Modification of Fish Behaviour by Parasites under Variable Flow Conditions

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A thesis submitted to Cardiff University for the degree of Doctor of Philosophy (PhD) in the School of Biosciences

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Douglas Adams (1979) The Hitchhikers Guide to the Galaxy

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Definition of Terms

Biological Terms

Term	Definition
Anguilla anguilla	European eel.
Anguillicoloides crassus	Nematode infecting the swimbladder of eels. Previously known as *Anguillicola crassus* before reclassification (Moravec 2006). However recently suggested to return to *Anguillicola crassus* (see Laetsch et al. 2012).
Complex lifecycle	Parasitic lifecycle in which there is movement between two or more hosts in the parasite life history (Parker et al. 2003).
Definitive host	Host in which the parasite reaches sexual maturity and reproduction (Schmidt and Roberts 1989).
Density dependent transmission	Transmission in which the rate of contact between infected and susceptible hosts increases directly with the density of the host population. Also known as mass action (McCallum et al. 2001).
Direct lifecycle	Parasitic lifecyle in which there is one host during the life history (Parker et al. 2003).
Direct transmission	A pathogen which transmission stages pass directly from one host to another, either via physical contact or a short distance through the environment (McCallum et al. 2001).
Ectoparasite	Parasite which lives on the outside of the host (Gosling 2005).
Endoparasite	Parasite which lives inside the body of its host (Gosling 2005).
Fork length (FL)	Total length of fish from most anterior tip of the body (tip of snout) to the centre of the fork on the caudal fin (Carlander and Smith 1945) .
Frequency dependent transmission	Transmission in which the rate of contact between infected and susceptible hosts is irrespective of the density of the population e.g. sexually transmitted disease (Begon et al. 2002).
Gasterosteus aculeatus	Three-spined stickleback.
Gyrodactylus bullatarudis	Common monogenean ectoparasite of the Trinidadian guppy originally described by Turnbull (1956).
Gyrodactylus gasterostei	Common monogenean ectoparasite on the three-spined stickleback.
Gyrodactylus turnbulli	Common monogenean ectoparasite on the Trinidadian guppy. Although originally described by Harris (1986) many papers published in the 1980s mistakenly identified this species as *G. bullatarudis*.
Intermediate host	Required host in which larval development must occur before the parasite is infective to its definitive host or additional intermediate hosts.
Mean parasite intensity	Mean number of individuals of a particular parasite species per infected host in a sample (Bush et al. 1997).
Parasite intensity	Number of individuals of a particular parasite species in the infected host (Bush et al. 1997).
Poecilia reticulata	Trinidadian guppy.
Prevalence	Number of individuals of a host species infected with a particular parasite divided by number of hosts examined. Usually expressed as a percentage (Bush et al. 1997).
Rheotaxis	Behaviour in which animals detect and orientate themselves to the flow of water. With positive rheotaxia, fish orientate themselves by positioning themselves with their head pointing upstream (Arnold 1974).
Sham infection	Method by which the fish are anaesthetised and manipulated so to simulate the infection process, without the transfer of parasites.
Shannon Weiner Index (*H*)	Diversity index proposed by Claude Shannon, also known as Shannon-Weaver Index or Shannon Entropy. Takes into account relative abundance of a species (Spellerberg and Fedor 2003).
Simpsons Diversity Index (*1-D*)	Diversity index proposed by Edward Simpson. Probability that two individuals randomly selected from a sample will be of different species. The lower the index, the less diverse the sample (Spellerberg and Fedor 2003).
Species richness	Number of species in sample. The more species, the 'richer' the sample (Spellerberg and Fedor 2003).

Standard length (SL)	Measurement from the most anterior tip of the body (tip of snout) to the end of the flesh on the caudal peduncle (Carlander and Smith 1945).
Station holding	The ability of a fish to maintain position in a current relative to the substratum (Gerstner 1998).
Transmission rate	Number of new host infections per area per unit time (McCallum et al. 2001).

Fluid Mechanic Terms

Term	Definition
Area mean velocity, $\bar{u}$	Mean velocity of a cross-sectional area of the channel defined as the volume rate of discharge, Q, divided by the cross section area, A (Douglas et al. 2005).
Control volume	A three-dimensional region selected for the purposes of fluid analysis to which specific fundamental physical laws can be applied.
Discharge, Q	Total volume of fluid flowing in unit time past a cross-section of a channel. Also known as flow rate (Douglas et al. 2005).
Turbulence length scale	Physical property which represents the size of the large dominant eddies in turbulent flows (Tennekes and Lumley 1972).
Turbulent Flow	Three-dimensional time-dependent motion characterised by rapid fluctuations superimposed on the mean velocity. In open channels, turbulent flow occurs when the Reynolds number (ratio of the inertial forces to the viscous forces) is >2000 (Bradshaw 1971; Douglas et al. 2005).
Turbulent kinetic energy (k)	Measure of the total turbulent energy production per unit mass and hence a bulk measure of the turbulence intensity.
Turbulent shear stress (Reynolds stress)	Caused by the irregular movement of fluid particles and their continuous exchange of momentum from one portion of fluid to another (Tennekes and Lumley 1972).
Velocity shear	Fluid particles in a turbulent flow experience different velocities depending on their spatial positions within a cross-section. These different particle velocities generate velocity shear.

References

Arnold GP (1974) Rheotropism in fishes. Biol Rev 49:515–576

Begon M, Bennett M, Bowers RG, et al. (2002) A clarification of transmission terms in host-microparasite models: numbers, densities and areas. Epidemiol Infect 129:147–53

Bradshaw P (1971) An introduction to turbulence and its measurement. Pergamon Press, Oxford

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al revisited. J Parasitol 83:575–583

Carlander K, Smith L (1945) Some factors to consider in the choice between standard, fork or total lengths in fishery investigations. Copeia 1945:7–12

Douglas JF, Gasiorek JM, Swaffield JA, Jack LB (2005) Fluid Mechanics, 5th ed. Pearson Education Limited, Harlow, Essex

Gerstner CL (1998) Use of substratum ripples for flow refuging by Atlantic cod, *Gadus morhua*. Environ Biol Fishes 51:455–460

Gosling PJ (2005) Dictionary of Parasitology. CRC Press, Taylor & Francis Group

Harris (1986) Species of *Gyrodactylus* von Nordmann, 1832 (Monogenea Gyrodactylidae) from poeciliid fishes, with a description of *G. turnbulli* sp. nov. from the guppy, *Poecilia reticulata* Peters. J Nat Hist 20:183-191

Laetsch DR, Heitlinger EG, Taraschewski H, Nadler SA, Blaxter ML (2012) The phylogenetics of Anguillicolidae (Nematoda: Anguillicoloidea), swimbladder parasites of eels. BMC Evol Biol 12:60

