept that upstream migrants follow the greater flow. However, experience with large hydropower also shows that the effectiveness of such strategies can be site specific. The apportionment of competing flows and configuration of attractant routes at low-head schemes is highly variable between sites, and the effectiveness of such general guidelines remains to be tested.

4.1.2 Physical barriers

Both the depleted stretch and weirs used by small hydropower schemes disrupt river connectivity to some extent for migrating fish. They can act as partial barriers, allowing some but not all species, populations or individuals to ascend (Powers & Orsborn, 1985; Kemp *et al.*, 2008; Kemp & O'Hanley, 2010; Lucas *et al.*, 2009). As the depth, head and velocity of water over these sections and in-stream structures change with discharge over them, the ease with which they can be traversed by fish with particular swimming and jumping abilities also varies. The concept of partial barriers has led to the development of tools for qualitative assessment of obstacles for fish passage based on current knowledge of swimming, jumping and climbing ability, and minimum depth requirements for a variety of species (Kemp *et al.*, 2008). Swimming performance varies between species and is influenced by body length, physiological condition, and water quality factors, particularly temperature (Wardle, 1975; Beamish, 1978; Blake, 1983; Beach, 1984; Videler 1993). Evaluations of leaping ability (e.g., Stuart, 1962; Powers & Orsborn, 1985; Holthe *et al.,* 2005; Ovidio *et al.*, 2007) have shown that at hydraulic drops or waterfalls, the factors influencing leaping success are head, depth of pool, hydraulic characteristics of the drop (e.g. the presence of a standing wave), and water velocities above and below the falls.

The operation of hydropower schemes has the potential to reduce passage opportunities during the migration periods by depleting flow and thus altering the hydraulic parameters discussed above. Fishways at low-head hydropower weirs establish or improve connectivity (from a fish viewpoint) by providing a route with more suitable hydraulic characteristics for a fish's movement capabilities and behavioural tendencies. For a fishway to function it must successfully attract fish to the entrance as well as provide an effective passage route. Here again there is the issue of competing attraction flows, particularly where there are large volumes of flow in regions away from the fishway, attracting fish to areas which are ultimately less easy to pass.

4.1.3 Potential effects on post-spawned fish

After spawning, a portion of adult Atlantic salmon and sea trout emigrate back to sea during winter and spring (Jonsson & Jonsson, 2009). The potential impacts of low-head hydropower scheme passage on these downstream migrants are analogous to those faced by smolts: damage from passage through turbines and associated infrastructure, and delay to migration, with implications of predation and loss of condition, and therefore survival. The migratory behaviour of kelts is not well understood, but there appears to be a dichotomous strategy of migration, whereby some kelts rapidly leave the river after spawning, whilst others overwinter in freshwater, migrating to sea in spring (Haltunen *et al.*, 2013).

4.1.4 Aims

In this chapter, telemetry data from fixed loggers at three small AST hydropower schemes with very differing site characteristics is used to attempt to identify the important commonalities and differences that influence Atlantic salmon passage behaviour and success at such schemes. Whilst mention is given to a small number of sea trout and brown trout captured and monitored at one site, the focus is on the larger overall sample of Atlantic salmon at all three sites.

Emphasis is given to the effect of turbine mediated modification of flows through alternative channels within the area of river between the points of water abstraction and return (hereafter referred to as the Scheme Affected Zone, SAZ) on behaviour and passage through this region. The present study is restricted to quantifying the passage behaviour at three different ASHT schemes, and although reference is made to obviously discernable differences in barriers between schemes, no formal analytical relation is made between barrier characteristics and fish passage behaviour.

4.2 Methods

4.2.1 Study sites

The movements of adult Atlantic salmon were monitored using logging radio and passive integrated transponder (PIT) receivers at three hydropower schemes.

Locations within Scotland are shown in Figure 4.1. Craigpot (57.258°N 2.620°W) and Strathdon (57.195°N, 3.039°W) hydropower schemes are both located on the River Don in Aberdeenshire, North East Scotland. Craigpot is located on the middle reaches of the river, and Strathdon on the upper catchment. Philiphaugh hydropower scheme (55.538° N, 2.875° W) is on the River Ettrick, a tributary to the River Tweed, South Scotland. For each scheme the layout, important structures and operational aspects affecting fish passage are detailed below. A plan view of each site is given in Section 4.2, where monitoring instrumentation is described.

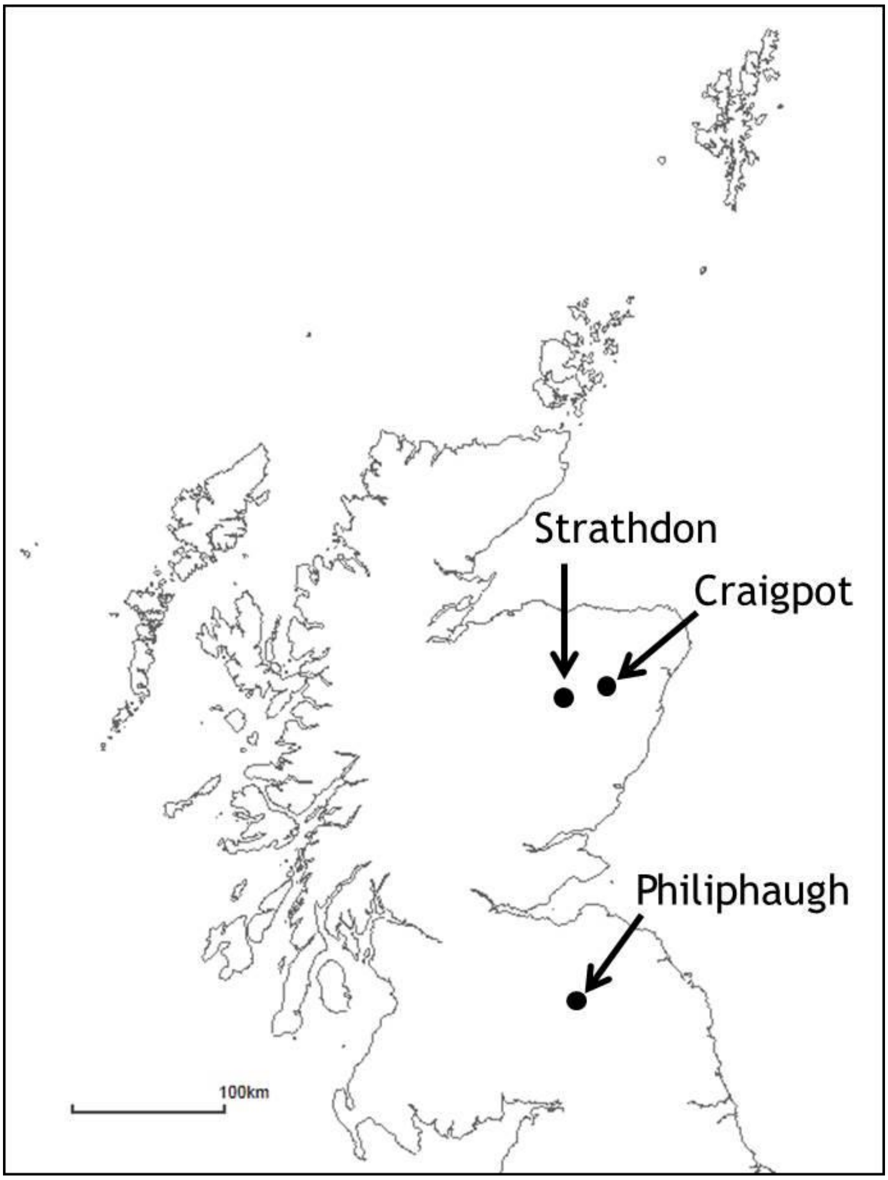


Figure 4.1. Location of the study sites.

4.2.1.1 Craigpot

For a full description of the layout and operational regime of Craigpot, the reader is referred to Chapter 2, section 2.2.1.

4.2.1.2 Strathdon

Strathdon hydropower scheme comprises a 70 kW capacity generator and maximum abstraction 2.9 m^3s^{-1} turbine, with its intake above a weir and perpendicular to the direction of river flow. The intake is a buried, 165 m long pipe which leads to the turbine house. The water exits the turbine into a 5 m wide, straight, open channel with a level bed of natural river gravel, which opens to the river 95 m downstream. At the time of this study there was no screen preventing upstream moving fish from entering this channel. The weir is a concrete construction, 23 m wide with a variable sloping profile (average slope 19 %), with the upper part steeper than the toe of the weir. Total head is 2.3 m. The weir is generally accepted to be impassable to adult Atlantic salmon, except perhaps at high flows. An upstream fish pass is located in the centre of the weir, and is of the pool and weir type, with four pools, 1.7 m wide and 1.65 to 2.03 m long, with equal step heights between adjacent pools. The depleted stretch of river between the weir and exit of the turbine outflow is 422 m long, with average bank to bank width 15.3 m, and total head of 1.5 m. This scheme differs from Craigpot in that only a minimum hands-off flow (HOF) of 1.52 m^3s^{-1} equivalent to Q_{90} at the site is required to pass down the depleted stretch. The turbine control system maintains this flow by adjusting a hydraulic sluice in the intake to ensure a minimum level in the pool above the weir, as monitored by a level sensor.

4.2.1.3 Philiphaugh

Whereas Craigpot and Strathdon have depleted stretches of several hundred metres between the impoundments and turbine outflows, Philiphaugh has two tubines mounted directly on the weir (known as Philiphaugh Cauld), so that only the flow over the weir itself is reduced by the abstraction. The weir is 120 m long, and the total hydraulic head has been reported as 2.7 m during a survey at Q_{41}, and 2.5 m at mean to low flows (Kibel, 2010). There is a hydraulic jump at the toe of the weir, which in profile has a variable slope (average 23%), with steeper toe and crest sections, and a flatter middle section. Fish attempting to pass the obstacle are able to jump the steep toe and swim up the flatter middle section, but not cross the crest section (Fishtek, 2010; personal observations, October 2014). Until the installation of the turbines and new fishway, fish passage was provided by a pool and weir fishway in the center of the weir. This is no longer operational.

The fish pass is a 1.8 m wide super active baffle Larinier fish pass, and is positioned directly adjacent to the turbines, 8 m from the left wall of the outflow basin, and approximately 70 m from the right hand edge of the channel. The pass has two rectangular section flights set at 15% gradient, separated by a 5.5 m length x 1.8 m width x 1.5 m depth (at Q_{90}) resting pool. The lower flight is 9.59 m long and the upper flight is 10.16 m long. The baffles are fixed to the base of the channel, are 0.1 m high, and are moulded polyethylene in a chevron pattern. At the upper end of the pass, the side walls extend for approximately 0.7 m beyond the edge of the fish pass. The turbine intakes are directly to the left beside this wall. To the right by 1.3 m, a steel piling extends 3 m upstream parallel to the fish pass walls. A series of stop log guides form a box around the top of the pass extending 2.7 m upstream, bounded by the piling on the right, and with its left edge in line with the left wall of the pass. A studded polyethylene eel and lamprey pass is affixed to the weir adjacent to the fish pass, and since the time of the study, a smolt chute has been installed near the southern end of the weir.

The maximum flow through both turbines combined is 12 m^3s^{-1}. The abstraction regime is equivalent to that at Strathdon. The only requirement is that a minimum flow of 1.81 m^3s^{-1}, equal to Q_{90} is maintained over the weir and fish pass. Above 14.26 m^3s^{-1} (Q_{30}) the turbines reach their design flow and flow over the weir increases. Below Q_{90} the turbines must cease to abstract. Flow through the turbines is limited by hydraulically powered sluice gates positioned at the leat intakes. These gates are controlled by a programmable logical controller, which uses as its inputs the water level from two level sensors: one upstream of the gate, and one between the gate and the turbine. There is an additional level sensor downstream of the turbines for control and power output calculation purposes. The variable speed turbines reduce their speed as flows reduce below Q_{30}, until such point (Q_{50}, 8.08 m^3s^{-1}) that controlling the speed can no longer maintain the necessary head above the weir, at which point one turbine will shut down and the other increases its speed to abstract the permitted amount.

4.2.2 Monitoring setup

The radio receivers (models varied between sites, reported below) monitored antennas arranged to observe fish movements, identified by coded radio tags, into and out of distinct zones at the schemes (approximated as the red shaded zones [measured in range tests] in Figures 4.2, 4.3, and 4.4). Receivers were housed in the turbine house where possible, and also in weatherproof boxes where the cable distances were too great to make this practical (greater than 500m). Broadly, the detection zones were defined as: the approach to the scheme from downstream (approach zone), the regions within which flows are modified by the scheme (SAZ), and the area above the scheme (exit zone). Because of the differences in site layout, the specific arrangement of antennas covering the SAZ varied somewhat: at Craigpot and Strathdon, the downstream entrances of each of the turbine and depleted stretch outflow were monitored with short range antennas, whereas at Philiphaugh, only the turbine and fish pass outflows were specifically monitored, the rest of the weir being within the range of the approach zone antenna. Thus the configuration at Philiphaugh was aimed specifically at investigating attraction to, and passage from the outflows without the ability to distinguish between broader approach movements and time spent attempting to ascend the weir. At the other two schemes, the aim was to observe total time in the turbine outflow and depleted stretch (from outflow to weir) combined. Radio detection zones at Craigpot and Strathdon were monitored using ATS R4520C receivers (at 150MHz) with antenna switching.

The setup at Craigpot in 2013 involved four detection zones monitored by a single receiver switching between antennas. This was modified in 2014 when more receivers were available to include more detection zones and reduce scan time between antennas: one receiver scanned the approach and exit zones, and another scanned each of the turbine and depleted stretch outflows (configurations differing between 2013 and 2014 are labelled in Figure 4.2). An additional frequency was scanned by these receivers. The set of tags in 2014 included this additional frequency in anticipation of a larger sample size, in order to reduce likelihood of code collisions for fish co-located at the site. For monitoring the downstream movement of kelts, additional antennas and zones were added in the intake channel (A5.1 and A5.2 in Figure 4.2), monitored by a dedicated receiver.

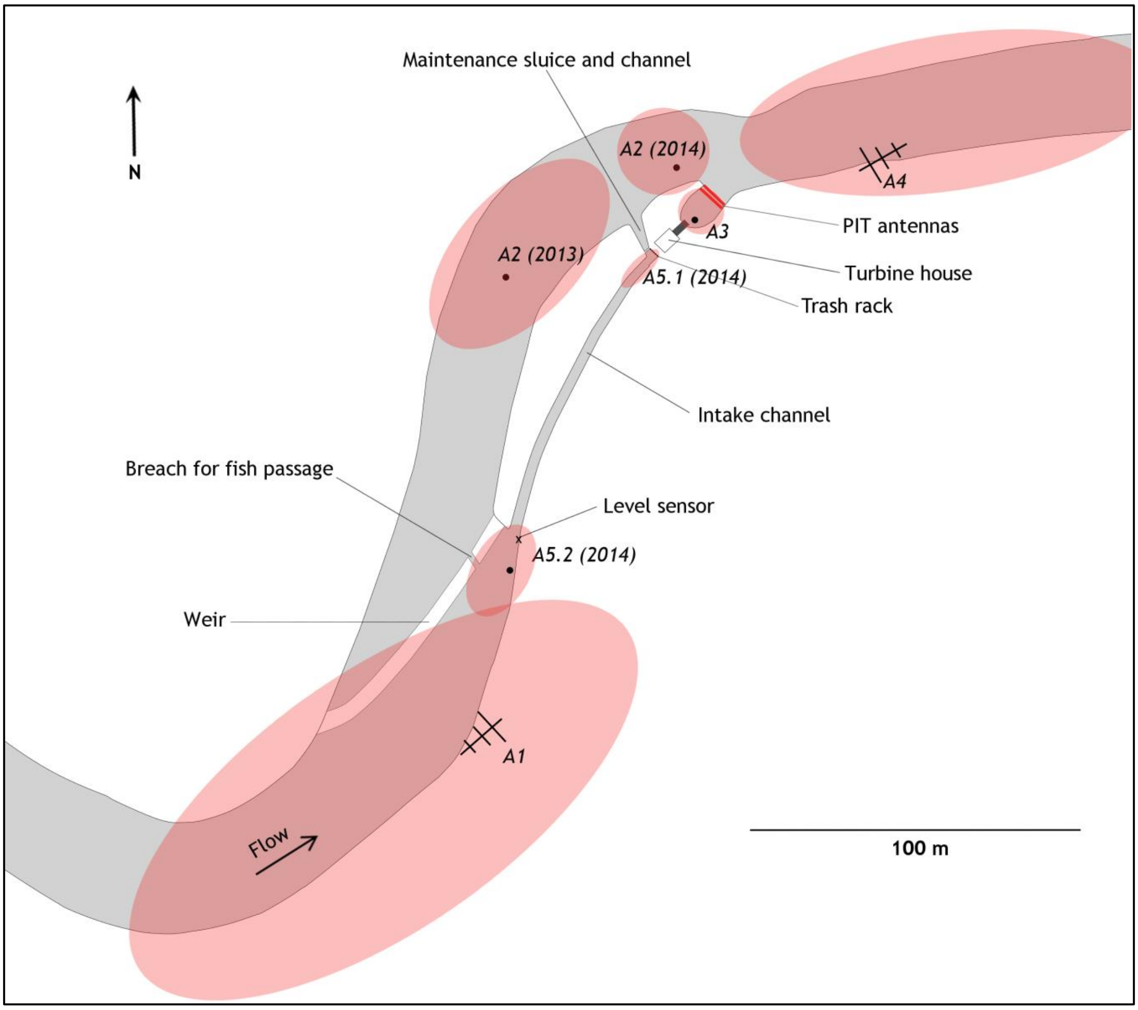


Figure 4.2. Craigpot site layout with radio and PIT monitoring configuration for adult migration study in 2013 and 2014. Red shaded areas approximate the zones monitored by radio antennas. The hatched symbols represent Yagi antennas, and black dots are dipole antennas. Red lines are PIT antennas. Here A4 is the approach zone, the region between A4 and A1 is the scheme affected zone, and A1 is the exit zone.

At Strathdon, six detection zones were monitored using three receivers, each switching between two antennas each. One receiver monitored the approach and depleted stretch outflow zones with a Yagi antenna and short range dipole antenna respectively, another at the downstream opening of the turbine outflow channel (underwater dipole) and a point midway up that channel (Yagi). The approach and exit zones at the weir were monitored with the third receiver and two Yagis. Receivers monitored two frequencies as for Craigpot in 2014 (150.102 and 150.162MHz).