McCallum H, Barlow N, Hone J (2001) How should pathogen transmission be modelled? Trends Ecol Evol 16:295–300

Moravec F (2006) Dracuncloid and Anguillicoloid Nematodes Parasitic in Vertebrates. Academia, Praha

Parker G A, Chubb JC, Ball MA, Roberts GN (2003) Evolution of complex life cycles in helminth parasites. Nature 425:480-484

Schmidt GD, Roberts LS (1989) Foundations of Parasitology, 4th Edition. Times Mirror/Mosby College Publishing

Spellerberg IF, Fedor PJ (2003) A tribute to Claude Shannon (1916 - 2001) and a plea for more rigorous use of species richness, species diversity and the “Shannon - Wiener” Index. Glob Ecol Biogeogr 12:177–179

Tennekes H, Lumley JL (1972) A First Course on Turbulence. MIT Press

Turnbull, ER (1956) *Gyrodactylus bullatarudis* n. sp. from *Lebistes reticulatus* Peters with a study of its life cycle. Canad J Zool 34:583-594

Publications

Publications associated with PhD thesis

- Newbold LR, **Hockley FA**, Williams CF, Cable J, Reading AJ, Auchterlonie N (in press) Non-native parasites alter European eel *Anguilla anguilla* swimming behaviour on encountering accelerating flow. J Fish Biol
- **Hockley FA**, Wilson CAME, Graham N, Cable J (2014) Combined effects of flow condition and parasitism on shoaling behaviour of female guppies *Poecilia reticulata*. Behav Ecol Sociobiol 68: 1513-1520
- **Hockley FA**, Wilson CAME, Brew A, Cable J (2014) Fish responses to flow velocity and turbulence in relation to size, sex and parasite load. J R Soc Interface 11:20130814

Publications not associated with PhD thesis

- van Oosterhout C, Mohammed RS, Xavier R, Stephenson JF, Archard GA, **Hockley FA**, Perkins SE, Cable J (2013) Invasive freshwater snails provide resource for native hermit crabs. Aquat Invasions 8:185-191
- Longshaw M, Stebbing PD, Bateman KS, **Hockley FA** (2012) Histopathological Survey of pathogens and commensals of white-clawed crayfish (*Austropotamobius pallipes*) in England and Wales. J Invertebr Pathol 110:54-59
- Longshaw M, Bateman KS, Stebbing P, Stentiford GD, **Hockley FA** (2012) Disease risks associated with the importation and release of non-native crayfish species into mainland Britain. Aquatic Biol 16:1-15
- **Hockley FA,** Williams CF, Reading AJ, Taylor NGH, Cable J (2011) Parasite fauna of introduced pumpkinseed fish *Lepomis gibbosus*: first British record of *Onchocleidus dispar* (Monogenea). Dis Aquat Organ 97:65-73

Abstract

Fish populations are increasingly under threat by anthropogenic habitat modification. As demands on rivers have increased through increased human activity, resultant watercourse manipulations have altered the natural flow regime. However, it is unclear how diseased fish react to variable flow conditions in terms of their behaviour and swimming ability. This thesis addresses fundamental questions about the interaction between flow hydraulics and fish behaviour using two popular model systems: Trinidadian guppies (*Poecilia reticulata*) and three-spined sticklebacks (*Gasterosteus aculeatus*). Firstly it was found that guppies shoaled less when a member was infected with *Gyrodactylus turnbulli* but the magnitude of this effect was greater in the absence of flow (Chapter 2). Over time, the shoaling behaviour of guppies in the static flow condition reduced as parasite prevalence increased. In the flow condition, however, this effect was not observed, which resulted in higher peak prevalence of the parasite (Chapter 3). Sticklebacks utilised the lower flow velocities near the bed boundary layer to improve anaerobic and aerobic swimming performance but their natural parasite fauna had little effect on their swimming ability (Chapters 4 and 5). Guppies of different size, sex and parasite load utilised different regions around boulders to refuge from undesirable hydraulic conditions (Chapter 6). Finally, the impact of an invasive nematode *Anguillicoloides crassus* on the swimming behaviour of downstream migrating European eels (*Anguilla anguilla*) was investigated (Chapter 7). The parasite reduced burst swimming ability of the eels, which may have a knock-on effect for migration. In summary, this thesis demonstrates the importance of flow heterogeneity within a river system to provide shelter for smaller or weaker fish in poor health. River managers need to carefully consider any adaptation of flow regimes to provide appropriate flow conditions for resident species displaying a range of microhabitat requirements.

Thesis Layout

This thesis consists of a general introduction to freshwater ecosystems and the ecological and economic importance of aquatic animal health (Chapter 1). The model host-parasite systems used in the thesis are introduced. This is followed by six experimental, self-contained chapters (Chapters 2-7), two of which are published (Chapters 2 and 6) and a third has been submitted for publication (Chapter 7). The remaining three are in preparation for submission for publication. Additional data collection is required for Chapter 3 prior to submission. The experimental chapters are followed by a general discussion (Chapter 8), which summarises the findings of the thesis and highlights important implications of the research for river managers and aquaculture. The appendices contain two additional experiments. The first follows up on the results of Chapter 4 (Appendix 1) and the second describes a field experiment with planned laboratory experiments (Appendix 2) combining methods from Chapters 5 and 7.

Chapter 1. General Introduction

Freshwater ecosystems

Despite covering less than 0.01% of the earth's surface, freshwater habitats play a vital role in economic development and ecosystem services (Vörösmarty et al. 2010; Mota et al. 2014). Globally, freshwater biodiversity has been increasingly threatened by a combination of climate change and human activity such as urban and agricultural land use and hydropower generation (Ormerod 2009; Vörösmarty et al. 2010; Hering et al. 2014). In Europe, the Water Framework Directive (WFD) was adopted in 2000 to set surface water quality standards (e.g. for bathing, outdoor recreation, industry and drinking), implement discharge controls and minimise the impact of anthropogenic pressures (Hering et al. 2010). The WFD triggered the reorganisation of water management by river catchment rather than administrative borders. Restoration measures for river catchments are orchestrated by River Basin Management Plans (RBMPs). Understanding how multiple stressors impact ecological status is vital for developing effective RBMPs and shaping future environmental policy (Hering et al. 2014).

During the 20th century over 45,000 large dams (>15 m high) have been constructed worldwide. The negative effects on river ecosystems caused by dams are well documented (Poff et al. 2007). Recent reports from European RBMPs suggest that one of the major stressors on European rivers is hydromorphological degradation which affects >40% of rivers and >30% of lakes (Kristensen 2012; Hering et al. 2014). In England and Wales, over 40% of river length is classed as 'severely modified' due to practises such as water abstraction, channel reinforcement, culverts, weirs, dams and sluices (Environment Agency 2010). River modifications affect flow patterns, often causing dramatic fluctuations in discharge such as a reduction in river flow when reservoirs are filled or sudden increase in flow through turbines to match energy demands (Murchie et al. 2008). The changes to flow regime can have significant effects on fish habitat, which in turn can have consequences for fish populations (see reviews by Bunn and Arthington 2002; Murchie et al. 2008). Although it is clear that modified flow can affect fish, the severity and direction of response varies widely (Murchie et al. 2008). Murchie et al. (2008) concluded that there was a paucity of knowledge about non-salmonid taxa, that few studies have evaluated the behaviour of fish during dynamic periods of flow and there is a need to examine factors known to co-vary with flow. In addition Murchie et al. (2008)

called for an interdisciplinary approach to integrate hydraulic modelling with biological responses in both field and laboratory studies.

Aquatic animal health

In addition to habitat modification and climate change, freshwater habitats are further threatened by overexploitation, invasive species and emerging diseases (Okamura and Feist 2011). Although population dynamics of infectious diseases are expected to respond to environmental change in various ways, the threat to freshwater species cannot be ignored. Freshwater environments have multiple roles in the transmission of disease, acting as a transport medium for waterborne pathogens or acting as a habitat for vectors, intermediate or definitive hosts (Johnson and Paull 2011). Anthropogenic change to freshwater fauna such as movement of fish stocks and associated pathogens, introduction of non-native species and movement of animal products will also drive disease emergence (Peeler and Feist 2011). With multiple stressors acting synergistically, freshwater systems may be the most sensitive to environmental change (Ormerod 2009).

The introduction of infectious disease in aquatic systems can be devastating both environmentally and economically. For example the introduction of *Gyrodactylus salaris* from Sweden to Norway in Atlantic salmon (*Salmo salar*) in the 1970's resulted in the collapse of wild salmon populations in 45 Norwegian rivers (Peeler et al. 2006). In Norway the annual loss of salmon is estimated to be 15-20% or 250-500 tonnes, equating to an annual loss of 34 million USD from fisheries and tourism, with a further annual expense of 23 million USD for surveillance and eradication (Bakke et al. 2007). In total, the parasite invasion is estimated to have cost Norway 450-600 million USD since its introduction 30 years ago (Bakke et al. 2007).

The World Organisation for Animal Health (OIE) regularly publishes the Aquatic Animal Health Code which aims to assure the safe of transport of aquatic animals and their products to avoid spread of infectious disease. *G. salaris* is one such parasite listed as a notifiable disease in the Aquatic Animal Health Code. Surveillance for notifiable fish diseases is compulsory in Europe under the EU Fish Health Directive (2006/88/EC). However, trans-boundary movement of aquatic animals through the import of live animals for aquaculture and aquarium trade will lead to associated introduction of disease which may threat native species (e.g. Hockley et al. 2011; Longshaw et al. 2012).

Diseased fish and the relationship with flow condition

Any physical or chemical disruption to freshwater ecosystems which affects the natural distribution patterns of aquatic biota will in turn affect the spread of exotic and endemic disease (Bunn and Arthington 2002). Research into the relationship between river hydraulics and fish disease is vital in supporting decisions by river managers, fish farmers and aquarium keepers to prevent disease introduction, spread and resultant mortality.

The effect of parasitism on fish behaviour has been widely studied both in the wild and the laboratory (see review by Barber et al. 2000). However the relationship between flow condition and fish disease has been largely overlooked. In general, infection prevalence and intensity is found to be higher in fish inhabiting low flows or stagnant water (Sousa and Grosholz 1991; Leniham et al. 1999; Barker and Cone 2000; Bodensteiner et al. 2000; Hallett and Bartholomew 2008). However, it is unclear whether residing in low flows is the effect or cause of disease. For example, parasites may be causing pathology which has an energetic drain on the host resulting in the fish seeking areas of low flow. Alternatively poorer swimmers may aggregate in low flow areas resulting in a higher transmission and infection status.

Study Species

Understanding how environmental factors influence parasite infection and transmission is facilitated by the use of model systems. A good host-parasite model system is one in which the ecology and life-history is well documented such that experimental studies can easily differentiate between “normal” and “abnormal” responses. The most successful model systems are those which have a wide geographical range and are common in the wild and/or can be easily and cheaply reared, bred or cultured in the laboratory. It is also important that the captive conditions do not constrain the natural behaviour of the species and that the size of the fish is considered. These model systems can then be used to provide data for epidemiological models or to develop specific methodology. Two fish species stand out as being the most popular and well established models for behavioural studies. These are the Trinidadian guppy (*Poecilia reticulata*) and the three-spined stickleback (*Gasterosteus aculeatus*).

The guppy is a well-established model organism used in several decades of research on evolutionary biology, ecology and behaviour (Fraser et al. 2011). For example, van

Oosterhout et al. (2007) used guppies as a model system to investigate the genetic and immunological impacts of captive rearing. Guppies are easily kept and bred in aquaria, are sexually dimorphic and have short generation times making them ideal for sexual selection studies (Amundsen 2003). In their natural environment in Trinidad, Venezuela, Guyana and Surinam, guppies inhabit a range of tropical habitats from fast-flowing mountain streams to lowland turbid rivers. Even within the same river catchment, guppy populations may be physically separated and subject to different pressures such as predation risk, overhead cover and flow discharge. This leads to large variation in their adaptation and evolution of life history traits (Fraser et al. 2011). Guppies are also an important economic species, being one of the most popular aquarium species and now have a worldwide distribution as a result of this trade.

The three-spined stickleback is a cold water fish found in freshwater, brackish and salt water and is widespread throughout the northern hemisphere. The fish are easy to catch in the wild, straightforward to rear in the laboratory (Barber and Scharsack 2010) and their biology and ecology are well documented (Katsiadaki 2007). Sticklebacks can act as a bioindicator for environmental pollution and are a major model organism for endocrine disruption research due to their strong sexual characters, behaviour and ability to determine genetic sex (Katsiadaki 2007). Sequencing of the stickleback genome (Kingsley 2003) has also facilitated the use of this fish in molecular studies of evolution and development (reviewed in Barber and Scharsack 2010).