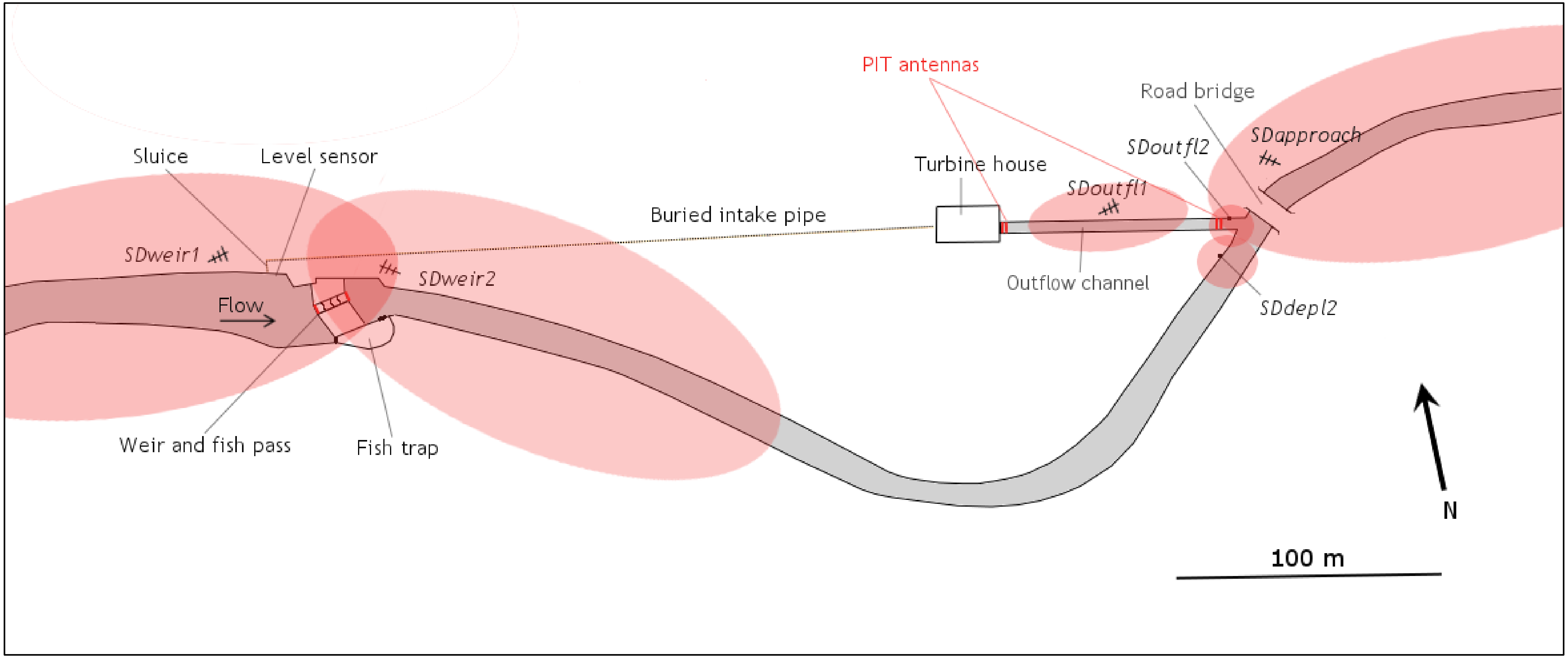


Figure 4.3. Strathdon site layout with radio and PIT monitoring configuration in 2014. Red shaded areas approximate the zones monitored by radio antennas (marked with symbols). The hatched symbols represent Yagi antennas, and black dots are underwater antennas. Red lines are PIT antennas. Here SDapproach is the approach zone, the region between this and SDweir1 the scheme affected zone, and SDweir1 is the exit zone.

At Philiphaugh, the two broad range zones covering the approach and exit pools below and above the weir were monitored using two Yagi 4-element antennas pointing downstream and upstream. These were connected to a Lotek SRX400 receiver equipped with a switcher that enabled the sequential monitoring of each zone at a fixed interval. The switching time was set at seven seconds, which was the maximum burst interval for the transmitters to be used. On 27 October 2014 the switching configuration was modified in order to scan an additional frequency due to coded tags at 173.800 MHz being deployed in addition to those at 173.845 MHz. This effectively doubled the period of time taken for the receiver to scan each antenna, as the receiver stepped between each frequency and each antenna every seven seconds. The short range zones at the upstream and downstream openings of the fish pass, and of the turbines (labelled FPU, FPL, TU and TL respectively in Figure 4.4) were monitored using underwater antennas connected to Sigma 8 Orion receivers. All underwater antennas were stripped coax cable with length 0.5 wavelengths in water (9.4 cm for 173 MHz). These were housed in PVC pipe affixed to wood. The pipe was pre-bent so that the antenna is held parallel to the direction of ascent of fish, and antennas were fixed at half water depth.

Antennas TL and TU (turbine lower and turbine upper, Figure 4.3) each comprised a pair of antennas joined with a BNC splitter so that there was an antenna on each side of the concrete wall dividing the flow. Initial tests with single antennas on each side of these walls showed that it was an effective block to the transmitted signal at the tested receiver gain. Tests with tags at each of these split antennas showed no evidence of a reduction in detection efficiency or range resulting from using the simple BNC splitters rather than more expensive isolating combiners.

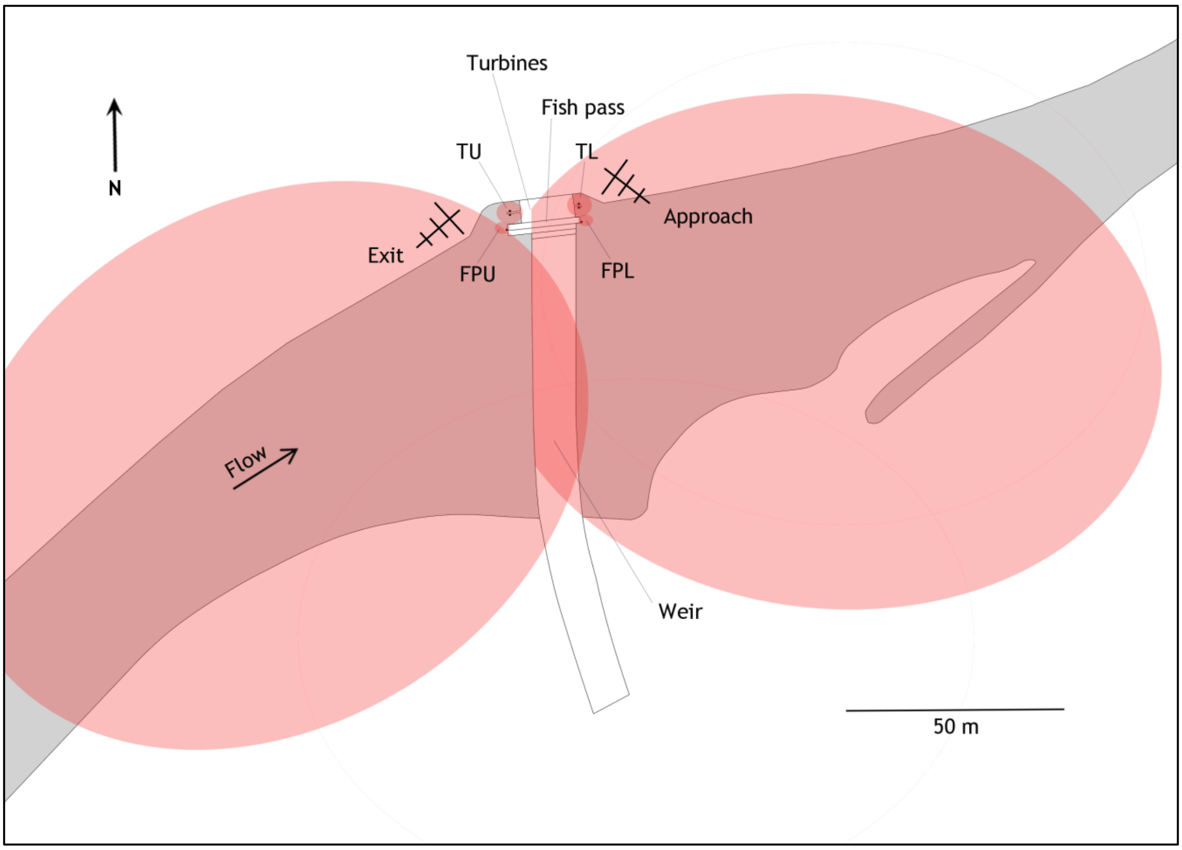


Figure 4.4. Philiphaugh site layout with radio monitoring configuration in 2014. Red shaded areas approximate the zones monitored by radio antennas. Here, TL and FPL collectively are the outflow zone.

Where possible, PIT detection equipment was used to gain high resolution data on movements of fish into and out of the turbine outflows. At Craigpot the turbine outflow was monitored by a single PIT reader driving two antennas spanning the entrance to the outflow and separated by a metre (see Section 2.2.2.1). At Strathdon, where the outflow is a long open channel the entrance to this channel was again monitored by a single reader driving two antennas separated by a metre, and an additional antenna was added immediately downstream of the turbine to detect fish ascending all the way to the top of this channel. In addition to this, PIT antennas were installed at the entrance and exit of the fish pass at Strathdon, in order to detect passage of PIT only tagged fish. At Philiphaugh, initial tests by the Tweed Foundation using PIT equipment (and a protocol developed through experience at Craigpot and Strathdon) showed very limited range of detection because of electrical interference from the larger turbine generators, and so PIT monitoring was not used at that site.

4.2.3 Fish capture, tagging and release

All procedures were carried out under UK Home Office Licence and complied with the UK Animals (Scientific Procedures) Act 1986. Several methods were used to capture fish for monitoring at the three sites. For Craigpot, fish were seine netted at Kemnay, 14 km downstream of the hydropower scheme, from August until November in 2013, and from June to November in 2014. When netting yielded no Atlantic salmon in 2014 additional fishing effort by rod and line was made during November and December. The original intent was to tag as many fish downstream of Craigpot as possible and then monitor those fish at Strathdon as they moved to spawning sites further up the catchment. Due to the lack of capture success in 2014, the fixed fish trap at Strathdon weir was used to catch the sample for that site. At Philiphaugh fish were captured in the shallows below the weir pool during high flows, by corralling followed by dip netting. Capture and tagging at Philiphaugh was carried out by the biologists of the Tweed Foundation.

After capture, fish were placed in a holding cage positioned within the flow of the river, or in an aerated bath, until tagging. Due to the large number of fish captured in the first trapping session at Strathdon (45 Atlantic salmon, 10 September 2014), these fish were transported by trailer tank to a broodstock holding pen in a small stream 270 m from the trap, from where they were tagged the following day. Prior to tagging fish were anaesthetised by immersion in a solution of benzocaine (*ca.* 30 ppm), prepared by drop-wise addition of 100g/l solution in alcohol to an induction tub of river water. Anaesthetisation followed the procedure described in Chapter 2, Section 2.2.4. The fish's gills were aspirated with fresh river water during surgical tagging, using either a gravity-fed hose into the mouth or a squeezable bottle. Fork length (mm), depth of body (taken dorsoventrally immediately anterior to the dorsal fin), head-width (immediately posterior to the eyes) and sex of the fish was recorded. Only fish that were judged to be in healthy condition and estimated to be greater than 1.25 kg in mass were tagged. A coded radio tag was selected, activated, and its unique code and frequency recorded before tagging. Percentage tag:fish mass burdens are reported in Results Section 4.3.1.

Transmission of radio tags was verified using a radio receiver, and for fish that were PIT tagged, the PIT ID was recorded prior to tag insertion. For the Atlantic salmon, radio tags were inserted oesophageally, and PIT tags surgically, whilst for sea trout both radio and PIT tags were surgically inserted. For those fish which were surgically tagged, a longitudinal incision (of 6 mm for the PIT tag or 16 mm for the small radio tag and PIT tag) was made on the left ventral side of the fish anterior to the pelvic girdle, with enough space between the incision and the pelvic girdle for the tag to sit against the body wall parallel to the antero-posterior axis. The tag was inserted and the incision closed with single interrupted sutures: one for the 6 mm incision, or three for the 16 mm incision. Dissolvable suture was used for closure (4-0 Vicryl Rapide, Ethicon Ltd, Livingston, UK). For those fish that were oesophageally tagged, the tag was placed in the mouth of the fish and gently pushed down the oesophagus using a tagging rod. An increased resistance to the push indicated that the tag was firmly lodged. Each fish was then visibly marked with a floy tag at the base of the dorsal fin, and the floy tag reference recorded.

After tagging, fish were moved to a tub of fresh water, where scale samples, (three scales from each flank) and an adipose fin clip (stored in alcohol in a referenced epindorph tube) were taken. Finally, the fish was weighed (except at Philiphaugh, where tagging operations were carried out by biologists of the Tweed Foundation, and this step was omitted for expediency) and allowed to recover in the river whilst supported by hand, ensuring that each fish was seen to swim off in a healthy condition. The time from introduction to anaesthetic until the last reintroduction to fresh water was recorded. Holding, induction and recovery tubs were refreshed with river water at frequent intervals for longer tagging sessions.

Fish tagged at Kemnay were released at the capture locations, 13.7 and 14.7 km downstream from Craigpot (57.224°N, 2.466°W and 57.231°N, 2.460°W). Fish captured by fixed trap at Strathdon were released 4.7 km downstream from the hydro scheme (57.215°N,3.000°W). The only two fish that were caught by rod and line at Craigpot in 2014 were transported 2.5 km downstream for release at 57.29°N, 2.591°W. At Philiphaugh, fish were released in the next pool downstream from the weir by 150 m, and out of range of the approach zone radio antenna.

4.2.4 Environmental and operational data collection

For each scheme, 15-minute interval river discharge (Q) as recorded by the nearest flow gauging station during the study period was obtained from SEPA. The gauging stations used for each scheme were: Craigpot – Alford station (57.242^{o}N, 2.720^{o}W), 9.86 km upstream; Strathdon – Culfork station (57.180^{o}N, 3.110^{o}W), 7.72 km upstream; and Philiphaugh - Lindean station (55.574^{o}N, 2.817^{o}W), 6 km downstream. Turbine operational data were provided by Highland Ecodesign and Mannpower Consulting for Craigpot and Strathdon, and by KC consulting for Philiphaugh. Fifteen-minute turbine log files for Craigpot comprised of the generator output (kW), turbine rotational speed (RPM) and weir crest level (mm) as measured by a level sensor above the weir. This was converted to flow over the depleted stretch (Qweir, m^3s^{-1}) and flow through the turbine (Qturb, m^3s^{-1}) using Equation 2.2, Section 2.2.1. For Strathdon, 15-minute generator output and level above weir was supplied. At present no relationship has been derived to calculate Qweir from weir level, but Qturb (m^3s^{-1}) was estimated as power output (kW) divided by 34.13, the relationship advised by the operator. For Philiphaugh, 15-minute average level above weir, and daily abstraction totals were supplied. Here turbine flow (m^3s^{-1}) was estimated as power output (kW) divided by the product of the turbine efficiency (the ratio of theoretical to actual power output, taken as 0.76), the head difference (taken as a constant 2.56 m) and the constant of acceleration due to gravity (9.81 ms^{-2}).

Temperature (T, ^{o}C) was logged at 15-minute intervals (Fourtek Picolite loggers, USA) at mid channel depth, in the intake channel at Craigpot and above the weir at Strathdon. Temperature loggers were installed at Philiphaugh but due to an unexplained malfunction with the loggers, no temperature data were logged for that site.

Sunrise and sunset times were calculated as in Chapter 2, Section 2.2.5. Here dawn and dusk were taken as the time at which the sun reaches 8 degrees below the horizon.

4.2.5 Data processing

The format and content of logged radio data differed between receiver types. The ATS receivers store the number of detections per transmitter code within a user defined interval – the store rate. This was chosen as the lowest resolution – one minute for short range antennas, and two or five minutes for broad range detection zones. The logger stores the code, with time of maximum signal strength within the interval, along with signal strength (on an arbitrary scale from 40 to 154), and the antenna number. In addition to scanning each antenna sequentially, these receivers also scan all antennas simultaneously for one scanning interval per scan cycle. Any detections in that interval are labelled as antenna zero. Records on antenna zero were not used in the analysis since they are not zone specific, but proved useful for confirming presence over false detection for short reads. The Lotek SRX 4000 was set to store continual detections within five minutes as a single record, to reduce data and thereby limit the possibility of reaching full storage capacity between downloads. Detections were stored as the transmitter code, signal strength (on an arbitrary scale from 0 to 235) time of detection and antenna number. The Sigma Eight Orion receivers store each transmission individually with the code signal strength and time of detection.

Log files from the radio receivers were collated into comma delimited format (*.csv) by site and receiver type. These were imported into R along with environmental and turbine operational data, where all joining, processing, manipulation and analyses were carried out.

Checking, cleaning, filtering and formatting the radio log files was an iterative process, which began with establishing the quality and extent of receiver coverage from the logged radio data. The process for data management and interpretation at Philiphaugh was the most complex because of instances of receiver down time, and so more description is given for that site. Both the Lotek and Sigma Eight receivers which were used at Philiphaugh store a status signal (every four or five seconds for the Orion receivers, and every hour for the Lotek receiver) which served as a check on the continuity of operation at this site. These signals were plotted over time for each receiver to identify periods of receiver down time and any fish presences which may have been only partially recorded due to this. These presences were later marked as censored (incomplete) at the time where they were truncated, so that they were treated appropriately within the analysis. The ATS receivers that were used at Craigpot and Strathdon do not log status signals, but these receivers were never found to have stopped recording during the study. This does not preclude the possibility of incomplete data due to receiver-antenna connection faults, but several range tests during the study showed no such problems. Despite this the data show that one fish was recorded above the weir without having been recorded at the lower antennas for that visit. This fish was excluded from the analyses of time in the monitored zones.

Where fish were tagged within range of the broad range antennas, detections of these fish less than an hour after release were removed. At both Strathdon and Philiphaugh there were records of transmitter codes that were not present in the tagging set. Since there was little possibility of tagged fish at these sites from another study, these “ghost” codes and along with logged noise signals could be either the result of electrical noise in the environment, or of co-located tag transmissions colliding to produce codes which were not in the tagging set, or error signals. These records were plotted with time in order to identify periods of uncertain data quality. Having identified these windows, they were checked in detail in order to ascertain a probable cause where possible, and whether data from these periods could be used or should be excluded from the analysis. This process also involved the plotting the individual fish detection series. Then false detections of real codes – for example detections which were not in line with the possible sequence of antenna visits – could be identified. All false detections and noise signals were then removed from the data.

In order to demarcate individual fish detection series into discrete visits, thresholds of gaps in detection were chosen based on the frequency distribution of the lag intervals between detections at each zone (Castro-Santos & Perry, 2012). When the log-frequency distribution is graphed as a histogram (Figure 4.5, for example), there is usually a rapid decrease in frequency of the shortest intervals with increasing duration, followed by a levelling off for longer intervals. The initial decrease is assumed to be the result of missed transmissions due to incomplete receiver efficiency, whilst the later more uniform part of the distribution is assumed to be actual behaviour: fish leaving the zone and returning at those intervals. Thus a visit separation threshold is chosen where frequency begins to stabilise. For the monitoring setup at these schemes, the separation threshold for the approach zone was always larger than for the scheme-affected or outflow zones, and thus this defined visits to the scheme as a whole.

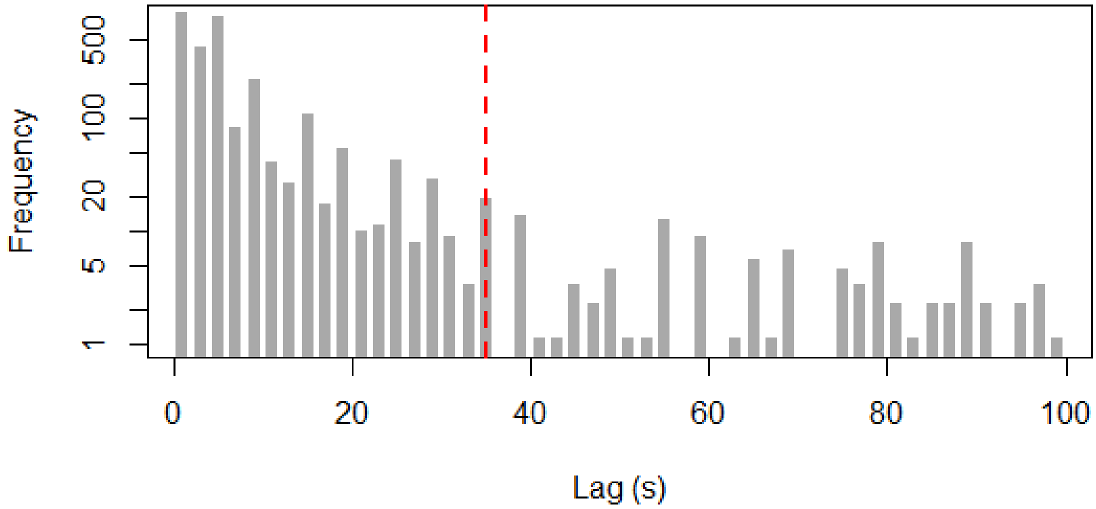


Figure 4.5. Log frequency distribution of lag intervals between subsequent detections for all detections in the zone below the turbines at Philiphaugh. Lag times are grouped into two second intervals. The x-axis is truncated to 100 seconds. The red dashed line indicates the chosen visit separation threshold.

The next task was to define presence intervals in each defined zone where fish transitioned between monitored zones. At Philiphaugh, where presences in the outflow zones were nested (spatially and temporally) in the approach zone, this was a case of splitting the detection series so that presences in short range zones were nested within, but not overlapping with, the presence in the broad range zone.

At Craigpot, the zones were not nested, but concurrent detections did occur. There are three potential causes for this:

1. actual overlap in antenna range;

2. very strong signals bleeding through the antenna switcher to be recorded at antennas other than the antenna being monitored; or

3. rapid movements of fish between zones resulting in records at more than one zone per minute.

Where possible these possibilities were addressed by processing the data with reference to the antenna setup: If detections between A2 and A1 overlapped, preference was given to A2 because during testing, the range of this antenna was entirely below the weir, whereas antenna 1 occasionally picked up signals from below the weir.

Entry to the scheme affected zone was defined as any upstream movement from the approach zone, as detected by radio antennas. More specifically: any detection at A2 or A3 was considered an entry. Start- and end- times for presence in the scheme affected zone were defined as follows:

1. If detection periods at A2 or A3 began prior to the end of a detection period at A4, time of entry was the earliest detection at A2 or A3.

2. If detection in A2 or A3 began after the end of a detection period at A4, time of entry was the time of last detection at A4 (the rationale for this is that if a fish is next detected in the combined outflow zones but is no longer detected at the broad zone, we can infer that it has actively moved from the broad zone towards the outflow areas).

3. If the end of a period in A2 or A3 was before the end of a period in A4, the exit from the scheme affected zone was taken as the first detection at A4.

4. If detection at A2 or A3 extended beyond detection at A4, and there was no return to A4, exit from the scheme affected zone was taken as the first detection at A1 where there were no further detections at A2 or A3.

5. Four minutes was identified as a threshold for new visits at the two outflow antennas. However where this lag was exceeded but there were

no new detections at the approach zone exceeding four minutes, this was considered a single visit as there was no confirmation of the fish having left the defined combined zones for more than the threshold.

At Strathdon these same rules were used for time of entry to and exit from the scheme affected zone. Here, presences in the approach and scheme affected zones at each antenna could be nested (near the entrances of the turbine and depleted stretch outflows) or mutually exclusive (once within either the depleted stretch or turbine outflow).

Visits comprising just one detection at the scheme as a whole (with all zones combined) were omitted from the data: they provide no information on length of visit and are likely stray detections from fish downstream of the approach, or false signals. At Philiphaugh, single detections at the outflow were retained and given a duration of one second, since these can be considered fleeting locations at this zone. At Craigpot and Strathdon, single detections at either zone within a longer visit were given a duration of one minute, since that was the resolution of the logged data.

Passage was confirmed by detection at the exit zone antenna, followed by no further detections at downstream antennas. The time of passage at Craigpot has already been defined above. At Strathdon this was taken as the first detection at the exit zone after the last detection at the antenna below the weir. Range testing at this site revealed that the downstream antenna did detect signals from near above the weir, whereas the upstream antenna had range more exclusively above the weir.

At Philiphaugh the short range antennas at the entrance and exit to the fishway allowed a more precise time of transition from the bottom to the top of the fishway: the last detection at the lower end before detection at the top of the fishway.

PIT data

Detections from the PIT loggers at Craigpot and Strathdon were collated into .csv files by site. These were imported into R along with environmental and turbine operational data, where all joining, processing, manipulation and analyses were carried out. These data were used to characterise residence times in the turbine outflows. Data from the PIT equipment at the fishway at Strathdon were used to confirm passage of PIT only tagged fish there.

Both Craigpot and Strathdon had a pair of antennas installed at the downstream end of the turbine outflow. Detections at these antennas were converted into presences following the visit separation threshold method described for the radio data. Entry to the outflow was determined by sequential detections first at the downstream antenna (A2) and then the upstream antenna (A1), and *vice-versa* for exit from the channel. An entry presence succeeded by an exit presence, or a single presence beginning and ending at A2, then comprised a visit.

Presences that had detection sequences inconsistent with a visit were excluded (115 out of 459 presences). The PIT readers used were not capable of detecting more than one transponder concurrently within range of the antennas. Hence this relatively high proportion of anomalous detection sequences are likely due to the presence of other PIT tagged fish blocking detection at the antennas in this highly frequented region. The selection of only apparently complete visit sequences limits the results,

4.2.6 Data analysis

The telemetry data from these studies may be considered to provide two basic responses of interest with respect to the effects of these schemes on fish passage: 1) passage success (whether or not a fish that approached the scheme passed it); and 2) the time that fish spend at the scheme before passing, or failing to pass (Castros-Santos & Haro, 2003; 2010; Castros-Santos *et al.*, 2009; Cooke & Hinch, 2013). These responses can be considered per fish, or per visit. An additional potential measure of the effort spent by a fish in passing is the number of separate attempts made. These responses may be considered separately, by regressing each on the measured environmental and operational covariates, and fish metrics, but by these methods, each analysis ignores the other, connected responses. A possible avenue is to include the other responses as covariates, for example, a logistic regression with passage success as the outcome, and cumulative attempt time, or attempt number as covariates. A concise way to describe this type of data and examine covariate effects, is *time to event*, or *survival* analysis. Examples of texts describing these techniques are Allison, 1995; Hosmer & Lemeshow, 1999; Kleinbaum & Klein, 2012, and specific application to the analysis of telemetry data on migratory delay to fish is made by Castros-Santos & Haro (2003), Moser *et al*. (2004) and Castros-Santos & Perry (2012) and others. Here the response considered is the rate at which an event of interest happens, for example, the rate at which a sample of tagged fish originating downstream of a hydropower scheme, passes upstream. The rate is estimated by calculating the proportional depletion of the sample population over time due to passage. A major advantage of this method is that all of the available data may be used – those fish that are not recorded passing contribute to the sample population – or risk set – until such time that they are removed by a different, competing event (departure downstream, death or receiver down time).

A possible alternative is the use of classification and regression models. Bendall et al. (2012) used these models to good effect to explain variance in the migration times over river sections for adult Atlantic salmon. This method uses algorithms to determine a set of if-then conditions that permit the accurate prediction or classification of migration times using a series of predictor variables. This is attractive because for each classification, the data are dichotomised around a value of a covariate that maximises explanatory power. The threshold value can be interpreted as the important threshold influencing the response. For example it can reveal the flow value above which migration speed differs most from migrants below that value. Whilst this method is of use for analysing migration times over defined zones, it does not have the advantage of incorporating competing events and censored data, which are key elements of fish passage at an obstacle.

This proportional depletion with time is estimated using the Kaplan Meier method (Chapter 2, Kleinbaum & Klein, 2012) and, when plotted, is the basic visualisation for this type of data. The curve shows the proportion of individuals at each time step which have not yet had the event (or the probability of an individual remaining until that time without undergoing the event), and the size of each step downward is the proportion of the remaining population that pass after that time (or the probability of the event happening at that time).

For the radio data, upstream transitions were plotted as increasing functions – i.e. the proportion of visits arriving in the next zone over time, given persistence in the previous zone, and rejections as decreasing functions – the proportion of fish remaining in the zone which have not left downstream. These figures are a useful way of rapidly assessing the passage status for each site. Each curve represents transition and rejection events over time, and thus the point where the curves intersect along the y-axis indicates the overall prevalence of these events relative to each other. The position of the intersect along the x-axis indicates how quickly these processes occur relative to each other. For example, an intersection at greater than 0.5 on the y-axis and near to the origin of time means that rate of transition dominates rejection, and transition occurs quickly overall. This suggests little resistance to transition (for example, Figure 4.6A). An intersection at the lower right hand side of the plot indicates that rejection is higher than transition, and also that visits persist within the time range considered. This could be considered the worst case in the context of passage upstream: where visits persist without success (for example Figure 4.6B).

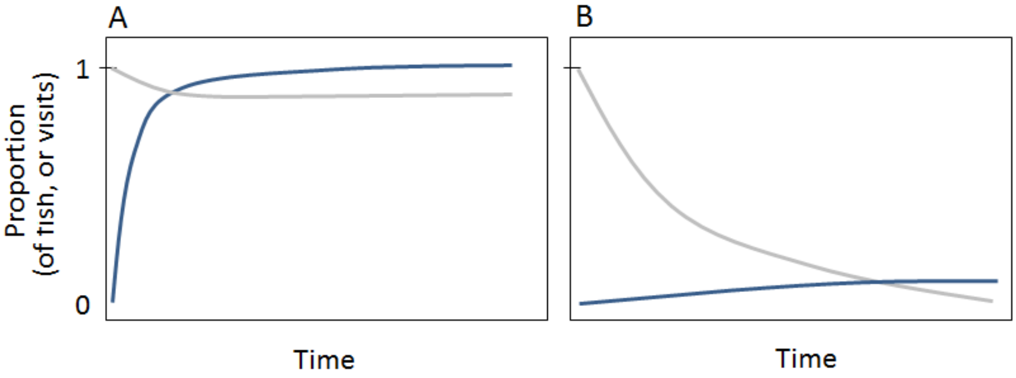


Figure 4.6. Exemplars for the cumulative rate of transition and rejection of a zone by a sample of fish, or number of visits by a sample of fish. The dark lines represent the proportion of the sample transitioning from the zone, and the grey lines represent the proportion of the sample remaining in the zone without rejecting it. A: rapid transition of a high proportion, and low rejection. B: slower transition of a low proportion, and high rejection. See text for explanation.

In practice these probabilities are computed by multiplying the sequential probabilities of the event happening, each estimated as the proportion of the population undergoing the event in the interval immediately after the observation, given that they remain until the end of the present interval (Equation 4.1, Kleinbaum & Klein, *op. cit.*). Thus for each observation time, the numerator of the probability for that time decreases by the number of individuals that underwent the event in the previous interval, whilst the denominator decreases by the number of individuals that are removed for any other reason than the event of interest (censored observations).

$$\hat{S}(t_f) = \prod_{i=1}^{f} \hat{P}\,[T > t_i | T \geq t_i] \qquad \text{Equation 4.1}$$

Another advantage of this form of analysis is that where there is more than one possible outcome (for example passage, or departure downstream), these can be considered as independent competing risks: the idea is that the rate of one potential outcome happening is not altered by the rate of any other event, but the sample population available for the outcome being examined is also depleted by other events through censoring. In the present analysis, two event rates were used for each of the two defined zones: 1) the rate of transition to the next zone (from approach to SAZ), or from SAZ to exit); and 2) the rate of rejection of these zones by downstream (and also lateral, in the case of the outflows at Philiphaugh) movement. For the transition event analyses, rejections were censored at their last observation, and for rejections, transitions were censored.

Cox Proportional Hazards (Cox PH) regression was used to investigate the relation between event rates and measured covariates. This is a semi-parametric model which, distinct from parametric forms of survival modelling does not require the fitting of a baseline hazard function. The baseline hazard may be viewed as the instantaneous probability of the event occurring with any covariate effects removed. Under Cox PH, only the *ratio* of hazards between a pair of covariate sets is estimated, so that the baseline hazard need not be specified. The model assumes that there is some baseline hazard which depends only on time, and that this is modified by an exponential term involving the covariates (Equation 4.2, Kleinbaum & Klein, 2012).

$h(t,\boldsymbol{X}) = h_0(t)e^{\sum_{i=1}^{p}\beta_i X_i}$ Equation 4.2. The Cox Proportional Hazard model.

Where:
*h(t,**X**)* is the instantaneous hazard, a function of time (t) and the vector of covariates (***X***),
h_0 is the baseline hazard function, which only depends on time
β_i is the coefficient describing the effect of the i^{th} covariate, $\boldsymbol{X}_i$.

By considering the ratio between two hazards with differing covariate values, the baseline term is cancelled out. The assumption, however, is that the effect of covariates is independent of time – in the case of fish passage, the ratio of the rate of passage between two individuals with differing covariate values is the same for all the time that they are present. For Cox PH regression, the data must be summarized into single records with a start and an end time. Here, the counting process format was used, where in addition to the transmitter code and start and end times, each record has a presence number, which allows for recurrent events (multiple visits) by the same individual, and a censoring indicator, which identifies each event type for each visit of each fish to a zone. This format is referred to hereafter as the event table, as distinct from the presence table, where each detection is a record. The time used in the analyses is then the difference between the start and end of each interval. These manipulations were achieved using the "dplyr" package in R (Wickham & Francois, 2014), and a counting function which generated presence numbers based on the chosen minimum separation time (modified from a sequence developed by Andrew Harbicht of Concordia University, and shared by Theodore Castros-Santos of the US Geological Survey).

The PH assumption does not mean that the hazard ratio may not vary over time for an individual because of time varying covariate values, only that the ratio for any two particular sets of covarites must be constant over time. Indeed, time-varying covariates may be introduced by splitting event table intervals into smaller intervals, and allowing covariates to vary between these. The beginning time of each new record is then equal to the end of the last, so that interval duration continues to increase across covariate changes rather than resetting to zero, which would indicate a new presence. New records due to covariate changes within one visit are given the censor zero to indicate that no event occurred. A user defined function split records over the dawn and dusk times, corrected start and end times of split records, and modified the censor appropriately. River flow, turbine operational data and temperature were averaged by the hour (excepting turbine flow at iliphaugh, which was available as daily mean) and joined to the event table. Here an outer join was used to replicate records for all covariate intervals, then only those records which were within or overlapping each hourly interval were retained, and finally the start and end times were modified where these were split by hours, and new "zero" censor indicators introduced for the split records.

Survival analyses were carried out using the "survival" and "coxme" packages in R. Transmitter code was included as a random effect, known as a frailty term, in order to account for any bias arising from repeated measurements of individuals with recurrent visits. Proportionality of hazards was checked using a goodness of fit test (function cox.zph in the "survival" package), and by examining plots of the Schoenfield residuals, which should appear approximately linear with time. Where the assumption was not met, log-log plots of the survivorship curves, stratified by the violating variable (split into quantiles if continuous) were used to identify points of violation. The form of the Cox PH model means that the vertical distance between these curves should be approximately constant. In order to address violations of proportional hazards (PH) the data were split into intervals within which the PH assumption was met, and a simple time interaction introduced using Heaviside functions. These are new variables which take on the value of zero outside the defined interval and the original value within it. Each interval is assigned a new variable so that the entire time range is covered. This is a simple way of introducing and testing whether covariate effects change over time. The process was carried out iteratively in order to meet the PH assumption for each model.

In the first instance univariate Cox PH regressions were carried out on each measured covariate, with addition of variables using Heaviside functions to meet the PH assumption where necessary. Different hydraulic measures were tested between the approach and scheme affected zones. The approach zones may be regarded as being unaffected by turbine abstraction or discharge, since they are below the outflow point. The exception is at Philiphaugh, where the approach zone included the extent of the weir, and so attraction to that area could well be mediated by turbine operation. Therefore, total flow was tested for the approach at Strathdon and Craigpot, and each of total flow, turbine flow and weir level were tested at Philiphaugh. Rates of transition and rejection from the scheme affected zone (Philiphaugh: outflow zone) were tested against turbine flow and weir flow where available (Craigpot), and weir level where not. The hypothesis here is that the time spent within the scheme affected zone (outflows) is influenced by the absolute flows through each channel. At Craigpot, where a weir flow was available, the ratio of these flows was also tested. The other tested covariates were: diel factor (day or night), temperature (Craigpot and Strathdon only), sex, length and Fulton's condition factor (Craigpot and Strathdon only, although depth:length ratio was tested for Philiphaugh). Models were then extended to two or more covariates with logical interactions for those covariates which were found to be significant, or near to significant ($p < 0.1$) in the univariate approach. Resultant models using the same datasets were compared using Akaike's information criterion (AIC) (Burnham & Anderson, 2010). At Strathdon, where temperature data were the most extensive, records without temperature were omitted from multiple covariate models to enable comparison by AIC between models with and without temperature.

4.2.7 Return movements of post-spawned fish

Following the monitoring of upstream adult fish movements, the radio receiving equipment was left in place in order to detect the downstream return movements of post-spawned adult Atlantic salmon (kelts). Additional detection zones were added at Craigpot, at the upstream entrance to the turbine channel, and near to the trash screen at the turbine (labelled A5.2 and A5.1 respectively in Figure 4.2). At Philiphaugh, the intake of the turbines was monitored using a short range underwater antenna. For all schemes, movements over the weir face could not be distinguished from movements through the designated fish pass.

Monitoring at Craigpot in the 2013/2014 season continued until 17 March, and in 2015, receivers were stopped on April 21 at both Craigpot and Strathdon. At Philiphaugh, the short range Orion receivers were removed in mid-December, but the Lotek receiver continued monitoring until June 2015

A visit separation threshold of 30 minutes was used at all schemes to define new presences of individual kelts. Two metrics were used to characterise passage behaviour: total duration from first to last detection at all receivers, and time until passage from the exit zone (above the weir) to the SAZ and approach zones. Several presences were recorded as single detections at the upstream zone, and these were taken as having a total duration of one minute. Where detections overlapped between the exit and downstream antennas, the same rules were applied as for upstream movements to assign time of entrance and exit from zones. In some cases, fish were not detected on downstream antennas, or final detections were at both upstream and downstream zones concurrently. This was likely either due to rapid movement downstream, or decreased antenna range after flood events. These cases were excluded from the calculation of passage times. Data on total duration and time until passage were sparse, and not normally distributed. Therefore comparison of these metrics between sites was limited to non-parametric Kruskall-Wallis, and *post-hoc* pairwise Mann-Whitney U-test comparisons between sites. For post-hoc tests, *p* values were adjusted for multiple comparisons using the Bonferroni correction.

In addition to the monitoring of post spawned fish using installed radio equipment, an experimental release into the turbine channel at Craigpot was carried out in February-March 2014. Whilst the sample of fish was very low (six), this experiment is reported briefly because it offers valuable insights into kelt movements in relation to such schemes. Six kelts (all females, mean FL = 704 mm, range = 610-796 mm) were captured by rod and line and PIT tagged with 23 mm PIT tags following the procedures described above. The kelts were released into the middle part of the turbine channel at Craigpot (between PIT antennas PLmid and PLbot in Figure 2.5, Chapter 2), in order to observe their movements in this channel and determine whether turbine passage occurs for kelts. Underwater CCTV monitoring was in place at the trash rack, as described in Section 2.2.2.3, Chapter 2. The first fish was released at midday on 22 February, and the following five were all released at 13:50 on 8 March.