Gyrodactylids are common ectoparasites of both guppies (*Gyrodactylus turnbulli* and *G. bullatarudis*) and three-spined sticklebacks (*G. gasterostei*) and are the best studied of all monogeneans (Bakke et al. 2007). Scott and Anderson (1984) first recognised these parasites as useful model systems to study disease dynamics within a laboratory in both short and long-term experiments. Gyrodactylids are a major driving force of many life history traits and are known to affect host behaviour such swimming ability, foraging (Bakke et al. 2007), sexual selection (Kennedy et al. 1987), social network structure (Croft et al. 2011), mortality (Cable and van Oosterhout 2007) and genetic structure (Fraser et al. 2010). The parasite short generation time and viviparous reproduction can lead to exponential population growth, which makes their population dynamics more akin to micro- rather than macroparasites (Johnson et al. 2011). The advantage of being able to count individual worm burdens on the skin of fish without euthanasia makes gyrodactylids an ideal and ethical system to study parasite population dynamics over

time. In addition the parasites are easy to culture, ubiquitous and highly diverse. Gyrodactylids are also economically important in the case of the aquaculture industry. *G. salaris* has had a devastating effect on salmon populations and species such as *G. turnbulli* and *G. bullatarudis* are commonly found infecting ornamental fish in the pet trade (Bakke et al. 2007).

Although model systems are important for answering general epidemiological and ecological questions, they may not be suitable for investigating interactions between specific parasites and their host. For example, the European eel (*Anguilla anguilla*) has experienced a major population crash in the last half century (ICES 2013) as a result of a number of contributing factors including habitat modification and infection with the invasive nematode *Anguillicoloides crassus* (see Feunteun 2002). High intensities of *A. crassus* cause more severe pathological damage in European eels compared to the native host Japanese eels (*Anguilla japonica*) (see Kirk 2003). In a case such as this, there are no suitable alternative models to investigate the interaction of environmental stressors and infection with *A. crassus* in European eel hosts.

Thesis aims

The aim of this thesis was to investigate the effect of hydraulic variation on the behaviour of fish using two model species (guppies and sticklebacks) and a species of high conservation importance (European eels). The behaviours of interest included shoaling, aerobic and anaerobic swimming performance, microhabitat use and migratory ability. Through the use of open channel laboratory flumes, the hydraulic conditions were manipulated to investigate the effect of velocity, turbulence and shear stress on the behaviour of infected fish. This bridged the gap between static laboratory conditions and variable flow conditions in the wild.

References

Amundsen T (2003) Fishes as models in studies of sexual selection and parental care. J Fish Biol 63:17–52

Bakke TA, Cable J, Harris PD (2007) The biology of gyrodactylid monogeneans: the "Russian-doll killers". Adv Parasitol 64:161–376

Barber I, Hoare D, Krause J (2000) Effects of parasites on fish behaviour: a review and evolutionary perspective. Rev Fish Biol Fish 10:131–165

Barber I, Scharsack JP (2010) The three-spined stickleback-*Schistocephalus solidus* system: an experimental model for investigating host-parasite interactions in fish. Parasitology 137:411–24

Barker DE, Cone DK (2000) Occurrence of *Ergasilus celestis* (Copepoda) and *Pseudodactylogryrus anguillae* (Monogenea) among wild eels (*Anguilla rostrata*) in relation to stream flow, pH and temperature and recommendations for controlling their transmission among captive eels. Aquaculture 187:261–274

Bodensteiner LR, Sheehan RJ, Wills PS, Brandenburg AM, Lewis WM (2000) Flowing water: an effective treatment for Ichthyophthiriasis. J Aquat Anim Health 12:209–219

Bunn SE, Arthington AH (2002) Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environ Manage 30:492–507

Croft DP, Edenbrow M, Darden SK, Ramnarine IW, van Oosterhout C, Cable J (2011) Effect of gyrodactylid ectoparasites on host behaviour and social network structure in guppies *Poecilia reticulata*. Behav Ecol Sociobiol 65:2219–2227

Environment Agency (2010) River habitats in England and Wales: current state and changes since 1995-96. Environment Agency, Bristol

Feunteun E (2002) Management and restoration of European eel population (*Anguilla anguilla*): An impossible bargain. Ecol Eng 18:575–591

Fraser B, Ramnarine I, Neff BD (2010) Temporal variation at the MHC class IIb in wild populations of the guppy (*Poecilia reticulata*) 64:2086-2096

Fraser BA, Weadick CJ, Janowitz I, Rodd FH, Hughes KA (2011) Sequencing and characterization of the guppy (*Poecilia reticulata*) transcriptome. BMC Genomics 12:202

Hallett SL, Bartholomew JL (2008) Effects of water flow on the infection dynamics of *Myxobolus cerebralis*. Parasitology 135:371–384

Hering D, Borja A, Carstensen J, Carvalho L, Elliott M, Feld CK, Heiskanen A-S, Johnson RK, Moe J, Pont D, Solheim AL, van de Bund W (2010) The European Water Framework Directive at the age of 10: a critical review of the achievements with recommendations for the future. Sci Total Environ 408:4007–4019

Hering D, Carvalho L, Argillier C, Beklioglu M, Borja A, Cardoso AC, Duel H, Ferreira T, Globevnik L, Hanganu J, Hellsten S, Jeppesen E, Kodeš V, Solheim AL, Nõges T, Ormerod S, Panagopoulos Y, Schmutz S, Venohr M, Birk S (2014) Managing aquatic ecosystems and water resources under multiple stress - An introduction to the MARS project. Sci Total Environ [in press]

Hockley FA, Williams CF, Reading AJ, Taylor NGH, Cable J (2011) Parasite fauna of introduced pumpkinseed fish *Lepomis gibbosus*: first British record of *Onchocleidus dispar* (Monogenea). Dis Aquat Organ 97:65–73

ICES (2013) Report of the Joint EIFAAC/ICES Working Group on Eels (WGEEL). Sukarietta, Spain, 18-22 March and Copenhagen, Denmark 4-10 September, 2013, ICES CM 2013/ACOM:18

Johnson MB, Lafferty KD, van Oosterhout C, Cable J (2011) Parasite transmission in social interacting hosts: monogenean epidemics in guppies. PLoS One 6:e22634

Johnson PTJ, Paull SH (2011) The ecology and emergence of diseases in fresh waters. Freshw Biol 56:638–657

Katsiadaki I (2007) The use of the stickleback as a sentinel and model species in exotoxicology. In: Ostlund-Nilsson S, Mayer I, Huntingford FA (eds) Biol Three-Spined Stickleback. CRC Press, Taylor & Francis Group, pp 353–372

Kennedy CEJ, Endler JA, Poynton SL, McMinn H (1987) Parasite load predicts mate choice in guppies. Behav Ecol Sociobiol 21:291–295

Kingsley D (2003) Sequencing the genome of threespine sticklebacks (*Gasterosteus aculeatus*). National Human Genome Research Institute White Paper