4.3 Results

4.3.1 Captured and tagged fish

During 2013, four out of five seine netting sessions (all at Kemnay) resulted in fish captures, with 23 Atlantic salmon and 15 sea trout caught and tagged with radio and/or PIT tags for monitoring through Craigpot. During 2014, seven sea trout were captured and tagged, in three out of 11 netting sessions at Kemnay. No Atlantic salmon were captured by seine net in 2014. Additional fishing effort by rod and line was made during November and December 2014, resulting in the capture of two salmon at Craigpot on 24 November 2014. Table 4.1 summarizes the tagged salmon. Three and two adult brown trout (mean FL = 474±106mm, range 314-603mm) were PIT tagged in 2013 and 2014 respectively. For monitoring at Strathdon a total of 69 Atlantic salmon were captured using the fixed trap in 2014 and tagged with radio and/or PIT tags (Table 4.2). At Philiphaugh, 31 Adult Atlantic salmon were captured by corralling and hand netting below the weir to be radio tagged (Table 4.3).

Table 4.1. Adult Atlantic salmon captured and tagged for monitoring passage at Craigpot in 2013 and 2014. Tag burden is the ratio of tag mass to fish mass in air. *Atlantic salmon captured in 2014 were caught by rod and line at Craigpot.

Tagging date	**Sex**	***N***	**Fork length**				**Mass**				**Tag burden**	
			[mean	**±**	**SD**	**(range), mm]**	**[mean**	**±**	**SD**	**(range), kg]**	**[mean**	**(range), %]**
						PIT + Radio tagged						
25/08/2013	F	3	740	±	43	(680 – 779)	4.11	±	0.80	(2.98 – 4.72)	0.62	(0.85 – 0.54)
	M	1	598				3.45				0.74	
21/09/2013	F	2	620	±	10	(610 – 630)	2.43	±	0.03	(2.40 – 2.45)	1.05	(1.06 – 1.04)
	M	2	825	±	45	(780 – 870)	5.49	±	0.81	(4.68 – 6.30)	0.46	(0.54 – 0.40)
13/10/2013	F	2	669	±	113	(556 – 782)	3.15	±	1.39	(1.76 – 4.54)	0.81	(1.44 – 0.56)
	M	1	696				3.42				0.74	
02/11/2013	F	2	780	±	6	(774 – 786)	5.73	±	0.97	(4.76 – 6.70)	0.44	(0.53 – 0.38)
	M	4	644	±	68	(559 – 750)	3.58	±	2.06	(1.52 – 5.64)	0.71	(1.67 – 0.45)
24/11/2014*	F	1	802				4.74				0.54	
	M	1	798				5.36				0.47	
Total		**19**	**699**	±	**91**	(**556** – **870**)	**4.00**	±	**1.53**	(**1.52** – **6.70**)	**0.64**	(**1.67** – **0.38**)
	F	**10**	**716**	**±**	**85**	**(556 – 802)**	**3.97**	**±**	**1.44**	**(1.76 – 6.70)**	**0.64**	**(1.44 – 0.38)**
	M	**9**	**702**	**±**	**98**	**(559 – 870)**	**4.34**	**±**	**1.52**	**(1.52 – 6.30)**	**0.59**	**(1.67 – 0.40)**
						PIT tagged only						
13/10/2013	F	1	590				2.24				0.0357	
	M	2	663.5	±	19	(645 – 682)	2.72	±	0.22	(2.5 – 2.9)	0.0294	(0.03 – 0.03)
02/11/2013	M	1	458				2.18				0.0367	
Total		**4**	**593.8**	**±**	**85**	**(458 – 682)**	**2.465**	**±**	**0.3**	**(2.2 – 2.9)**	**0.0325**	**(0.04 – 0.03)**
	F	**1**	**590**				**2.24**				**0.0357**	
	M	**3**	**595**	**±**	**98**	**(458 – 682)**	**2.54**	**±**	**0.31**	**(2.2 – 2.9)**	**0.0315**	**(0.04 – 0.03)**

Table 4.2. Adult Atlantic salmon captured and tagged for monitoring passage at Strathdon in 2014. Tag burden is the ratio of tag mass to fish mass in air.

Tagging date	**Sex**	***N***	**Fork length**			**Mass**			**Tag burden**	
			[mean	**± SD**	**(range), mm]**	**[mean**	**± SD**	**(range), kg]**	**[mean**	**(range), %]**
2014: PIT + Radio tagged										
10/10/2014	F	10	726	± 36	(652 – 785)	3.65	± 0.76	(2.36 – 4.82)	0.72	(0.52 – 1.07)
10/10/2014	M	11	668	± 91	(514 – 808)	2.67	± 1.00	(1.10 – 4.20)	1.10	(0.60 – 2.30)
27/10/2014	M	2	675	± 87	(613 – 736)	2.62	± 0.96	(1.94 – 3.30)	1.04	(0.77 – 1.30)
06/11/2014	F	2	704	± 12	(695 – 712)	3.33	± 0.07	(3.28 – 3.38)	0.76	(0.75 – 0.77)
12/11/2014	F	1	694			3.70			0.68	
12/11/2014	M	1	846			5.64			0.45	
Total	**F**	**13**	**720.3**	± **33**	(**652** – **785**)	**3.60**	± **0.67**	(**2.36** – **4.82**)	**0.01**	(**0.01** – **0.01**)
	M	**14**	**681.9**	± **96**	(**514** – **846**)	**2.88**	± **1.21**	(**1.10** – **5.64**)	**0.01**	(**0.00** – **0.02**)
2014: PIT tagged only										
10/10/2014	F	10	674	± 36	(588 – 722)	2.84	± 0.35	(2.10 – 3.30)	0.03	(0.02 – 0.04)
10/10/2014	M	14	624	± 95	(507 – 842)	2.15	± 1.02	(0.90 – 4.76)	0.04	(0.02 – 0.09)
06/11/2014	F	1	614			2.18			0.04	
06/11/2014	M	4	623	± 41	(585 – 666)	2.10	± 0.58	(1.56 – 2.84)	0.04	(0.03 – 0.05)
12/11/2014	F	6	588	± 55	(536 – 689)	1.87	± 0.22	(1.56 – 2.14)	0.03	(0.03 – 0.04)
12/11/2014	M	6	630	± 58	(544 – 692)	2.32	± 0.76	(1.04 – 3.20)	0.03	(0.02 – 0.06)
19/11/2014	F	1	662			2.62			0.03	
Total	**F**	**18**	**641.1**	± **57**	(**536** – **722**)	**2.46**	± **0.54**	(**1.56** – **3.30**)	**0.00**	(**0.00** – **0.00**)
	M	**24**	**625.5**	± **78**	(**507** – **842**)	**2.18**	± **0.88**	(**0.90** – **4.76**)	**0.00**	(**0.00** – **0.00**)

Table 4.3. Adult Atlantic salmon captured and tagged for monitoring passage at Philiphaugh in 2014. Fish mass was not recorded, hence there is no measure of tag burden.

Tagging date	**Sex**	**N**	**Fork length**							
			[mean	**±**	**SD**	**(**	**range), mm]**			
2014: Radiotagged only										
08/10/2014	F	3	786.7	±	10	(	775	–	795	)
08/10/2014	M	2	850	±	99	(	780	–	920	)
09/10/2014	F	4	752.5	±	68	(	665	–	830	)
09/10/2014	M	3	785	±	49	(	730	–	825	)
22/10/2014	F	2	837.5	±	25	(	820	–	855	)
22/10/2014	M	1	810	±	NA	(	810	–	810	)
23/10/2014	F	2	742.5	±	46	(	710	–	775	)
23/10/2014	M	2	635	±	35	(	610	–	660	)
23/10/2014	F	2	787.5	±	103	(	715	–	860	)
23/10/2014	M	1	600	±	NA	(	600	–	600	)
27/10/2014	F	5	721	±	55	(	655	–	790	)
27/10/2014	M	1	820	±	NA	(	820	–	820	)
27/10/2014	M	2	747.5	±	117	(	665	–	830	)
31/10/2014	F	1	785	±	NA	(	785	–	785	)
Total	**F**	**19**	**762.9**	**±**	**60**	**(**	**655**	**–**	**860**	**)**
	M	**12**	**754.2**	**±**	**100**	**(**	**600**	**–**	**920**	**)**

4.3.2 Radio tag detections and passage success

Table 4.4 summarizes the outcomes for approach and passage of tagged fish at each scheme. Table 4.5 summarizes environmental and operational conditions during radio tagged fish presences. The timeline of flow and temperature durig visits is shown in Figure 4.6. During the monitoring there were no extended continual detections of transmitters within the scheme that could be interpreted as death or tag loss. All presences were consistent with movement over time, and no transmitters remained beyond the periods depicted in Figure 4.6, until the downstream movement of radio tagged post-spawned fish. Tagged fish that were not recorded approaching the schemes were assumed to have remained downstream or ascended tributaries downstream.

Eleven radio tagged fish were recorded approaching Craigpot hydropower scheme from downstream: eight salmon in 2013 (five males and three females, mean fork length (FL) ±SD (range) = 704.3±123.4 (559-870) mm), two female sea trout (455 and 470 mm) and a male salmon (798 mm) in 2014. All except two of these fish ascended beyond the scheme in one visit lasting a mean time of 1.91 h (range=0.71-5.83) from first detection at A4 to last detection at A1. The one fish which did not ascend made four visits to the scheme between 15:37 on 14 November 2013 and 09:24 the following morning. Several excursions were made upstream into the scheme affected zone during this time. This fish was later located by manual tracking at a location 1 km downstream and was found there on four occasions (17 Nov, 21 Nov, 29, Nov and 5 Dec) until it was tracked moving further downstream. Both sea trout which approached the scheme ascended upstream, taking one and two attempts respectively.

At Strathdon 27 radio tagged Atlantic salmon were logged approaching the scheme, of which fifteen passed upstream. At Philiphaugh the number of passing fish was fifteen out of 28 recorded approaching. The number and duration of visits to these two schemes varied considerably, with one to five visits per fish at Strathdon, and one to sixty-four at Philiphaugh, lasting between nine minutes and five days at Strathdon, and from less than a minute to three days at Philiphaugh.

Table 4.4. Summary of detections of radio and PIT tagged fish at the three hydropower schemes. * Passage of PIT only tagged fish at Strathdon was determined using data from the PIT equipment installed on the fish pass.

	Craigpot				**Strathdon**			**Philiphaugh**		
Number tagged	Atlantic salmon			Sea trout	Atlantic salmon			Atlantic salmon (Radio tagged only)		
PIT+Radio	19			5	27			31		
PIT only	4			17	42			-		
Number approaching scheme										
PIT+Radio	9			2	20			28		
PIT only	4			4	31			-		
Number confirmed passing scheme										
PIT+Radio	8			2	15			15		
PIT only	-			-	24*			-		
Number of visits per fish (radio only)	Mean	Median	Range	Each fish	Mean	Median	Range	Mean	Median	Range
Passing	1.1	1	1-2	1,1	2.4	2	1-5	14.9	14	2-26
Not passing	2 (N=1)	-	-	-	5	3	2-12	23.2	21	1-64
Time from first to last presence (radio only) (hours)										
Passing	1.67	1.92	0.73-6.37	0.65,0.72	13.3	7	0.2-47.8	80	72.5	0.18-237
Not passing	16.87 (N=1)	-	-	-	22	11.5	1.9-44.5	179	139	0.02-501
Time per visit (radio only) (hours)										
Passing	1.54	1.05	0.07-6.37	0.65,0.72	803.6	418	9-2866	1771	1444	2.25-4286
Not passing	2.17	2.17	1.78-2.55		808.8	138	4-7423	1497	1070	0.73-4861

Table 4.5. Summary of measured covariates during fish presences at the three schemes. Variables are: Discharge (Q) from the nearest gauging station, turbine flow (Qturb), weir flow (Qweir), turbine:weir flow ratio (Qturb:Qweir), level above weir (Hw) and temperature (T). The differing temperatures between approach zone and SAZ reflects overall temporal differences in occupation of these zones by fish. Qweir and Qturb:Qweir could not be calculated for Strathdon and Philiphaugh, since no stage-discharge relationship has been derived for these sites.

		Craigpot				Strathdon				Philiphaugh			
Variable		Mean	Median	Range		Mean	Median	Range		Mean	Median	Range	
Q	Approach	12.83	12.72	10.05	21.28	2.44	1.70	1.56	11.77	41.91	26.88	8.92	160.50
(m^{3}s^{-1})	SAZ	14.85	12.96	10.05	25.75	3.94	2.68	1.56	32.97	37.98	22.12	8.92	160.50
Qturb	Approach	3.09	3.14	2.56	3.38	1.32	1.32	0.00	1.81	5.64	6.11	0.00	10.12
(m^{3}s^{-1})	SAZ	3.09	3.03	2.56	3.55	1.37	1.34	0.00	1.92	5.90	6.11	0.00	10.12
Qweir	Approach	9.74	9.42	7.22	18.72	-	-	-	-	-	-	-	-
(m^{3}s^{-1})	SAZ	11.76	9.61	7.22	22.22	-	-	-	-	-	-	-	-
Qturb:Qweir	Approach	0.34	0.35	0.14	0.39	-	-	-	-	-	-	-	-
	SAZ	0.30	0.35	0.14	0.39	-	-	-	-	-	-	-	-
Hw	Approach	10120	10120	10100	10190	299.6	266.7	238.9	712.8	531.9	450.5	247.5	1066
(mm)	SAZ	10140	10120	10100	10220	361.7	313.1	238.5	897.4	497.8	393.2	280.5	1066
T	Approach	9.3	10.0	6.5	10.0	7.8	8.0	5.0	11.0	-	-	-	-
(^{o}C)	SAZ	8.1	7.8	6.5	10.0	7.7	7.5	4.0	11.0	-	-	-	-
		Ratio				Ratio				Ratio			
Ratio of night:day presence duration	Approach	1.76				1.61				1.53			
	SAZ	1.73				1.72				1.19			

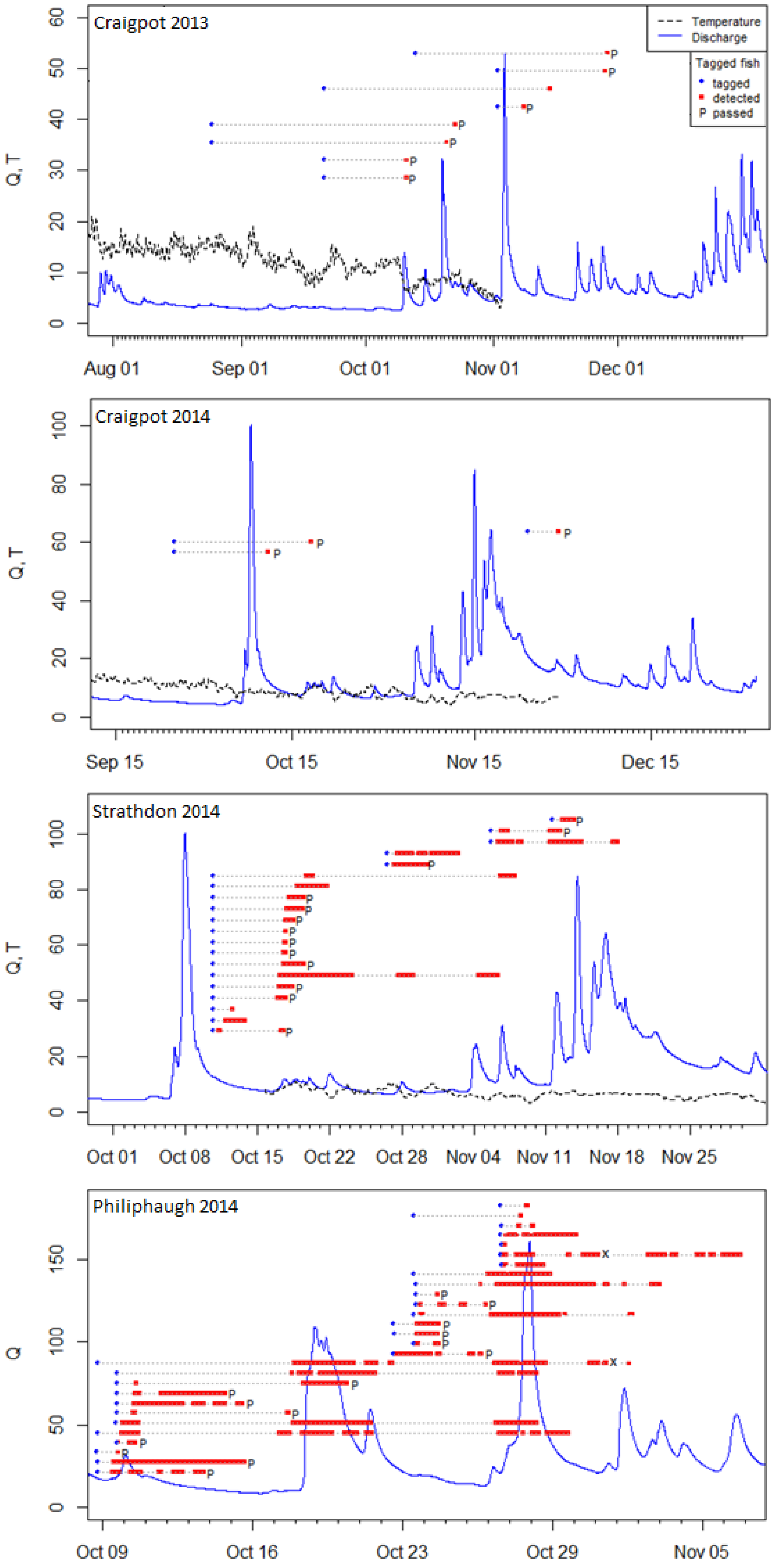


Figure 4.6. Presence of radio-tagged fish in range of radio receivers at Craigpot, Strathdon and Philiphaugh. Each line of points represents an individual fish. The first two fish to be detected at Craigpot in 2014 were sea trout. Visits ending in passage (P), and the two censored presences at Philiphaugh (X) are labelled, Temperature data do not span all presences due to loss of loggers.

4.3.4 Results of time to event analyses

Figure 4.7 shows the Kaplan Meier estimates for cumulative rate of transition and rejection for visits to the approach and scheme affected zones. At Craigpot, the rate of transition from the approach zone upstream dominates the rejection rate, with the majority of fish entering the SAZ within 25 minutes, whilst all rejections happened within 40 minutes (Figure 4.7A). This indicates that most fish ascended moved into the SAZ quickly on first arrival, rather than holding below the scheme. However the plateau in the proportion of transitions between 25 and 165 minutes shows that two fish did hold in the approach and then passed upstream. Once in the SAZ (Figure 4.7B), there is a slight delay of 15 minutes before the first successful upstream passage, which is due to the time taken to travel the depleted stretch. The proportion of successful transitions climbs steadily between 15 and 40 minutes, with relatively few fish moving back downstream during that time. The two remaining fish that did not ascend in that time then moved back downstream.

At Strathdon, there is both a swift rate of transition and rejection of the approach zone, indicated by the steepness of both curves in Figure 4.7C in the first 15 minutes. Transition does, however dominate rejection, and those fish that persist continue to enter the SAZ rather than leaving downstream, shown by the decaying rise of the transition curve, and no further drop in rejections after 60 minutes. However, in the SAZ (Figure 4.7C), rejections overpowered successful upstream passages. The greatest proportion of rejections occurred in the first 200 minutes, but persisted steadily beyond that. Most ascents happened within 500 minutes, but again persisted steadily beyond.

The initially steep transition curve at Philiphaugh (Figure 4.7E) shows that most fish were locating the turbine and fish pass outflows quickly, within 60 minutes. Those that persisted beyond that time continued to find these zones. There was also a continual rejection downstream, but the high initial transition dominated. Once at the outflows, however, the dominant tendency was to leave without ascending upstream, with only a small portion of visits persisting and resulting in passage (Figure 4.7F).

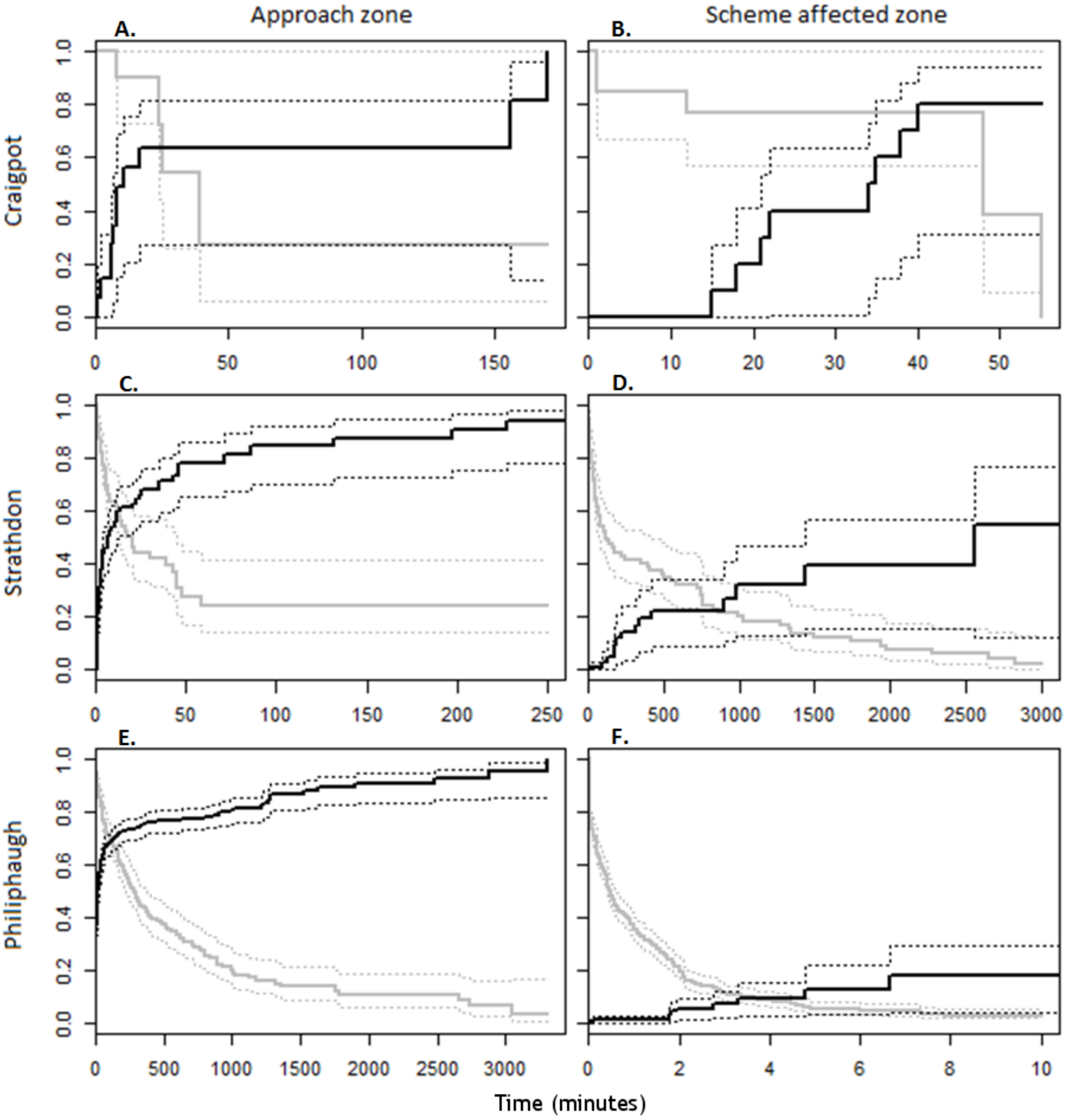


Figure 4.7. Cumulative proportion of visits that transitioned (black lines) and remained without rejecting (grey lines) approach and scheme affected zones at the three schemes. Transitions were visits to the approach zone that ended in arrival in the scheme affected zone, or visits to the scheme affected zone which ended with a passage upstream. Rejections were those visits to the approach zone that terminated in downstream exit, or visits to the scheme affected zone which ended with arrival back in the approach zone. Dotted lines are 95% confidence intervals. Plots for Strathdon, and for the Philiphaugh affected zone are truncated for better display, although the transition proportion changed very slightly after time of truncation.

4.3.4.1 Univariate models

Craigpot

There were no significant associations between tested variables and rate of exit from the approach zone, either by movement upstream or rejection downstream (univariate Cox Proportional Hazard models on each of total flow, diel factor, temperature, sex, length and condition factor, $p > 0.05$, Table 4.6). The strongest non-significant association was with condition factor $p = 0.09$), which suggested a faster rate of approach to the scheme affected zone with decreasing condition: a 1% decrease in transition rate per 1% increase in condition factor.

The rate of upstream passage from the scheme affected zone was related to weir flow (univariate Cox Proportional Hazard model, $p = 0.05$) with passage rate increasing by 17% per 1 m^3s^{-1}. Turbine flow was not a significant predictor of passage or rejection times in the scheme affected zone. Considering the effect of turbine:weir flow ratio in a separate regression showed that this was significantly negatively related ($p = 0.03$), with $1.5x10^4$ faster rate of passage for every 1% decrease in flow ratio. There was no effect of turbine flow on passage or rejection rates.

Table 4.6. Results of univariate Cox Proportional Hazards models for adult salmon movements at Craigpot. Abbreviated variables are: total discharge (Q), temperature (T), flow over the weir (Qweir) and flow through the turbine (Qturb). "Condition" is Fulton's condition factor.

Craigpot																	
	Time to transition								**Time to reject**								
Variable	AIC	*n*	events	*p*	exp(*coef*)	95% CI			Variable	AIC	*n*	events	*p*	exp(*coef*)	95% CI		
	Approach zone																
Q	42.76	25	11	0.15	1.15	0.95	-	1.39	Q	13.28	25	4	0.40	0.77	0.39	-	1.51
Daytime	44.22	25	11	0.50	1.72	0.36	-	8.37	Day	10.42	25	4	0.74	0.64	0.05	-	9.06
T	22.63	13	6	0.47	0.80	0.43	-	1.48	-	-	-	-	-	-	-	-	-
Sex	43.98	25	11	0.39	1.78	0.47	-	6.67	-	-	-	-	-	-	-	-	-
Length	44.43	25	11	0.58	1.00	1.00	-	1.01	Length	11.65	25	4	0.28	1.01	0.99	-	1.03
Condition	42.08	25	11	0.09	0.00	0.00	-	4.17	-	-	-	-	-	-	-	-	-
	Entry zone																
Qweir	26.77	22	8	**0.05**	1.17	1.00	-	1.36	Qweir	16.46	22	5	0.30	0.77	0.47	-	1.26
Qturb	24.20	22	8	0.92	1.22	0.03	-	59.35	Qturb	17.75	22	5	0.48	4.80	0.06	-	370.50
Qt/Qw	23.45	22	8	**0.03**	6.3x10^{-7}	0.00	-	0.18	Qt/Qw	16.39	22	5	0.28	6.6x10^{5}	1.3x10^{-4}	-	3.4x10^{13}
Daytime	22.74	22	8	0.60	1.82	0.20	-	16.63	Day	18.20	22	5	0.78	1.39	0.14	-	14.22
T	9.80	10	5	0.27	0.56	0.20	-	1.57	-	-	-	-	-	-	-	-	-
Sex	26.26	22	8	0.08	7.11	0.82	-	61.55	Sex	18.19	22	5	0.77	1.44	0.13	-	15.96
Length	29.09	22	8	0.19	1.00	1.00	-	1.01	Length	15.50	22	5	0.25	0.98	0.96	-	1.01
Condition	28.27	22	8	0.11	1.5x10^{-4}	0.00	-	7.01	Condition	12.15	22	5	0.18	5.1x10^{62}	2x10^{-30}	-	9.3x10^{154}

Strathdon

Rate of approach at Strathdon was 1.79 times greater in the day than in the night (*p* = 0.01), and rate of rejection of the approach zone by females was twice that of males (*p* = 0.02). There was no significant relation of movement into and out of the approach zone for any other variables (Table 4.7). Upstream passage from within the SAZ was 8.3 times faster at night time within the first 170 minutes of a visit, but not related to diel factor after that, and was also 1.62 times faster per degree increase in temperature (*p* = 0.05). Although weir level and turbine flow was not related to upstream passage, there was significant association between rejection rate of the SAZ and these variables (*p* = 0.02 for both). Rejection decreased by a factor of 0.37 per extra m^3s^{-1} turbine flow, and was very slightly decreased by 3% with every extra cm of upstream pool depth. Here again, temperature had an effect (*p* = 0.02) with an 84% increase per degree.

Table 4.7. Results of univariate Cox Proportional Hazards models for adult salmon movements at Strathdon. Abbreviated variables are: total discharge (Q), temperature (T), level above weir (Hw), flow over the weir (Qweir) and flow through the turbine (Qturb). Interactions with time are marked with the interacting interval (e.g. Q(t<50) denotes flow during first 50 minutes of a fish presence. “Condition” is Fulton’s condition factor.

Strathdon																	
	Time to transition								**Time to reject**								
Variable	AIC	*n*	events	*p*	exp(*coef*)	95% CI			Variable	AIC	*n*	events	*p*	exp(*coef*)	95% CI		
	Approach zone																
Q	716.55	225	87	0.13	1.09	0.97	–	1.21	Q	400.77	225	54	0.76	0.97	0.79	–	1.18
Daytime	711.94	225	87	**0.01**	0.56	0.36	–	0.88	Daytime	399.44	225	54	0.53	1.20	0.67	–	2.15
T	670.13	194	83	0.52	1.05	0.91	–	1.20	T	382.65	194	51	0.40	0.92	0.76	–	1.12
Sex	717.73	225	87	0.97	0.99	1.67	–	4.34	**Sex**	409.86	225	54	**0.02**	1.99	4.07	–	13.08
Length	717.23	225	87	0.81	1.00	1.00	–	1.00	Length	413.08	225	54	0.11	1.00	0.99	–	1.00
Condition	716.50	225	87	0.15	4.16	0.59	–	29.35	Condition	402.00	225	54	0.09	16.63	0.65	–	426.58
	Entry zone																
Hw	69.83	1002	14	0.49	1.00	0.99	–	1.00	Hw	595.00	1002	83	**0.02**	1.00	0.99	–	1.00
Qturb (t>170)	66.71	1002	14	0.70	2.55	0.02	–	304.88	Qturb	595.96	1002	83	**0.02**	0.37	0.16	–	0.86
Qturb (t<170)	66.71	1002	14	0.13	0.04	0.00	–	2.75								–	
Day (t>170)	72.97	1002	14	**0.01**	0.12	0.02	–	0.62	Day (t>170)	587.51	1002	83	**0.00**	0.19	0.08	–	0.45
Day (t<170)	72.97	1002	14	0.98	1.03	0.14	–	7.48	Day (t<170)	587.51	1002	83	0.14	1.51	0.87	–	2.63
T	70.09	990	14	**0.05**	1.62	1.00	–	2.61	T	549.42	990	78	**0.02**	0.84	0.72	–	0.97
Sex	68.71	1002	14	0.71	1.43	0.22	–	9.12	Sex	602.91	1002	83	0.13	1.41	0.90	–	2.21
Length	66.36	1002	14	0.55	1.00	0.98	–	1.01	Length	604.23	1002	83	0.32	1.00	1.00	–	1.00
Condition	67.62	1002	14	0.44	31.08	0.00	–	$2.1\mathrm{x}10^{5}$	Condition	601.11	1002	83	0.70	1.55	0.16	–	14.76

Philiphaugh

Rate of approach to the fishway and turbine outflows at Philiphaugh was significantly negatively related to total discharge (*p* = 0.02), but only in the first 50 minutes of a presence, and the effect was negligible (Table 4.8). Approaches were faster in the day than at night, but the rates varied with time: 11.11 times faster in the day within the first 50 minutes, and then reducing to 1.79 times faster. In contrast to approach rate, rate of rejection in the day was 0.61 than at night (*p* = 0.05), but only within the first 50 minutes. At Philiphaugh, the zone labelled as the approach also included the extent of the weir outside of the area directly in front of the outflows, and therefore weir level is likely to affect searching behaviour in this area, and so this was also tested. Rate of approach was significantly related to weir level during the first ten minutes of a visit (*p* = 0.0059), with the rate of transition to the outflows increasing by 0.1% per mm increase in weir level. Turbine flow was not related to approach or rejection rates of this zone, although there was a near to significant (*p* = 0.058) increase in rate of rejection with increasing turbine flow for visits lasting more than 30 minutes. Rate of passage from the outflows was not significantly related to any tested variables at the 5% level, but the strongest association was with weir level (*p* = 0.07), with a 0.5% decrease in this rate per mm increase in level (Table 4.9).

Table 4.8. Results of univariate Cox Proportional Hazards models for adult salmon movements in the approach zone at Philiphaugh. Abbreviated variables are: total discharge (Q), temperature (T), level above weir (Hw), flow over the weir (Qweir) and flow through the turbine (Qturb). Interactions with time are marked with the interacting interval (e.g. Q(t<50) denotes flow during first 50 minutes of a fish presence. “Condition” is Fulton’s condition factor.

Philiphaugh

	Time to transition						**Time to reject**						
Variable	AIC	*n*	events	*p*	exp(*coef*)	95% CI	Variable	AIC	*n*	events	*p*	exp(*coef*)	95% CI
	Approach zone												
Q (t>50)	4523.7	3119	418	0.06	0.99	0.98 – 1.00	Q (t>50)	1648.0	3119	177	0.13	0.99	0.99 – 1.00
Q (t<50)	4523.7	3119	418	**0.02**	1.00	1.00 – 1.01	Q (t<50)	1648.0	3119	177	0.28	1.00	1.00 – 1.01
Hw(t<10)	4520	3737	418	0.006	1.001	1.00 – 1.00	Hw (t<50)	1649.3	3737	177	0.15	1.00	1 – 1.002
Hw (t>10)	4520	3737	418	0.062	1.00	1.00 – 1.00	Hw (t>50)	1649.3	3737	177	0.24	1.00	1 – 1
Qturb	3403	1744	330	0.160	0.9507	0.9 – 1.02	Qturb (t<30)	1143.8	1916	131	0.98	1.002	0.8 – 1.184
							Qturb (t>30)	1143.8	1916	131	0.06	1.113	1 – 1.243
Day (t>50)	4459.8	3119	418	**<0.001**	0.09	0.05 – 0.19	Day (t>50)	1652.8	3119	177	0.12	0.71	0.46 – 1.09
Day (t<50)	4459.8	3119	418	**<0.001**	0.56	0.41 – 0.75	Day (t<50)	1652.8	3119	177	**0.05**	1.62	1.00 – 2.64
Sex	4577.2	3119	418	0.30	1.51	0.69 – 3.30	Sex (t<100)	1674.7	3119	177	0.17	1.73	0.79 – 3.78
							Sex (t>100)	1674.7	3119	177	0.91	0.95	0.40 – 2.29
Length	4577.8	3119	418	0.95	1.00	0.99 – 1.01	Length	1644.7	3119	177	0.11	1.00	0.99 – 1.00

Table 4.9. Results of univariate Cox Proportional Hazards models for adult salmon movements in the combined outflow zone at Philiphaugh. Abbreviated variables are: level above weir (Hw), and flow through the turbine (Qturb). Interactions with time are marked with the interacting interval (e.g. Q(t<50) denotes flow during first 50 minutes of a fish presence.

Philiphaugh																	
	Time to transition								**Time to reject**								
Variable	AIC	*n*	events	*p*	exp(*coef*)	95% CI			Variable	AIC	*n*	events	*p*	exp(*coef*)	95% CI		
	Entry zone																
Qturb	114.62	825	14	0.32	0.86	0.64	–	1.16	Qturb	3191.1	714	335	0.69	1.02	0.94	–	1.10
Hw	115.79	1057	14	0.07	0.99	0.99	–	1.00	Hw (t>0.02)	4228.0	905	423	0.37	1.00	1.00	–	1.00
							–		Hw (t<0.02)	4228.0	905	423	0.20	1.00	1.00	–	1.00
Day	109.64	1057	14	0.68	0.67	0.10	–	4.33	Day	4231.3	905	423	0.49	1.11	0.82	–	1.51
Sex	107.35	1057	14	0.12	3.84	0.70	–	21.05	Sex	4260.2	905	423	0.12	1.48	0.90	–	2.42
Length	108.39	1057	14	0.32	0.99	0.98	–	1.01	Length	4260.4	905	423	0.51	1.00	1.00	–	1.00

4.3.5 Multiple covariate models

Tables 4.10 to 4.12 show the results of Cox PH regressions considering two or more variables, with univariate models also presented for comparison of AIC scores. The small sample size at Craigpot limited the exploration of the effects of multiple variables, particularly at the approach zone. For time to pass the scheme from the scheme affected zone, the best fitting model as scored by AIC used diel factor alone, whilst for time to reject this zone, a combination of the turbine:weir flow ratio and condition factor produced the lowest score. At Strathdon, Q and T together produced the best fitting model for movements both into and out of the approach zone, whilst at Philiphaugh (where T was not available), Q and day provided the best fit. For passage from the entry zone, Q and weir level was the best combination at Strathdon, whilst at Philiphaugh, although this combination was the second-lowest AIC score, a combination of turbine flow and sex provided the lowest AIC. Interestingly, when an interaction between weir level and turbine flow was considered, this produced significant results, although this was not the best scored model. At Strathdon, rejection of the scheme affected zone was best modelled with turbine flow, weir height and diel factor, whilst at Philiphaugh just turbine flow and weir height had the lowest AIC.

Table 4.10. Comparison of univariate and multivariate (shaded) Cox PH models for Craigpot approach (**A**) and entry (**B**) zones. Covariates with a significant effect ($p<0.05$) are in bold. Non-converging models are not shown. Abbreviated variables are: total discharge (Q), temperature (T), level above weir (Hw), flow over the weir (Qweir) and flow through the turbine (Qturb). Interactions with time are marked with the interacting interval (e.g. $Q(t<50)$ denotes flow during first 50 minutes of a fish presence. "Condition" is Fulton's condition factor.

A

Variables	AIC	*N*	events
Approach zone			
	Time to transition		
Daytime	44.22	25	11
T	22.63	13	6
Length	44.43	25	11
Condition	42.08	25	11
	Time to reject		
Length	11.65	25	4

B

Variables	AIC	*N*	events
Entry zone			
	Time to transition		
Qw	26.77	22	8
Qt	24.20	22	8
Qt/Qw	23.45	22	8
Daytime	22.74	22	8
Length	29.09	22	8
Condition	28.27	22	8
Qt+Qw	28.11	22	8
Qt+Qw+Condition	27.30	22	8
	Time to reject		
Qw	16.46	22	5
Qt	17.75	22	5
Daytime	18.20	22	5
Length	15.50	22	5
Qt+Qw	16.91	22	5
Qt+Qw+Sex	18.88	22	5
Qt+Qw+Condition	16.03	22	5
Qt/Qw+Condition	14.12	22	5

Table 4.11. Comparison of univariate and multivariate (shaded) Cox PH models for Strathdon approach (**A**) and entry (**B**) zones. Models for rejection of the entry zone (**C**) are overleaf. Covariates with a significant effect (*p*<0.05) are in bold. Non-converging models are not shown. Abbreviated variables are: total discharge (Q), temperature (T), level above weir (Hw), flow over the weir (Qweir) and flow through the turbine (Qturb). Interactions with time are marked with the interacting interval (e.g. Q(t<50) denotes flow during first 50 minutes of a fish presence. “Condition” is Fulton’s condition factor.