Kirk RS (2003) The impact of *Anguillicola crassus* on European eels. Fish Manag Ecol 10:385–394

Kristensen P (2012) European waters: assessment of status and pressures. Luxembourg : Publications Office of the European Union. EEA Report No 8/2012, Denmark

Leniham HS, Micheli F, Shelton SW, Peterson CH (1999) The influence of multiple environmental stressors on susceptibility to parasites: An experimental determination with oysters. Limnol Oceanogr 44:910–924

Longshaw M, Bateman K, Stebbing P, Stentiford GD, Hockley FA (2012) Disease risks associated with the importation and release of non-native crayfish species into mainland Britain. Aquat Biol 16:1–15

Mota M, Sousa R, Araújo J, Braga C, Antunes C (2014) Ecology and conservation of freshwater fish: time to act for a more effective management. Ecol Freshw Fish 23:111–113

Murchie KJ, Hair KPE, Pullen CE, Redpath TD, Stephens HR, Cooke SJ (2008) Fish response to modified flow regimes in regulated rivers: research methods, effects and opportunities. River Res Appl 24:197–217

Okamura B, Feist SW (2011) Emerging diseases in freshwater systems. Freshw Biol 56:627–637

Ormerod SJ (2009) Climate change, river conservation and the adaptation challenge. Aquat Conserv Mar Freshw Ecosyst 19:609–613

Peeler E, Thrush M, Paisley L, Rodgers C (2006) An assessment of the risk of spreading the fish parasite *Gyrodactylus salaris* to uninfected territories in the European Union with the movement of live Atlantic salmon (*Salmo salar*) from coastal waters. Aquaculture 258:187–197

Peeler EJ, Feist SW (2011) Human intervention in freshwater ecosystems drives disease emergence. Freshw Biol 56:705–716

Poff NL, Olden JD, Merritt DM, Pepin DM (2007) Homogenization of regional river dynamics by dams and global biodiversity implications. Proc Natl Acad Sci U S A 104:5732–5737

Scott ME, Anderson RM (1984) The population dynamics of *Gyrodactylus bullatarudis* (Monogenea) within laboratory populations of the fish host *Poecilia reticulata*. Parasitology 89:159–94

Sousa WP, Grosholz ED (1991) The influence of habitat structure on the transmission of parasites. In: Bell SS, McCoy ED, Mushinsky HR (eds) Habitat Struct Popul Community Biol Ser Vol 8. Springer Netherlands, pp 300–324

van Oosterhout C, Smith AM, Hänfling B, Ramnarine I, Mohammed RS, Cable J (2007) The guppy as a conservation model: implications of parasitism and inbreeding for reintroduction success. Conserv Biol

Vörösmarty CJ, McIntyre PB, Gessner MO, Dudgeon D, Prusevich A, Green P, Glidden S, Bunn SE, Sullivan CA, Liermann CR, Davies PM (2010) Global threats to human water security and river biodiversity. Nature 467:555–61

Chapter 2. Combined effects of flow condition and parasitism on shoaling behaviour of female guppies *Poecilia reticulata*

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This chapter is adapted from the published article in the journal Behavioural Ecology and Sociobiology (Hockley FA, Wilson CAME, Graham N, Cable J (2014) Combined effects of flow condition and parasitism on shoaling behaviour of female guppies Poecilia reticulata. *Behav Ecol Sociobiol 68: 1513-1520).*

Abstract

For fish, living in groups can provide benefit of protection from predators and some parasites, more efficient foraging for food, increased mating opportunities and enhanced energetic benefit when swimming. For riverine species shoaling behaviour can be influenced by various environmental stressors, yet little is known how flow rate might influence the shoaling of infected fish shoals. In view of the increasingly unpredictable flow rates in streams and rivers, this study aimed to assess the combined effect of flow condition and parasitism on the shoaling behaviour of a model fish species. Shoal size, shoal cohesion and time spent shoaling of female guppies (*Poecilia reticulata*) were compared when infected with the directly transmitted ectoparasite *Gyrodactylus turnbulli* under flow and static conditions. Flow condition was an important factor in influencing shoaling behaviour of guppies with the fish forming larger shoals in the absence of flow. When a shoal member was infected with *G. turnbulli* shoal cohesion was reduced but the magnitude of this effect was dependent on flow condition. In both flow and static conditions, bigger fish formed larger shoals than smaller counterparts. Future changes to stream hydrology with more frequent flooding and drought events will affect the shoaling tendency of fish. During high flow events infected fish may not be able to keep up with shoal mates and therefore have a higher risk of predation. Additionally, these findings may be important for aquaria and farmed species where an increase in flow rate may reduce aggregation in fish.

Introduction

Many fish spend part or all of their lifetime living in groups forming either loosely aggregated shoals or polarised synchronised schools (Pitcher and Parrish 1993; Stumbo et al. 2012). The costs and benefits of grouping behaviour, environmental influences on shoaling and social interactions within shoals have been widely studied by animal ecologists (Pitcher and Parrish 1993). The physical constraints of being able to observe the natural behaviour of fish or recapture individuals in the wild have led to many laboratory or mesocosm experiments. Such experiments, by design, remove complexity from the study system to concentrate on a small number of potentially influencing factors. This has often resulted in shoaling studies using standard fish tanks with minimal water movement which do not reflect the flow conditions that fish experience in the wild.

Considering that riverine fish are subjected to a variety of flow conditions, from large-scale tidal or riverine currents to small-scale microhabitat variations, surprisingly few studies have investigated how shoaling preferences differ under different magnitudes of flow. In a recent study, Chicoli et al. (2014) demonstrated that shoal structure, stimulus detection and transmission of information between shoal members differed between flow and no-flow conditions in a small laboratory flume. Garner (1997) found that minnows (*Phoxinus phoxinus*) shoaled less during a high flow rate and the shoals failed to return to their original size within an hour following the flow event. Dominance hierarchies of three-spined stickleback (*Gasterosteus aculeatus*) shoals became increasing unstable as flow rate increased (simulating spate conditions) or water levels decreased (simulating drought) (Sneddon et al. 2006). In contrast, juvenile chub (*Leuciscus cephalus*) shoaling was not affected by flow rate unless in the presence of a predator where they exhibited greater aggregation at a higher flow rate (Allouche and Gaudin 2001). This indicates that shoaling differences with respect to flow could also depend on other influencing abiotic or biotic factors.