A

Variables	AIC	*N*	events
Approach zone			
Time to transition			
Q	716.55	225	87
Daytime	711.94	225	87
T	670.13	194	83
Condition	716.50	225	87
Q+Daytime	667.80	194	83
Q+T	670.17	194	83
Q+Daytime+Q:Daytime	669.39	194	83
Q+T+Q:T	670.51	194	83
Time to reject			
Q	400.77	225	54
Daytime	399.44	225	54
T	382.65	194	51
Length	413.08	225	54
Condition	402.00	225	54
Q+Daytime	378.78	194	51
Q+T	382.11	194	51
Q+Condition	380.45	194	51
Q+**Sex**	388.80	194	51
Q+Daytime+Q:Daytime	376.28	194	51
Q+T+Q:T	383.57	194	51

B

Variables	AIC	*N*	events
Entry zone			
Time to transition			
Hw	69.83	1002	14
Qturb (t>170)	66.71	1002	14
Qturb (t<170)	66.71	1002	14
Daytime (t>170)	72.97	1002	14
Daytime (t<170)	72.97	1002	14
T	70.09	990	14
Length	66.36	1002	14
Condition	67.62	1002	14
Qt+Hw	71.66	990	14
Qt+Hw+Daytime(t<170)+**Daytime(t>170)**	83.48	990	14
Qt+Hw+Daytime+Qt:Daytime	78.57	990	14
Qt+Hw+T	73.46	990	14
Qt+Hw+T+Qt:T	76.60	990	14
Qt+Hw+T+Hw:T	76.95	990	14
Qt+Hw+T+Daytime(t<170)+Daytime(t>170)	85.08	990	14
Qt+Hw+**T**+Daytime+Qt:Daytime	85.05	990	14

Table 4.11C. Comparison of univariate and multivariate (shaded) Cox PH models for Strathdon. Covariates with a significant effect ($p<0.05$) are in bold. Non-converging models are not shown. Abbreviated variables are: total discharge (Q), temperature (T), level above weir (Hw), flow over the weir (Qweir) and flow through the turbine (Qturb). Interactions with time are marked with the interacting interval (e.g. Q(t<50) denotes flow during first 50 minutes of a fish presence. “Condition” is Fulton’s condition factor.

C

Variables	AIC	*N*	events
Entry zone			
Time to reject			
Hw	595.00	1002	83
Qturb	595.96	1002	83
Daytime (t>170)	587.51	1002	83
Daytime (t<170)	587.51	1002	83
T	549.42	990	78
Length	604.23	1002	83
Condition	601.11	1002	83
Qt+Hw	548.92	990	78
Qt+Hw+Qt:Hw	550.40	990	78
Qt+Hw+**Daytime(t<170)**+Daytime(t>170)	537.29	990	78
Qt+Hw+Daytime+ Qt:Daytime	550.85	990	78
Qt+**Hw**+**T**	542.90	990	78
Qt+Hw+Qt:Hw	550.40	990	78

Table 4.12. Comparison of univariate and multivariate (shaded) Cox PH models for Philiphaugh approach (**A**) and outflow (**B**) zones. Covariates with a significant effect (*p*<0.05) are in bold. Non-converging models are not shown.

Philiphaugh			
Variables	AIC	*N*	events
Approach zone			
Time to transition			
Q (t>50)	4523.66	2703	418
Q (t<50)	4523.66	2703	418
Daytime (t>50)	4459.77	2703	418
Daytime (t<50)	4459.77	2703	418
Q(t<50)+Q(t>50)+**Day(t<100)+Day(t>100)**	4446.74	2703	418
Time to reject			
Q (t>50)	1648.05	2703	177
Q (t<50)	1648.05	2703	177
Daytime (t>50)	1652.77	2703	177
Daytime (t<50)	1652.77	2703	177
Q(t<50)+Q(t>50)+Day(t<50)+Daytime(t>50)	1651.44	2703	177

Philiphaugh			
Variables	AIC	*N*	events
Entry zone			
Time to transition			
Qturb	114.62	825	14
Hw	115.79	1057	14
Daytime	109.64	1057	14
Sex	107.35	1057	14
Length	108.39	1057	14
Qt+Hw	117.3913	825	14
Qt+Hw+Qt:Hw	120.4374	825	14
Qt+Sex	112.4428	825	14
Hw+Sex	122.8584	1057	14
Hw+Sex+Hw:Sex	120.2298	1057	14
Time to reject			
Qturb	3191.11	825	335
Weir level (t>0.02)	4227.95	1057	423
Weir level (t<0.02)	4227.95	1057	423
Daytime	4231.25	1057	423
Qt+Hw	3193.278	825	335
Qt+Hw+Qt:Hw	3194.879	825	335

4.3.6 Time in the turbine outflow from PIT detections at Craigpot and Strathdon

Craigpot

Of the 50 fish PIT tagged downstream of Craigpot, nine were recorded entering the turbine outflow by PIT logger P2: four salmon and two sea trout in 2013, and two sea trout and a brown trout in 2014. The brown trout detected in 2014 was tagged at Kemnay in 2013. In addition, a post-spawned fish tagged in March 2014, and a fish tagged at Strathdon upstream in October 2014 were detected entering the outflow in November 2014 (see Section 4.3.5 below). Three fish were excluded from the analysis of outflow entry: the detections of the brown trout and the salmon which was tagged upstream were not consistent with upstream movements, and a sea trout which was detected before the addition of a second antenna loop allowed the inference of entry to the outflow.

The Atlantic salmon made just one (n=4),or two (n=1) visits each, lasting from 0.76 to 4.9 minutes (mean=2.20, median=1.57, for the four fish which had defined visits). The sea trout made one (n=3), two (n=1) and unknown multiple (n=1, the sea trout arriving before installation of the second loop) visits. Only one sea trout visit comprised a complete visit sequence, and this lasted 1.93 minutes.

Strathdon

The PIT reader monitoring the entry to the turbine outflow detected 35 PIT-tagged fish, of which 28 could be identified as having visits beginning and ending downstream. Based on these data, each fish made between 1 and 48 visits (Figure 4.8, mean = 5, median=2), ranging from single detections at A2 to a 20 hour visit within the outflow (Figure 4.9, mean=2.21 h, median = 0.48 h). Total time in the outflow ranged from zero (single detection) to 198.7 hours (Figure 4.10, mean=11.11 h, median=2.52 h). The exclusion of incomplete detection series means that visit numbers and total times per fish will be negatively biased.

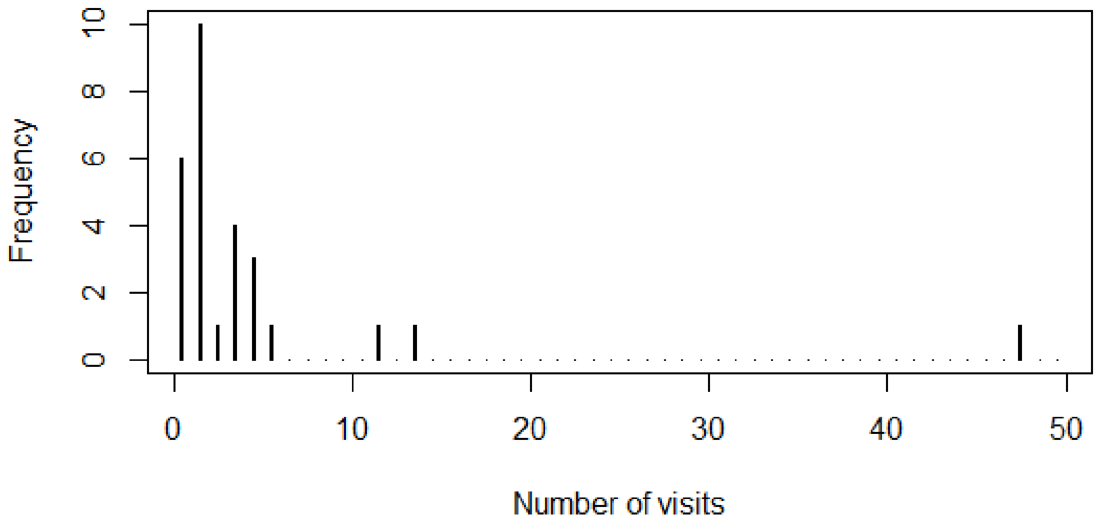


Figure 4.8. Number of visits by each PIT tagged fish to the outflow channel, as detected by PIT readers at Strathdon.

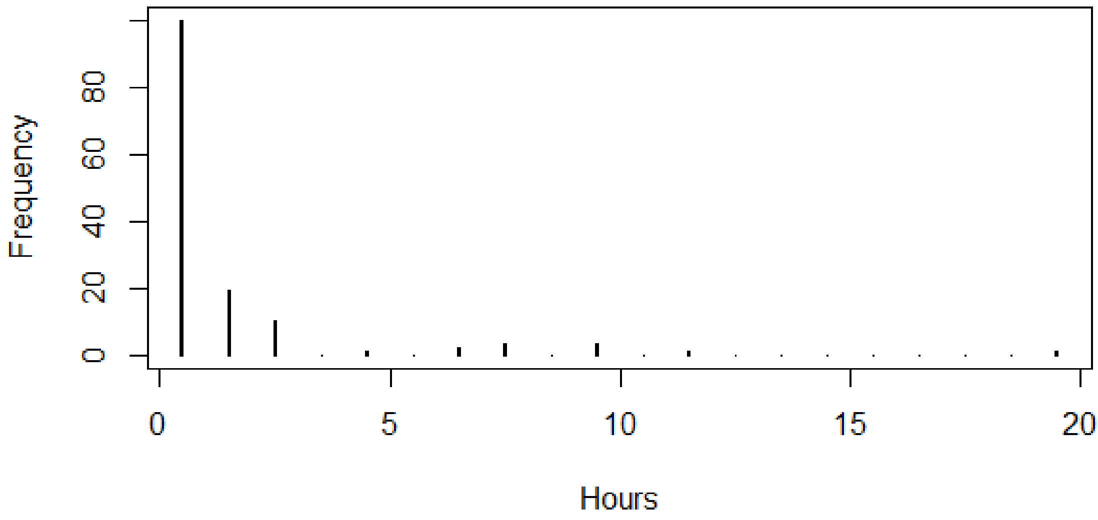


Figure 4.9. Visit durations by PIT tagged fish in the outflow, as detected by PIT readers at Strathdon.

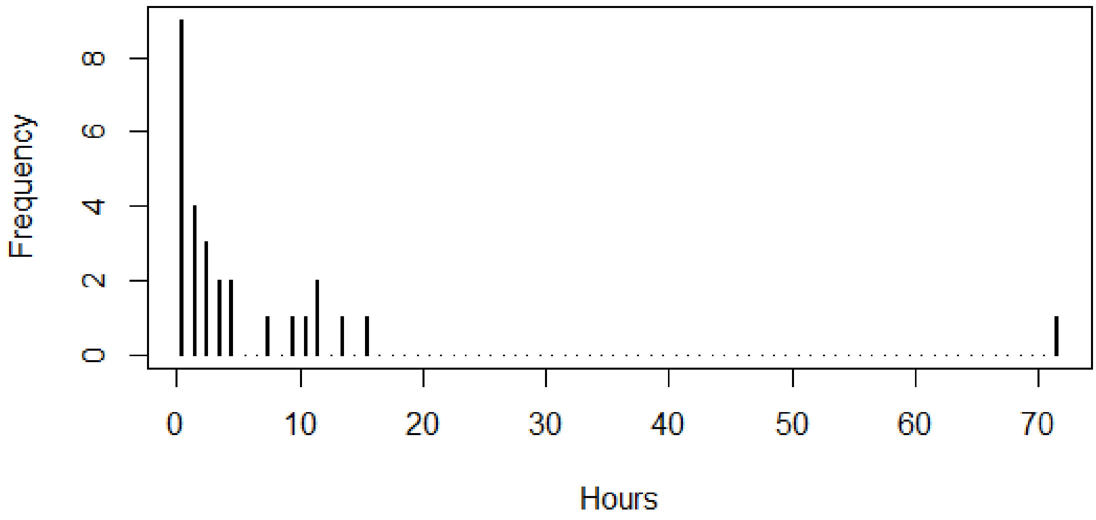


Figure 4.10. Total time spent by PIT tagged fish in the outflow, as detected by PIT readers at Strathdon.

4.3.7 Return movements of post-spawned fish

At all three hydropower schemes, radio tagged fish were detected moving downstream with the radio equipment. Full coverage could not always be confirmed for this period, but these data provide information about downstream movements at the schemes even if they cannot be used as reliable proportional returns. Whilst it cannot be certain that all of these downstream moving fish had spawned, these results are considered in the context of the movements of post-spawned fish, as it is felt that that is the most likely case.

At Craigpot, 19 upstream migrants were recorded returning downstream (Table 4.13): two salmon that had been tagged and ascended in 2013; two sea trout that were tagged and ascended in 2014; and 19 salmon that had been tagged 42 km upriver at Strathdon in 2014, which then descended to Craigpot. Ten salmon kelts were recorded returning past Strathdon after ascending beyond it (Table 4.14), and at Philiphaugh, six salmon kelts were recorded as they moved back down past the scheme (Table 4.15).

Qualitative differences in behaviours were evident between schemes. At Craigpot, several individuals were recorded on multiple occasions at the exit zone (the same zone defined as ‘exit’ in Section 4.2.5 for upstream migrants), and these were separated by periods greater than 30 minutes. From the detection series it was not clear whether these separate presences were recurrent approaches to the upper end of the scheme, or movements within the scheme between monitored zones and the unmonitored areas. Nevertheless, up to 38 separate presences were recorded (median = 1, mean = 5.2). By contrast, downstream passages at Strathdon involved just single visits. At Philiphaugh five of the six downstream returners visited the scheme, just once, and the other made two visits to the upstream end.

The duration of visits was generally longest at Craigpot (mean = 162.39 h) followed by Philiphaugh (mean = 15.20 h) and Strathdon (mean = 7.93), although these durations were not statistically different between sites. (Kruskal-Wallis test $H(2) = 1.12$, $p = 0.57$). Where it was possible to determine time until passage from the exit zone, these times were significantly different between schemes (Kruskal-Wallis test $H(2) = 10.21$, $p = 0.0061$). Time until passage was significantly shorter at Strathdon (mean = 0.64 h) than at Craigpot (21.1 h) (Mann-Whitney U-test $W(2) = 64$, Bonferroni corrected $p = 0.0238$), and also than that at Philiphaugh (mean = 23.05 h) (Mann-Whitney U-test $W(2) = 10$, Bonferroni corrected $p = 0.0459$).

At Craigpot, during the 2014-2015 migration period, and also at Philiphaugh, receivers were set to detect presences of kelts at the turbine intakes. Four fish were recorded entering the intake channel at Craigpot, travelling all the way to the zone at the trash screen. At Philiphaugh three fish spent time at the turbine intake. Of these intake presences, one at Craigpot and two at Philiphaugh could be interpreted as possible turbine passages, since they were not subsequently detected at short range zones above the barrier thereafter. However, this could also have been the result of missed detections in these short range zones.

Of the six PIT tagged fish which were experimentally introduced to the turbine channel, no turbine passages were recorded, as determined by no detections at the PIT antenna below the turbine. The single fish released at midday on 22 February exited the top of the channel at 20:47 and was not detected again until it returned to be detected at the turbine outflow on 14 November of the same year, presumably whilst making a repeat spawning migration. One kelt in the second release on 8 March exited the turbine channel immediately, but returned 23 days later, at 21:52. It then made three movements from the entrance of the channel to the trash rack and back, including a full departure from the channel of 2 h, all of which took place over the space of 2.4 h. Another fish was recorded in the channel sporadically over the course of 26 days eventually passing the depleted stretch (recorded at Pdepl) at 23:20 on 3 April. The remaining three kelts were recorded for between one and four hours by readers in the channel, but were not detected exiting in either direction, probably due to signal blockage by other PIT tagged fish. One of these fish was captured by an angler 22 days later, 17 km downstream, and identified by its floy tag. Video monitoring at the trash rack resulted in the night time observation of two kelt approaches. In these instances the fish came within 15 cm of the rack before leaving the field of view, which was confined to approximately 1.5 m in front of the trash rack. These approaches lasted less than ten seconds each.

Table 4.13. Kelts moving downstream past Craigpot hydropower scheme. Duration of presence is from first to last detection of downstream movement per fish.Time to pass is from first arrival at the exit zone to presence in the SAZ. *Time in the intake channel is calculated from entry to this channel until last detection inside it, and so may be shorter than actual time. ** Final detection for this fish was simultaneous at exit and entrance zones.

Species	Length	Sex	Start	End	Duration (h)	Time to pass (h)	Entries to turbine channel	Time in channel*
sa	610	m	28/11/2013 23:06	29/11/2013 11:34	12.47	12.10		
sa	637	m	27/12/2013 21:33	19/01/2014 06:32	536.98			
sa	695	f	01/01/2014 01:05	01/01/2014 01:05	0.00			
sa	598	m	17/01/2014 20:29	17/01/2014 22:34	2.08	70.40		
sa	749	f	29/10/2014 00:15	31/10/2014 20:26	68.18	31.27		
sa	785	f	01/11/2014 03:19	01/11/2014 07:39	4.33		2	1.78
sa	714	f	13/11/2014 05:24	13/11/2014 05:54	0.50	0.50	1	0.35
sa	652	f	13/11/2014 14:56	14/11/2014 15:17	24.35	21.10		
sa	694	f	16/11/2014 17:17	16/11/2014 17:20	0.05			
sa	726	f	17/11/2014 09:52	17/11/2014 09:55	0.05			
sa	514	m	17/11/2014 20:40	17/11/2014 20:42	0.03	0.03		
sa	716	f	17/11/2014 21:39	17/11/2014 21:39	0.00			
sa	738	f	19/11/2014 06:57	19/11/2014 06:57	0.00			
sa	605	m	23/11/2014 09:07	23/11/2014 12:21	3.23	1.20	1.2	0.76
sa	712	f	26/11/2014 06:02	26/11/2014 06:23	0.35			
sa	751	f	29/11/2014 08:09	18/02/2015 21:51	1957.70	1957.70**	2	0.5
sa	798	m	30/11/2014 16:49	17/12/2014 21:56	413.12			
st	455	f	31/10/2014 04:47	31/10/2014 21:02	16.25			
st	470	f	09/11/2014 20:09	11/11/2014 15:53	43.73	23.62		
Mean					162.29	235.32		0.85

Table 4.14. Kelts moving downstream past Strathdon hydropower scheme. Duration of presence is from first to last detection of downstream movement per fish. Time to pass is from first arrival at the exit zone to presence in the SAZ.

Species	Length	Sex	Start	End	Duration (h)	Time to pass (h)
sa	712	f	23/11/2014 06:34	23/11/2014 06:42	0.13	0.02
sa	514	m	17/11/2014 14:59	17/11/2014 14:59	0.00	-
sa	610	m	14/11/2014 11:55	17/11/2014 14:23	74.47	2.37
sa	658	m	11/07/2014 07:23	11/07/2014 07:40	0.28	0.00
sa	726	f	16/11/2014 22:44	17/11/2014 01:58	3.23	3.10
sa	605	m	20/11/2014 16:52	20/11/2014 17:02	0.17	0.02
sa	738	f	11/12/2014 16:15	11/12/2014 16:20	0.08	0.02
sa	694	f	11/12/2014 15:11	11/12/2014 15:16	0.08	0.00
sa	785	f	28/10/2014 16:01	28/10/2014 16:14	0.22	0.05
sa	716	f	11/10/2014 04:33	11/10/2014 05:12	0.65	0.22
Mean					7.93	0.64

Table 4.15. Kelts moving downstream past Philiphaugh hydropower scheme. Duration of presence is from first to last detection of downstream movement per fish. Time to pass is from first arrival at the exit zone to presence in the SAZ. Times in turbine intake marked “T” had detection series consistent with turbine passage, but this could not be confirmed. * Two upstream presences lasting 4h51mins and 12h47mins.