Abiotic influences known to affect shoaling include temperature (Weetman et al. 1999), vegetation cover (Allouche and Gaudin 2001) and demography (Song et al. 2011) but there are also many biotic factors which influence shoaling including sex (Griffiths and Magurran 1998; Ruhl and McRobert 2005; Richards et al. 2010), size (Paxton 1996; Hoare et al. 2000a) and disease (reviewed by Barber et al. 2000). Fish shoals tend to be size assorted to minimise phenotypic oddity (Hoare et al. 2000a). There is evidence for

increase in shoaling tendency in larger fish (Pitcher et al. 1983; Paxton 1996; Hoare et al. 2000a) due to their higher conspicuousness, higher calorific value to predators (Rodgers et al. 2011), better foraging ability (Hoare et al. 2000a) and development of discriminatory ability by larger fish to select larger shoals (Ledesma and McRobert 2008).

Previous studies have shown that parasites such as *Gyrodactylus turnbulli* on guppies (*Poecilia reticulata*) can influence population dynamics, as demonstrated in both laboratory and mesocosm experiments (Richards et al. 2010; Croft et al. 2011; Richards et al. 2012). When considering infectious disease, the costs and benefits of group living can be complex, typically varying with host taxa and the mode of parasite transmission. For parasites which actively seek out their hosts, the benefits of shoaling can be similar to that of anti-predator defence. The larger the group size, the lower the chance of a particular individual becoming infected (Poulin and Fitzgerald 1989). However for directly transmitted parasites, host-host contact within a social group could increase the spread of disease and therefore cause avoidance behaviour of group members. For example, three-spined sticklebacks avoided shoaling with conspecifics infected with the ectoparasite *Argulus canadensis* in a choice experiment (Dugatkin et al. 1994). Avoidance behaviour has also been observed for hosts infected with parasites with indirect life cycles; for example, banded killifish (*Fundulus diaphanous*) chose to shoal with unparasitised groups over those that were infected with the trematode *Crassiphiala bulboglossa* (see Krause and Godin 1996). However, regardless of transmission route, it is likely that often the anti-predator and foraging benefits of group living will outweigh the risk of infection (Barber et al. 2000). Some studies have even shown infected fish to shoal more. For example, rainbow trout (*Oncorhynchus mykiss*) infected with the trematode *Diplostomum spathaceum* showed enhanced shoaling tendencies compared to uninfected controls (Mikheev 2009).

Of the few studies which have examined the effect of flow rate on host-parasite interactions, generally there is a higher prevalence and intensity of parasites in low flow conditions. This may be due to an increased rate of contact between parasite and host, reduced physiological condition of the host due to increased sedimentation, hypoxia and lower food availability making them more susceptible to infectious disease (Leniham et al. 1999), or due to infected fish having lower energy reserves so selecting areas with low flow. For example, American eels (*Anguilla rostrata*) had higher prevalence and intensity

of the copepod *Ergasilus celestis* and monogenean *Pseudodactylogyrus anguillae* at a water velocity <5 cm s^{-1} (Barker and Cone 2000) and oysters *Crassostrea virginica* experienced a higher prevalence and intensity of the protist *Perkinsus marinus* at the base of reefs where flow rates were lower (Leniham et al. 1999). Similarly, free-living infective stages of the protozoan *Myxobolus cerebralis* were at a higher density in a low flow system (water velocity 0.02 cm s^{-1}) compared to a relatively faster flow system (water velocity 2 cm s^{-1}) and this was also reflected in the infection prevalence and duration in rainbow trout fry (*Oncorhynchus mykiss*) (see Hallett and Bartholomew 2008). High flow rates lowered mortalities of channel catfish (*Ictalurus punctatus*) associated with *Ichthyophthiruis multifiliis* (see Bodensteiner et al. 2000). In mammalian hosts, the highest infectivity of *Schistosoma mansoni* cercariae was detected at water velocities of 30-40 cm s^{-1}. Infections were reduced at velocities >40 cm s^{-1} due to increased turbulence affecting cercarial penetration and at <30 cm s^{-1} perhaps due to fewer contacts between the parasite and hosts (Sousa and Grosholz 1991). At present, such data is difficult to interpret as little is known about the changes in infected host behaviour with respect to flow (Hockley et al. 2014). The changes in host behaviour would in turn affect transmission potential of directly transmitted parasites and therefore better explain the reasons for the increase in parasitism at low flows. The combined effect of parasitism and flow on shoaling behaviour of fish, to our knowledge, has never been investigated.

In this study, the effect of flow on the tendency of fish to shoal was compared to static conditions typically used in laboratory experiments. Further, the size of fish and infection with parasites on the shoaling behaviour of fish was also investigated using the host-parasite model system guppies (*P. reticulata*) and the directly transmitted ectoparasitic monogenean *G. turnbulli*. Guppies are a popular model species in ecological and evolutionary studies and their shoaling behaviour is particularly well studied (Magurran 2005). Previous studies have shown that parasites such as *G. turnbulli* can influence population dynamics, as demonstrated in both laboratory and mesocosm experiments (Richards et al. 2010; Croft et al. 2011; Richards et al. 2012). It was predicted that shoaling would be reduced by both an increased flow rate and the presence of an infected individual and that larger fish would have a higher tendency to shoal.

Methods

Study system

Experiments were conducted between January and April 2012 using offspring from wild-caught guppies (*Poecilia reticulata*). Guppies were originally caught in the Tunapuna River, Trinidad and maintained in aquarium facilities at Bristol University, UK, before being transferred to Cardiff University, UK in October 2005. Female guppies (13.6-27.3 mm standard length) were size matched in groups of six individuals. Each shoal was housed in a 6 L aerated tank for a minimum of 12 days to familiarise (Griffiths and Magurran 1997) with different shoals visually isolated from each other. The shoals were maintained under a 12 h light: 12 h dark regime at a varying temperature range of 22-24°C, fed on a diet of fish flakes (Aquarian®) and bloodworm, with half water changes every second day. An isogenic strain of *G. turnbulli* (Gt3) isolated from ornamental guppies in 1997 was used for all experimental infections.

Experimental design

Observations of shoaling behaviour took place in a glass walled unidirectional recirculating open channel flume in the Hydro-environmental Research Centre (HRC), Cardiff University, UK. The channel measured 10 m length x 0.29 m width and had a tailgate weir at the downstream end to allow for control of the surface water profile. Discharge was adjusted by controlling the power provided to the pump through a control box. Honeycomb flow straighteners 2 cm thick were used at both ends of the flume. The channel was set at a negative gradient of 1 in 1000. Uniform flow was established by measuring the flow depths at nine points along the channel and calculating total energy head of the flow (E) using the equation $E = z + y + u^2/2g$ where y is the flow depth (0.135 m), u is the area mean velocity ($u=Q/A$), g is acceleration due to gravity, Q is discharge and A is the cross-sectional flow area of the channel. The variation of energy head with the distance along the flume was calculated for each weir setting and uniform flow defined as where the slope of the total energy head line equalled the channel bed slope. Chlorides were removed from the water by the addition of Haloex at 0.02 ml L^{-1} and water was heated between the range of 24-26°C using a 3 kW Electro Titanium Digital heater. A 2 cm^2 grid was drawn on one side of the flume side wall in order for the observer to estimate nearest neighbour distance between the shoaling fish.

A static (no flow) and flow (flow action) condition with an area mean velocity of 0.125 ms^{-1} (discharge 0.0048 m^3s^{-1}) was chosen for the trials. The flow velocity chosen was similar to that found in guppy streams in Trinidad (Reznick et al. 2001). Both conditions had a uniform flow depth of 0.135 m along the channel. Shoals of six female fish were used in both the flow action trial (n=10) and the static condition trial (n=13). On Day 1 each shoal was acclimatised in the flume for 30 min where they were observed to exhibit normal behavioural before pre-infection trials took place. A focal fish from the shoal of six was randomly assigned and then observed for a total of 10 min. During the first 5 min, the nearest neighbour distance (NND) ± 1 cm and size of shoal (number of fish in shoal) was recorded every 10 s for the focal fish. During the second 5 min period, the total time the focal fish spent shoaling was recorded. These observations were then repeated for two randomly selected non-focal fish from within the shoals. Shoaling was defined as being within four body lengths of the nearest neighbour (Pitcher et al. 1983) and NND was measured to a maximum of 20 cm, with distances any greater not scored. The identity of the focal fish was retained by a second observer carefully following that fish for the duration of the trial.

On completion of the first flume trials, one individual from each shoal from half the flow action trials (n=6) and half from the static condition trials (n=8) were infected with *G. turnbulli* following standard procedures (e.g. Richards et al. 2010). This was achieved by anaesthetising the individual with 0.02% MS222 and bringing it into contact with a heavily infected donor fish until four worms had transferred from donor to recipient (taking <5 min). Transfer of the parasites was observed continuously under a stereo-microscope. The remaining focal fish from the flow action trials (n=6) and static condition trials (n=5) acted as controls and were sham infected by placing them under anaesthetic and manipulating them under a microscope without transfer of parasites. Non-focal fish in all shoals were also sham-infected so all fish experienced the same degree of handling. All fish were then housed individually in 1 L pots to allow the infection to develop for three days but to prevent parasite transfer between fish. Although physically isolated, shoal members remained in visual contact throughout in order to maintain familiarity. The shoal groups were isolated from other shoals throughout the duration of the study.

On Day 4, infection was confirmed by restraining each individual in a small amount of water in a crystalizing dish under a stereo-microscope. All uninfected fish were sham-

screened to maintain equal handling stress. The shoals were then placed back in the open channel flume for a repeated behavioural trial post-infection. The 10 min observations for the focal and two randomly chosen non-focal fish were repeated after 30 min acclimatisation period. On completion of the trials, all fish were screened under anaesthetic for any parasite transmission and accurate worm counts. Mean intensity of *G. turnbulli* was 11.4 (range 1 – 29) after a three day infection. These comparatively low numbers are reflective of burdens observed in the wild (Harris and Lyles 1992; Fraser et al. 2010). There was no evidence of secondary pathology (e.g. fin clamping) in the fish at any time during the experiment and there was no evidence of parasite transmission between fish during the 30 min post-infection trials.

Statistical analysis

The effect of flow condition, parasitism and host standard length on shoaling behaviour parameters (shoal cohesion, shoal size and proportion of time shoaling) was assessed using Generalised Linear Mixed Models (GLMMs) with model selection and model averaging based on corrected Akaike Information Criterion (AICc) using the lme4 library (Bates et al. 2013) within the R statistical interface (R Core Team 2013) based on methods described in Burnham and Anderson (2002).

The model fixed effects were shoal infection status (binary factor whether there was a member of the group infected or uninfected), individual infection status (binary factor whether the individual fish was infected or uninfected), flow condition (flow action or static) and fish standard length (mm). Two-way interactions were also included to account for any differences in parasitism and standard length in different flow conditions. Individual infection status was nested within group infection status as a random term. As each fish was tested twice, the identity of individual fish was included in the models as a random factor to account for repeated measures. Time of day (to the nearest hour) was also included as a random term to account for any temporal effects of shoaling.

Shoal cohesion (measured as the mean nearest neighbour distance, NND up to 20 cm) and mean shoal size (number of fish in shoal) were $\log_e+1$ transformed to normalise the data. The shoal cohesion GLMM was fitted using an inverse-Gaussian error structure and log link function, the shoal size GLMM was fitted using a Gaussian error structure with square root link function and the proportion of time shoaling fitted with a binomial error

structure and logit link function. The fixed and random effects were included in global GLMMs with all plausible explanatory variables and interactions. Because the fixed effects were measured on different scales, the variables were standardised using the arm library (Gelman and Su 2013). The most important explanatory variables were determined by selecting the top most plausible models which fell within 2.5 AICc of the best model and model averaging the top models using the MuMIn library (Barton 2013) as described by Grueber et al. (2011). The output then gives the relative importance of each explanatory variable which is the sum of the Akaike weights for each variable for the models in which it appears across the top models, with the higher the value (closest to 1) giving a higher relative importance compared to the other variables (Burnham and Anderson 2002). The variables were considered significant if the 95% confidence intervals did not bound zero.

Results

Flow condition was an important predictor for the size of *P. reticulata* shoals, with significantly larger shoals being formed in static conditions (mean 2.99, SE 0.15 fish) compared to the flow action condition (mean 2.35, SE 0.13 fish) (Table 2.1, Figure 2.1). Additionally, the size of the fish was an important predictor of shoal size, with a significant positive correlation between fish standard length and number of individuals in the group (Table 2.1, Figure 2.1).

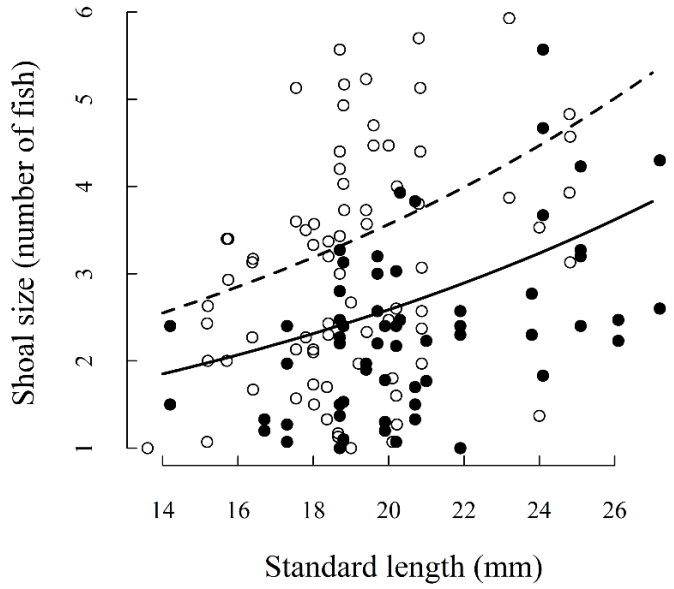

Figure 2.1. Size of guppy *Poecilia reticulata* shoals in flow (closed circles) and static (open circles) conditions. The predicted data trends generated by the top-scoring generalised linear mixed model are shown for flow (solid line) and static (dashed line) conditions.