Species	Length	Sex	Start	End	Duration (h)	Time to pass (h)	Time at turbine intake (h)	Time at fishway intake (h)
sa	665	m	13/11/2014 20:35	14/11/2014 07:28	10.88	10.86	0.79	
sa	760	f	07/12/2014 12:56	09/12/2014 15:46	50.83	13.9	13.83 T	11.6
sa	610	m	09/04/2015 15:53	09/04/2015 15:58	0.08			
sa	755	f	18/10/2014 06:55	18/10/2014 06:56	0.02			
sa	820	f	06/11/2014 20:51	08/11/2014 17:19	44.47*	44.4	0.05 T	0.0006
sa	830	f	07/05/2015 11:56	07/05/2015 11:56	0.00			
Mean					15.20	23.05	0.79	5.8

4.4 Discussion

Small, low-head hydropower schemes have the potential to disrupt movements important to the lifecycle of salmonids (*sensu* Anderson *et al.*, 2015). Studying upstream migrating adult Atlantic salmon movements at three sites with different characteristics offered the opportunity to draw generalities about these effects. The observed passage behaviours of adult Atlantic salmon at these three sites exhibited some commonalities and also some distinct differences, both in the magnitude and variability of the measured responses. In the following discussion these trends are described and explored in terms of potential causative mechanisms, particularly in the context of differing site characteristics and flow management. Treatment is then given to potential extensions to the analyses which may further elucidate the status of fish passage at these sites, and the factors which might influence this process. Conceivable sources of bias, and useful improvements to study design are considered. Finally, the implications of these findings are considered in the general context of the impacts of these types of hydropower schemes on wild salmonids on their spawning migration.

4.4.1 Trends in passage behaviour

Although the study at Craigpot involved the smallest sample of monitored fish, the observed behaviours here appear cause for least concern, with the majority of fish passing in a single visit lasting under one hour, with only brief excursions of a few minutes into the turbine outflow. This is not to say that Craigpot does not pose an obstacle at all, and indeed the observed effects of flow, and turbine:weir flow ratio on passage rates suggest that the structures and abstraction do modify fish behaviour to some extent. The flow ratio in particular was a strong predictor for time until passage from within the SAZ. A logical explanation is that at higher turbine:weir flow ratios, fish are more likely to be attracted to the turbine outflow, and may spend longer exploring this route before locating and ascending the depleted stretch. The absence of effect of turbine flow on passage or rejection rates also indicates that it is the turbine and weir flows in relation to each other that are important, rather than absolute turbine flow. The effect of weir flow is also logical, since with increasing flow, a greater number of potential passage routes over the depleted stretch and weir become available with increasing depth and wetted width, and so less time may be spent navigating this section. Additionally, as flow increases beyond the point where the turbine can abstract at full capacity, the attraction flow of the depleted stretch will increase to overpower that of the turbine outflow. A potential confounding effect is that at higher flows fish may have greater motivation to move, and move more quickly. In any case, these data suggest that Craigpot appears to become less of an obstacle with increasing flow and so does not inherently counter any natural increase in fish movement at higher flows.

By contrast to Craigpot, fish observed at Strathdon and Philiphaugh generally visited multiple times and spent substantial periods of time at the schemes (within the SAZ at Strathdon, and within the weir pool at Philiphaugh). At Strathdon, the results from the single and multiple covariate time to event analyses indicate that flows influence behaviour in so much as they modify the degree of persistence within the scheme (increasing with both turbine flow and weir level), rather than by changing the approach or passage rate. This suggests greater motivation to migrate at higher overall discharge, coupled with less restrictive depth conditions within the scheme. The results from the PIT monitoring in the outflow at Strathdon suggest that much of this persistence is within the outflow channel, with repeated visits lasting several hours.

At Philiphaugh, the rate at which fish located the outflow was positively (albeit only to a minor degree) related to both total flow and weir level, during the first part of a presence, and the strength of this relation slightly decreased later in the presence. The only other near to significant flow effects were an increase in rate of approach zone rejection with increasing turbine flow after 30 minutes, and decrease in rate of passage with increasing weir level. The time dependent effect of flow on outflow location could represent distinct behaviours: those fish that are actively exploring the different flow regions may find the outflows faster at higher total or weir flows, and those fish that were holding or making persistent attempts away from the outflow are not so affected. The near to significant decrease in passage from the outflows with weir level is interesting because it indicates that having located the outflows, increasing flow over the weir has the opposite effect to expected - rather than increasing attraction and passage at the fish pass it decreases it. The other near to significant positive effect of turbine flow on rate of approach zone rejection after thirty minutes is also counter intuitive – one might expect faster location of the turbines at higher turbine flow (which is not seen), or decreased rejection of the approach at higher weir levels (also not seen), but a decrease in rejection of the approach zone with increasing turbine flow is unexpected.

The fact that hydraulic variables (turbine flow and weir height) are not significantly associated with persistence in the approach, or with transition and rejection of the outflows is surprising. A possible interpretation is that at this scheme, the rate of passage is not sensitive to these variables within the ranges observed. Alternatively, it may be that the statistical models used do not adequately capture underlying relationships between these variables and movement rates. The Cox PH regressions used here were limited to testing for gross linear changes in movement rates over covariate ranges observed. It is probable that a linear relation with each of the flows is not really the case. More likely is that there are variable, perhaps asymptotic relationships that have optimal conditions for each rate of movement.

4.4.2 Potential extensions to the analysis

The analysis of fish passage behaviour in this study was limited by the resolution of the telemetry equipment to movement between zones of interest. Others (e.g. Noble et al., 2014) have used sophisticated acoustic telemetry arrays to collect fine scale movement data, and related this to information on hydraulics at a similar resolution, measured using acoustic Doppler profiling technology. Whilst this approach would undoubtably have provided useful information here, the coarser resolution approach was chosen to be within time and resource constraints.

The process of passing an obstacle may be considered as a combination of sub-processes: for example the location of a potential passage route, followed by attempted passage through that route. The rates of component events may each have differing relationships with flow. At Philiphaugh, although we have only monitored a broad approach zone and the turbine and fish pass outflows, there is a complex and dynamic set of attraction flows and resistances to passage, all in the immediate vicinity of the weir. By contrast the other sites present a false attraction flow some distance downstream from the weir. At all sites, the parallel competing attraction flows are related to each other via the abstraction regime of the turbine.

Figures 4.11 and 4.12 are conceptual illustrations of how an exemplar HOF-only abstraction regime might act to influence proportional discharge at different flow regions, based on those at Philiphaugh. Here, turbine abstraction may only begin where river flow exceeds Q_{90} (the HOF). Above Q_{90}, a flow equivalent to Q_{90} is required to be maintained through the fish pass and over the weir. All flow exceeding the flow equivalent to Q90 may be abstracted, up to a maximum at Q_{30}, above which the flow over the weir and fish pass increase, but turbine flow stays the same. Proportional discharge at each area (Figure 4.12) could be considered a proxy for attractive power, and is conjectured to influence the rate at which salmonids arrive at each region. This model suggests that at low flows, the fishway would be efficient at attracting fish. As flows increases beyond Q_{80} in this model, flow from the turbine (the black line in Figure 4.12) begins to dominate the other flows because this is not a proportional abstraction regime. At the highest flows (above Q_{10} in the depicted model), the spill over the weir (the red line in Figure 4.12) overpowers flow from both the turbine and the fish pass.

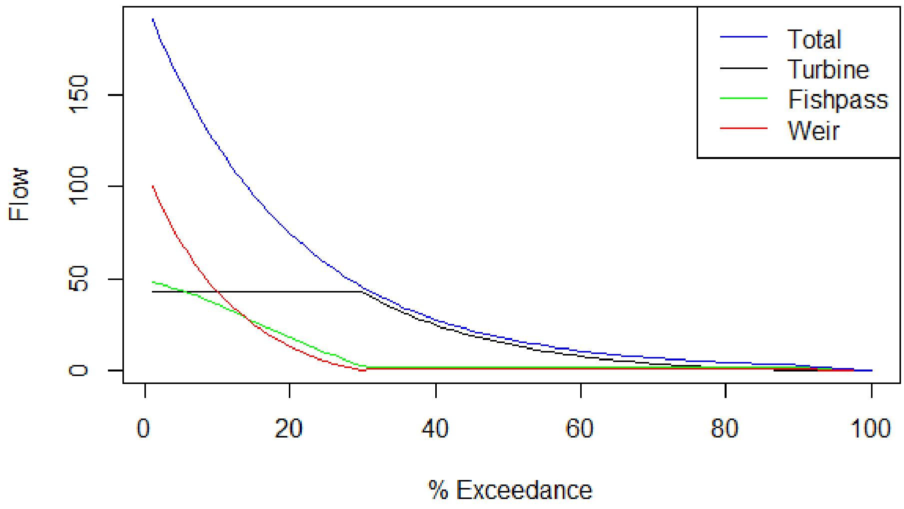


Figure 4.11. An exemplar flow exceedance curve, and the apportionment of this flow through alternative routes under a simulated “hands off” flow abstraction regime. “% Exceedance” is the proportion of time (as a percent) that the flow is above the flow indicated by the (blue) line for total discharge (uppermost smooth curve). The lines for the turbine, fishpass and weir indicate the flow which would be going down these routes given the flow on the total discharge exceedance curve.

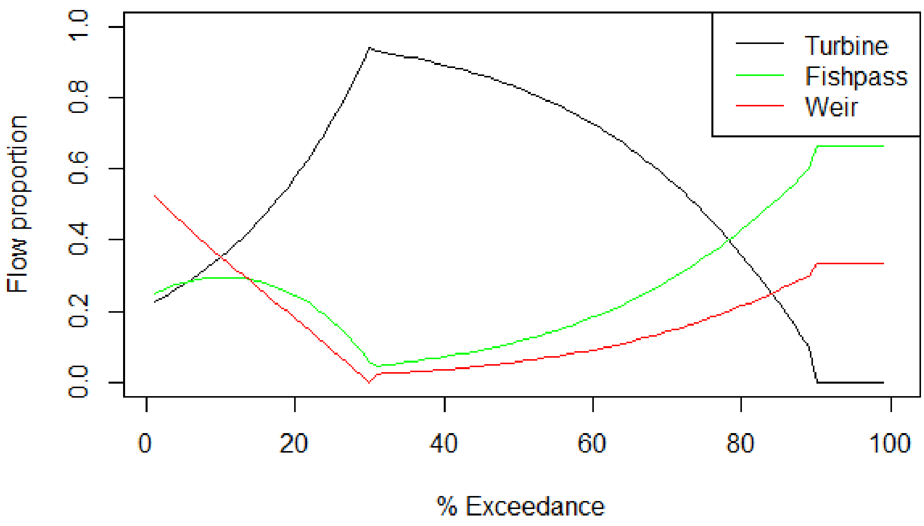


Figure 4.12. Flow proportions going through alternative routes under a simulated “hands off” flow abstraction regime as illustrated by Figure 4.11.

Flow management regimes should aim to balance power production with provision of flows that encourage efficient attraction and passage at fish passage routes. This requires the matching of hydrodynamic conditions that promote attraction with those that promote passage, at the river flows that fish attempt to make passage. For example the hypothetical case presented in (Figures 4.11 and 4.12) suggests that efficient attraction would occur below Q_{80}, based purely on the assumption of attraction to the area with highest discharge. The modification of the turbine abstraction to be a fixed proportion of total flow above the HOF, may enable the preservation of efficient attraction for a higher range of flows.

The next step in attempting to understand how competing and sequential sub processes contribute to overall passage, would be to identify and analyse each process using the time-to-event framework. The data collected at all three schemes may provide further insights if entry and exit from the competing outflow and depleted stretch routes are treated separately, and this is a logical extension to the analysis. At Strathdon the information from the radio antenna directly below the weir, and the PIT antennas in the turbine outflow and fish pass may also be used to further discretize fish movements with the aim of resolving regions of delay or resistance to movement.

Whilst the current analysis does not identify optimal conditions (e.g. flows) for passage (rather identifying the important covariates correlating with movement rates) a possible extension would be the flow frequency method used by Solomon et al. (1999), and refined by Greest et al. (2006). Here the cumulative frequency of flows where fish are available to migrate is compared to the cumulative frequency of flows during which actual migration occurs, allowing the actual usage of flows to be determined.

4.4.3 Return movements of post-spawned fish

Little is known about the downstream migration patterns of kelts. The short passage times at Strathdon in comparison to Craigpot is likely due to the tendency of kelts to exit small streams in the upper catchment quickly, but then continue through the lower catchment in such a way as to conserve energy (Haltunnen *et al.,* 2013). Interpreting the highly variable times spent at Craigpot and Philiphaugh in relation to possible scheme effects is challenging because kelts are known to display different behavioural patterns in their migration. For example Hubley *et al.* (2008) observed brief periods of continuous migration, but also of holding and backtracking. From the experimental release of six kelts into the turbine channel, it seems that these fish avoided passage through the trash rack and turbine (more likely avoiding passage beyond the trash rack, as supported by video observations), sometimes making repeated excursions into the channel, and eventually exiting upstream and passing down the depleted stretch (although only confirmed in one case). This difference in behaviour in comparison with smolts (Chapter 2), which passed through the turbine, is in accordance with previous findings that kelts are better able to locate bypasses and avoid turbine passage (Scruton *et al.* 2007), possibly because of their stronger swimming abilities (Booth 1997). Amongst the naturally migrating radio tagged fish, several were observed to have approached the turbine intakes at Craigpot and Philiphaugh. Haltunnen *et al.* (2013) suggested that optimizing each leg of the migration to the lowest possible risk and the highest possible gain is likely important for the overall survival. Delay and energy expenditure in turbine intakes could act to reduce onward survival.

4.4.4 Limitations and potential sources of bias

Ideally at each scheme, the approach and entry zones of each identified attraction flow region would have been monitored, though in the event this was not possible under resource and technical constraints. By combining those radio detection zones which were within the scheme affected area at Craigpot and Strathdon, a degree of consistency was achieved for cross-scheme comparison. The two regions considered at Philiphaugh differ somewhat in that the approach zone includes regions from which fish may attempt to pass, and of the attraction flows, only the turbine and Larinier fishway outflows were monitored specifically. This means that although we have fine resolution data on attraction to this region, we cannot fully understand the role of the weir face in competing with this zone.

A source of bias which is relevant to all studies of this type is uncertainty about the motivation of fish to pass. If it was known that all fish approaching a site were equally intent on ascending beyond it, then our observed rates of movements would reflect the actual covariate effects on those movements. In reality, we cannot know this motivation, and will not capture the full set of factors influencing movement behaviour: for example the effect of prior migration history on holding behaviour (Hinch & Rand, 2010).

At Strathdon, we have the particular problem that the site is in the upper part of the catchment, and has spawning habitat in both the outflow and depleted stretch. Spawning activity increases at higher flows (Webb *et al.*, 2001; Vollset *et al.*, 2016) and so this may be a confounding influence. It is possible that a portion of visits to the scheme were spawning activities. Without continual visual observation we cannot identify and account for spawning behaviour. It is conceivable that an aggregation of fish at such a site near to spawning time may also induce behaviours distinct from earlier migration patterns. These issues may be mitigated by selecting sites where these possibilities are minimized, or by increasing monitoring effort to account for them.

The rationale for considering three sites in concert was to gain general insights and contrast outcomes using differing site characteristics. It should be recognized that there could well be other reasons for these differences which arise from variation in unknown, unmeasured inter-site differences. Position within the catchment in combination with timing and migration history are likely to contribute to behaviour. In these studies, fish were captured in autumn/winter and their prior movements are unknown, other than limited observations of colour and sea-lice marks which can indicate time since entry to freshwater.

The location of fish capture relative to the scheme also differed between sites: whereas at Craigpot fish were for the most part captured 14 km downstream (with the exception of the two salmon captured at Craigpot in 2014), at Philiphaugh and Strathdon all fish were captured within the SAZ. These differences in relative capture location may be a source of bias. The fish captured at Kemnay were more likely to have been naïve to Craigpot because of the distance downstream, whereas those captured within Strathdon and Philiphaugh are known to have already been at these schemes at least once. On one hand, it could be argued that prior experience of a barrier may increase ability to pass it, but conversely, if there is individual variability in motivation or ability, those fish with a higher propensity to pass quickly may not have been available for capture, having already passed upstream.

Additionally, at Strathdon and Philiphaugh, the act of capturing fish and moving them a small distance downstream at a time of high flow when motivation is highest may have disadvantaged them. These are not issues that can be entirely resolved, but the larger samples at these sites, and the extent of recurrent visits and visit times suggest that these biases are likely a minor component of the observed behaviours. Ideally capture would be at first entry to the river, but the methods used were necessary compromises in order to attain the sample. The very fact that Strathdon and Philiphaugh provided convenient opportunity for efficient fish capture represents basic evidence of their status as partial barriers to migration (capture was also attempted at Craigpot, with no success), below which substantial aggregation occurs. An advantage of capture at a scheme is that there is greater likelihood that these fish are attempting to ascend beyond the scheme than any captured further downstream, and so the data return per capture effort is better.

A widespread limitation of these types of impact studies is a lack of baseline information, because for the most part the motivation for research comes only after the development, and any baseline information is incidental. It cannot be known what the behaviour of migrating fish would be in these river reaches in the absence of any hydropower infrastructure or flow modification, although reference can be made to behaviour at unaltered sites (e.g. the comparison of smolt migration on adjacent rivers with and without barriers, M.Newton, 2014, pers. comm.). It should be acknowledged that rates of movement and spawning activity of salmonids do depend on hydrologic and geomorphologic factors which vary between sites (Gibbins *et al.* 2002; Moir *et al.* 2004; Tetzlaff *et al.*, 2008). In the case of these small, low-head hydropower installations, the differentiation of new anthropogenic and natural effects on fish activity is inherently difficult because new developments usually use historic impounding structures, and these themselves often make use of natural falls or constrictions. In the absence of appropriate baselines, one can only describe the current status, and measure this against a desired status in order to decide whether the situation is acceptable.

4.4.5 Implications for management

It is striking that the proportion of fish which passed (given that they approached the scheme) was so much higher at Craigpot than at the other two schemes. The more detailed information from the time to event analyses further supports this difference, by showing that fish at Strathdon and Philiphaugh are spending longer at the schemes during multiple visits. What is surprising is that there were few, and unclear, positive hydraulic effects on rate of movement. Setting aside any potential biases or limitations in study design, one may consider some basic distinctions between these sites.

1) Craigpot has a variable abstraction regime, which is less of a modification to flow under all conditions than the hands off flow regime employed at Strathdon and Philiphaugh.
2) The barrier and depleted stretch at Craigpot present little challenge to adult salmonids under all but the lowest of flows, whereas the weirs at Strathdon and Philiphaugh are near impassable at all but the highest flows, aside from within the fish pass. Furthermore, the increase in

hydraulic attraction and decrease in resistance to passage is fairly uniform across the width of the barrier at Craigpot with increasing flow. At Philiphaugh and Strathdon, the relative attraction of the barrier away from the fish pass is much greater at higher flows, but these areas remain virtually impassable.

3) The outflow channel at Craigpot is a short *cul de sac*, whereas at Strathdon it is a long channel with spawning habitat, and at Philiphaugh it is in line with the rest of the weir.

It seems plausible that at Craigpot the combination of a short but impassable outflow, a low gradient alternative route with minimally obstructive weir and least modification of flow would have the best overall passage statistics. At Strathdon the combination of two longer alternative routes, one impassable and one partially passable, and with substantially competing flows from each, would produce more exploratory behaviour and lengthen time until passage, and potentially also encourage spawning within the scheme or downstream. Lastly at Philiphaugh, it is proposed that the hydraulic complexity at the higher flows - when passage motivation is greatest- results in more effort and time spent exploring and attempting to pass in regions away from the fish pass. This ‘overpowering’ of the fish pass at high discharge has been observed elsewhere (Bjornn & Peery, 1992; Quinn *et al.*, 1997; (Lundqvist *et al.*, 2008).

The impacts of delay, increased swimming effort, or stress resulting from repeated or sustained effort to cross partial barriers have many consequences which are interrelated, and may be immediate, latent or cumulative (Johnsson *et al.*, 2012). Aggregations at barriers increase likelihood of predation; physiological stress or physical injury can also result in susceptibility to predation or disease (Mathers *et al.*, 2002) and access to spawning grounds further upstream is reduced for a portion of the population. Although spawning behaviour has thus far been considered as a confounding factor for observing passage behaviour, it may also, at least partly, be an effect of limited passage opportunity and success. This has several potential population level effects: the potential for desiccation or freezing of redds (Malcolm *et al.*, 2012) in the outflow, overcutting by later arriving spawners and density dependent mortality of juveniles (Elliot, 1994; Milner *et al.*, 2003; Einum & Nislow, 2011) due to crowded spawning below the obstacle.

All these effects have consequences at the population level. In the next section (4.5), conclusions are drawn based on these studies which may be used to develop mitigation measures, and generalizations drawn for the design and placement of such schemes where salmonid stocks are an important consideration. It should be noted however that the decision as to whether these conclusions should apply will depend on management aims and catchment specifics. At Craigpot, for example, it is desirable to make the scheme as 'transparent' to fish migration as possible, since the Atlantic salmon population in this catchment is not at capacity (Don District Salmon Fishery Board, 2015), the scheme is midway up the catchment and there is abundant spawning habitat upstream. The same can be said of Philiphaugh, but at Strathdon we have the particular case that, at low flows when the barrier is more of an obstacle, useful spawning opportunities actually exist *within* the scheme itself. If the outflow channel was screened off to reduce delay by attraction to the turbine, then the spawning habitat therein would not be available. But in very low flow years this may be an important compensatory alternative spawning location for fish which are unable to ascend the weir. However, given the large extent of spawning habitat above the scheme, improving connectivity by reducing attraction to the outflow is likely the more desirable option.

4.5 Conclusions and recommendations

- Where the outflow and depleted stretch are distinct alternatives (as at Craigpot and Strathdon); the time that upstream migrants spend at the scheme during those flows where fish are moving, is likely to be related to proportion of flow going through the turbine. Therefore reduction of delay is likely contingent on maintaining adequate attraction flows to the upstream passage route.

- A short outflow, as is the case at Craigpot, appears to provide only a temporary distraction from movement upstream. Where there is a long outflow, the use of behavioural or physical barriers that shorten the area of outflow accessible may reduce delay at this area.

- Further to these observations, it is probable that the variable abstraction regime at Craigpot promotes fast passage over a HOF-only regime, though this requires more investigation for reliable evidence. Where delay to migration is seen at HOF-only schemes, the situation may be improved by maintaining attraction to fish passage routes via a variable abstraction regime.

- Where the fishway and turbine are on the weir, as is the case at Philiphaugh, the influence of flows on time at scheme is unclear, but further exploration of time spent at the alternative attraction flows may resolve this.

Chapter 5

General Discussion

5.1 Passage route and potential for delay of salmonid smolts at ASHT schemes

In Chapter 2, the proportion of migrating juvenile salmonids exposed to turbine passage at an ASHT scheme was estimated as 28% and 22% using Radio and PIT telemetry techniques respectively, and the splitting of the sample of smolts through alternative routes was in proportion to flow. This has important implications for flow management for the protection of fish at similar low-head schemes, and conforms in general to findings at large hydropower schemes (Coutant & Whitney, 2000), and for other species (eels, for example; Jansen *et al.*, 2007), where downstream migrants orient with the bulk flow.

Atlantic salmon smolts did not appear to be unduly delayed during nocturnal migration, with passage times of 28.8±39.9 mean±*se* minutes over the weir and depleted stretch, which was the slower of the two routes (but not significantly so). A few smolts were, however, present for substantially longer when this presence involved daytime. It was not clear whether this halting at the scheme during periods of daylight was simply the natural diel pattern of migration (Thorpe & Morgan, 1978, Riley, 2012; 2014), and which just happened to occur within the monitored areas, or whether it was due in part to the infrastructure at the scheme. Incidental visual observations were made of smolts aggregating in the turbine channel in front of the trash rack during the day. This indicates that this structure presents a behavioural barrier (Haro *et al.*, 1998; Kemp *et al.*, 2005; Enders *et al.*, 2009; Vowles *et al.*, 2014a) but the telemetry data showed that the tagged sample generally passed quickly at night. The results indicate that this type of hydropower scheme does not present a hindrance to downstream migrating smolts. Further research should consider the diversity of configurations, management regimes and site specific hydraulics in relation to smolt passage.

5.2 The effects of ASHT passage on salmonid smolts

Having thus shown that substantial proportions of juvenile migrating salmonids do pass through an ASHT, Chapter 3 tested whether this could cause harm to these fish. No severe injuries were found, nor was there a significant prevalence of a more subtle measure of damage – scale loss – associated with turbine passage. These results are a clear indication that smolts can pass through these turbines unharmed, with the caveats that: sample size limited power to detect low prevalence effects; that the impacts on the hatchery origin smolts used may differ from wild smolts; and that site specifics could influence outcome. Nevertheless this is valuable evidence that supports previous claims that ASHTs do not cause injury to smolts, except for limited scale loss to a small proportion of smolts (Kibel, 2007), provided that turbine design and operation conform to current regulatory guidelines (EA 2013, SEPA 2014). In summary, the current guidelines, requiring no screening for smolts, limiting rotational speeds and providing protection of the leading edge of the turbine blades appear sufficient. However a precautionary approach should be taken, and more evidence should be gathered for a variety of turbine specifications and situations.

For other species, available evidence on impacts of ASHT passage remains limited. Whilst Bracken & Lucas (2013) found a low damage rate for downstream migrating lamprey transformers and ammocoetes (1.5%), these remain a concern, because the relatively weak swimming abilities of this taxa, allied to their slender shape and small size may put them at greater risk from pinching and grinding between the turbine blades and trough. Eels too could be at greater risk from grinding because the freshwater phase tends to be thigmotactic (i.e. structure oriented, (Russon *et al.*, 2010)), whilst seaward migrating silver eels could be at affected differently because of the behaviourial, physiological and morphological adaptations that are preparations for marine migration (Rousseau *et al.*, 2008). Available evidence for eels (Kibel & Coe, 2008) indicates low rates of damage, but the migratory silver phase was not tested. Studies of downstream eel passage through Archimedes pumps in Belgium assessed mortality rates in the range 8-30%, with injuries reported as bruising, skin discolouration, and possible broken vertebrae, which were attributed mostly to grinding (Buysse *et al.*, 2014; 2015). The risks from Archimedean screws are likely to differ substantially between pumping and turbine modes, but these findings do demonstrate the potential for damage from these devices.

Further to testing for visible signs of damage, the study in Chapter 3 explored the use blood biochemistry correlates to investigate possible subtle damage. This novel application was less conclusive, in part because of limited sample size, but also because of a general lack of existing knowledge on the effective application of these methods to fish. Even in areas such as clinical pathology for aquaculture these techniques are not yet standardised (Braceland *et al.*, 2016). Nevertheless this study aspect was an important first step towards the exciting prospect of rapidly measuring subtle trauma to fish with biomarkers, without the complications of extended post-trauma observation (e.g. Normandeau Associates, 2009; Amaral *et al.*, 2015), or behavioural testing protocols (e.g. Cada *et al.*, 2003). It has, however, highlighted the need for further experimental validation of such techniques to enable their effective use in field settings. This should include testing of the intriguing effects of temperature and treatment lag which these results suggested, and which have been shown to be important in the limited number of studies using these measures to test for mechanical trauma (Yousaf & Powell 2012; Grizzle *et al.* 1992; Grizzle & Lovshin, 1994; 1996).

The latter part of Chapter 2 investigated onward survival after ASHT scheme passage, and found no association between measures of migration success and passage through the turbine or alternative route. This is evidence that ASHT passage does not cause rapid mortality compared with the alternative route, although the sample did not allow for detection of low prevalence effects. These types of field telemetry studies are a useful option for assessing latent or subtle effects of turbine passage because: a) they measure the most relevant outcome – the survival of fish in the natural environment; and b) they incorporate the host of environmental factors which may interact with the effects of passage to cause mortality (e.g. predation or disease (Mesa, 2002; Budy & Thiede 2002; Ferguson, 2006) without the need to understand them. However this comes with the risk that subtle effects are masked by environmental conditions for the particular period of study, and so multiple replicate studies over time are needed to gain reliable results. Telemetry studies on migrating fish are abounding, in many cases for the purpose of investigating human impacts (e.g. low-head barriers - Gauld *et al.* (2013); - recreational angling - Havn *et al.* (2015) – aquaculture – I.Moore (2016, pers.comm.), offshore renewables – M. Newton (2016, pers. comm)). These provide opportunities for the correlation of in river stressors (such as turbine passage, descaling, delay, catch and release angling), or indicators of stress or fitness (blood chemistry parameter levels, metabolic scope, condition), with onward survival, and such measures should be included where possible in order to enhance our understanding of the interplay of environmental conditions, human stressors and biological characteristics of migrants, and their onward survival (*sensu* McCormick *et al.*, 2009).

5.3 Adult salmonid interactions with ASHT schemes

Lastly, Chapter Four investigated the impacts of three ASHT schemes on adult salmonid migration. Impact, as quantified by proportional passage, time to ascend and number of visits by individuals, was highly variable both between and within sites. The fundamental finding, that halting and oscillatory behaviour, and aggregation of migrants, can occur at such schemes, is valuable evidence that small, low-head hydropower schemes can impact on the upstream movement of migratory salmonids, in ways which are analogous to those found at large hydropower schemes (e.g. Thorstad & Økland, 2003). The complement to this evidence is that none of the sites were found to be complete barriers to fish movement. This information should be viewed in the context of prior, existing and desirable fish passage situations for such sites. For example the modification of an existing weir for hydropower may improve fish passage status over the prior situation (Anderson *et al.*, 2014). Also even where passage is hindered, it may be that a sufficient proportion of migrants can access spawning grounds to meet management targets. Meanwhile weirs which have been in existence for a long time may provide valuable functions in the wider ecological context, such as predation opportunities for piscivorous animals. Obstacles to migration also constitute selective pressures on migrating populations (McLaughlin *et al.*, 2013). In some situations, weirs could play a role in balancing species interactions, and reducing or slowing the spread of invasive species (Holthe *et al.*, 2005; Fausch *et al.*, 2006, Rahel, 2013). Whilst it is often desirable to improve longitudinal connectivity for fish, it is important to evaluate such possibilities, in case of unintended consequences (McLaughlin *et al.*, op.cit).

It would appear from the studied sites that low-head diversion type schemes (Craigpot and Strathdon) can present very differing obstacles, and it is speculated that, at Strathdon, it is the long length of the attractant turbine outflow channel, the greater passage resisting characteristics of the barrier, and the HOF-only abstraction regime that make it a greater obstacle overall. The on-weir scheme at Philiphaugh also has a highly 'passage resistant' barrier, a HOF-only regime, and had high impact relative to Craigpot. However it is recognized that the motivation of fish to ascend upstream of these schemes cannot be accounted for. This study is, nevertheless, a step towards providing evidence about scheme configurations which impact on fish movements to greater or lesser extents. Configurations with short outflows are preferable for ensuring efficient upstream passage. Variable abstraction regimes appear to better balance attraction flows. It is recommended that the abstraction proportion is managed to provide opportunity for efficient passage (sufficient attraction flow to - and favourable hydraulics in - the passage route) at the river flows most utilized for migration.

By using the time-to-event framework, this study also showed that the rate of upstream fish passage at Craigpot relates to total flow, and to flow management. Passage times were, however, found to be generally fast regardless of flow condition. At Strathdon, the motivation to pass appears, as expected, to be greater at higher flows, but the rate at which upstream passage occurred did not increase. This was possibly due, in part, to fish being attracted to the scheme outflow. Such behaviour has previously been reported at a run of river schemes (Arnekleiv & Kraabøl, 1996). At Philiphaugh, the relationship between fish movement and flow, and flow management, was not clear. It is speculated that it is the range of competing attractant flows, including high levels of spill over the adjoining weir, which causes this. Similar effects have been reported to occur at large hydropower schemes (Lundqvist *et al.* 2008; Bjorrn and Peery, 1992; Quinn *et al.*, 1997). However, it was clear that the rates at which fish were attracted to the turbine and fishway flows far exceeded the rate of upstream passage from these areas.

Post-spawned fish movements at these schemes were highly variable in terms of the time spent at the scheme. This could have arisen from differing migration strategies that optimise survival given the location within catchment and fish condition (Hubley, 2008; Halttunen *et al.*, 2013). However, kelts did spend time at the approach to turbines, and this has implications in terms of energy cost, delay and predation risk. It appears that a long, open intake channel is more likely to result in delay for downstream moving kelts, and this should be avoided, possibly by coarse screening at the offtake. This could also have consequences for populations because the likelihood of repeat spawning is negatively related to energy depletion in the previous spawning event (Fleming, 1996; Jonsson, 1997; Wertheimer & Evans), and this may extend to exertion during downstream migration. No incidences of turbine passage were confirmed, and kelts were observed avoiding the entrance to the turbine, and passing back upstream out of the intake channel at Craigpot. This is consistent with findings by Scruton *et al.* (2007), who found that kelts were better able to avoid turbine entrainment than smolts. Whether protecting kelts is important is a question in need of attention. Whilst the proportion of repeat spawners is small (0.84% and 1.48% on the nearby rivers North Esk and Dee respectively (Malcolm *et al.*, 2010), they are more fecund, and produce larger eggs (Hatch *et al.*, 2014), and this influences the survival of offspring. They may also act to stabilize population fluctuations (Halttunen, 2011). This is because repeat-spawners can contribute proportionately more to the production of a new generation than their relatively low numbers would indicate, due to their larger size and their resultant higher fecundity (Niemelä *et al.*, 2006).

5.4 Cumulative impacts

The potential impact of single hydropower schemes on migratory fish populations can be increased if additional installations are present within the same river or catchment (Robson *et al.*, 2011; Fraser *et al.*, 2015). The cumulative effects of multiple hydropower schemes, as well as other in-stream barriers, within a single river have been investigated for a variety of diadromous fish species. Within the UK these studies have been restricted to Atlantic salmon (Gowans *et al.*, 2003) and river lamprey (Lucas *et al.* 2009). A small number of studies have also been carried out in mainland Europe (Chanseau *et al.*, 1999; Aarestrup & Koed, 2003; Winter *et al.*, 2006; 2007; Larinier, 2008; Pedersen *et* *al.*, 2012) and the USA (Bjorn *et al.*, 2000; Williams *et al.*, 2001; Moser *et al.*, 2002).

The impact of successive ASHT installations on salmonid passage was not specifically tested within the present study. Tracking data for the scheme at Craigpot and Strathdon showed that Atlantic salmon were able to ascend each scheme, but ascents of both schemes did not occur. It is clear, however, that downstream migrating smolts and kelts can negotiate passage. Whilst it is possible that the cumulative impact of low head schemes may be less than those of 'conventional' run-of-river schemes, this is an issue which not only merits further study, but could be an essential requirement for the installation of multiple schemes in rivers designated for nature conservation, such as SSSIs and SACs for migratory fish.

5.5 The future for anadromous salmonids in the face of a changing climate and shifting energy generation paradigms

The remarkable plasticity and spatial variation in the life history strategies of salmonids have been shaped by the environments in which they live and migrate. To maximise survival, these fish have developed strategies which allow them to survive in environments which display significant seasonal and interannual environmental variation, and these adaptations have developed over many generations. For anadromous salmonids local adaptations include, amongst others, the time taken to reach the smolt stage; smolt emigration times; marine feeding location and periodicity at sea; the timing of their return to freshwater; and the time of arrival in river spawning locations (Todd *et al.*, 2012, Otero *et al.*, 2014).

Freshwater, marine and terrestrial environments are currently undergoing unprecedented change due to climate warming (Munoz-Mas *et al.,* 2016) and the impacts of this can include phenological changes, biogeographical range shifts, reduced population fitness and population loss (e.g. Perry *et al.* 2005; Bradshaw & Holzapel, 2006; McGinnity *et al.*, 2009). Climate-mediated changes in the marine environment may also have significant implications for the survival of Atlantic salmon at sea (e.g. Todd *et al.*, 2010; Jonsson *et al.*, 2016; Renkawitz *et al.,* 2016) which are already at historically low levels (ICES, 2016). Whilst the relative influences of changes to freshwater and marine environments are interrelated and difficult to dissect, change is occurring. On average, the initiation of smolt emigration has occurred 2.5 days earlier per decade since the 1970s throughout the basin of the North Atlantic (Otero *et al.*, 2014). The run timing and age structuring of adults is also changing at a broad scale. In Scotland, this has manifested as a later arrival of adult fish, and a higher proportion of grilse, apparently related to elevated sea surface temperatures (Otero *et al.*, 2011; Todd *et al.*, 2012).

Whilst much work has focussed on adaptive responses to climate warming, in areas such as thermal tolerance (Enders & Boisclair, 2016) and the ability of aquatic organisms to acclimate to a range of higher summer temperature scenarios (Munoz-Mas *et al.*, 2016; Santiago *et al.*, 2016), relatively few studies have examined the impact of changing flow conditions and increasing temperatures on the performance of migratory salmonids (Fenkes *et al.*, 2016).

The installation or modification of obstacles to migration for hydropower may favour those individuals which have the ability to ascend rivers to reach spawning areas above them. Such modifications may therefore exert selective pressures which could have the potential to drive adaptation processes within individual populations over time. This could be achieved, for example, through the disruption of evolutionarily selected arrival times at migration destinations, or by changing environmental cues which initiate upstream and downstream movement (Wilcove & Wikelski, 2008; Marschall *et al.*, 2011). Within the River Don system such structuring is not apparent. Recent genetic analyses (Coulson *et al.*, 2012) showed that Atlantic salmon from most of the nine sites sampled within the catchment exhibited weak to no genetic differences from one another. However, before it can be concluded that there are little to no genetic differences within these systems, a much more detailed survey, using more precise genetic markers, should be undertaken

Such selection pressures have already been explored in relation to man-made structures, such as fish ladders (Volpato *et al.*, 2009). The specific impact of low head hydro as a structuring force within migratory salmonids populations within the UK has been unexplored. The legislative requirement to install and maintain adequate fish passage at such sites suggest that this may not become an issue. However, the combined effects of changing environmental pressures, and human alterations to migration corridors is an area for further exploration.

The global shift from the use of fossil fuels to sustainable energy sources such as small hydropower is a positive and desirable trend. In the past the use of long term stores of non-renewable energy has led to the long-term impacts of climate change, effects which were probably inconceivable near the start of their large scale exploitation in the last century. The present trend towards exploiting river and coastal currents for energy generation using hydrokinetic devices is likely at some scale to influence migratory animals which make use of these currents, including anadromous salmonids. This new era of renewables development requires an understanding of impacts as they occur, if these developments are to be ecologically sustainable.

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