Table 2.1. Summary of the averaged model predictors explaining variation in shoaling behaviour of *Poecilia reticulata* infected with *Gyrodactylus turnbulli*. Semicolons indicate interactions, shaded rows indicate confidence intervals which do not bound zero and therefore are considered significant. SE= standard error, CI=confidence intervals.

Dependent Variable	Predictor	Estimate	Adjusted SE	95% CI	Relative importance
Shoal cohesion (nearest neighbour distance)	(Intercept)	0.225	0.126	(-0.023-0.473)	
	Flow	-0.017	0.074	(-0.163-0.128)	0.90
	Individual infected	-0.011	0.105	(-0.196-0.217)	0.90
	Flow : Individual infected	-0.302	0.149	(-0.595- -0.008)	0.90
	Shoal infected	0.170	0.076	(0.022-0.319)	0.78
	Flow : Shoal infected	0.227	0.161	(-0.088-0.542)	0.26
	Standard Length	0.007	0.076	(-0.142-0.156)	0.10
Shoal size	(Intercept)	1.167	0.066	(1.138-1.296)	
	Flow	0.102	0.036	(0.032-0.173)	1.00
	Standard Length	0.100	0.036	(0.029-0.170)	1.00
	Shoal infected	-0.025	0.021	(-0.065-0.016)	0.34
	Individual infected	-0.017	0.023	(-0.061-0.027)	0.14
	Flow : Shoal infected	-0.040	0.040	(-0.118-0.037)	0.12
	Flow : Standard Length	-0.045	0.073	(-0.188-0.098)	0.15
Proportion time shoaling	(Intercept)	1.596	10.652	(-19.282-22.474)	
	Standard Length	0.769	0.486	(-0.183-1.721)	0.61
	Individual infected	4.742	161.942	(-312.658-322.142)	0.34
	Flow	1.205	32.555	(-62.601-65.011)	0.28
	Flow : Individual infected	16.481	385.141	(-738.381-771.343)	0.19
	Shoal infected	-0.335	0.459	(-1.236-0.566)	0.17

The interaction between flow condition and infection with *G. turnbulli* was the most important predictor for shoal cohesion (nearest neighbour distance) of the guppy shoals (Table 2.1). When a member of the group became infected with *G. turnbulli* there was a reduction in shoal cohesion, however magnitude of decrease was dependent on flow condition and whether shoaling behaviour was observed at the group or individual level. At the group level, infection with *G. turnbulli* increased mean nearest neighbour distance from mean 3.09 cm (SE 0.32 cm) to 5.17 cm (SE 0.60 cm) in the static condition but this was not observed under flow action with only a minor increase from mean 3.56 cm (SE 0.35 cm) to 3.73cm (SE 0.30 cm) (Figure 2.2A). At the individual level, the increase in nearest neighbour distance was less apparent from 3.71 cm (SE 0.33 cm) to 3.89 cm (SE 0.89 cm), whereas under flow action the increase was much larger from 3.50 cm (SE 0.28 cm) to 4.6 cm (SE 0.62 cm) (Table 2.1, Figure 2.2B).

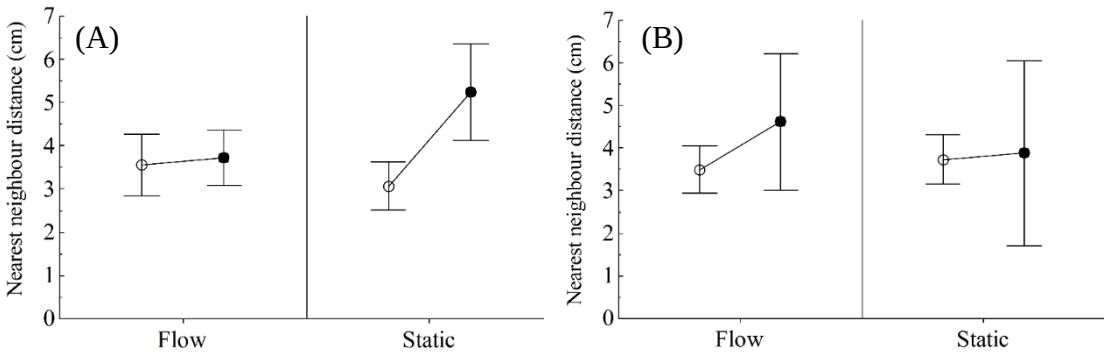

Figure 2.2. Mean shoal cohesion (nearest neighbour distance) in guppy *Poecilia reticulata* shoals uninfected (open circles) and infected (closed circles) with *Gyrodactylus turnbulli* in flow and static conditions. Error bars show 95% confidence intervals. Plots in (A) show overall changes in shoal behaviour in response to member being infected and (B) show change in shoaling behaviour for individuals.

During the 5 min observation period, guppies spent a large proportion of their time shoaling (mean 229.63, SE 8.02 s). Larger guppies spent more time shoaling, with standard length having the highest relative importance (0.61) however, the 95% confidence intervals bounded zero so this is not considered significant (Table 2.1). Parasite infection and flow condition had little effect on the time shoaling with 0.35 and 0.29 relative importance respectively (Table 2.1).

Discussion

This is the first study to investigate the interaction between flow condition and parasitism on the shoaling behaviour of fish and has demonstrated that flow action is an important variable to consider when observing shoaling activity. In the absence of water flow, *P. reticulata* shoals were larger and shoals containing an individual infected with *G. turnbulli* displayed a reduction in shoal cohesion. The size of the fish was also an important predictor for shoaling, with larger fish forming larger shoals in both flow conditions.

The difference in shoal size of *P. reticulata* between the two flow conditions may be explained by energetic allocation. It is more energetically costly to keep up with and maintain large shoals in addition to swimming against a current. When there is no water movement, energy can be allocated to social interaction. In the wild, when there is minimal water movement predators may also be able to allocate more energy to hunting rather than station holding and so the guppies would respond to the enhanced predation risk through increased shoaling (Pitcher and Parrish 1993). The effect of flow rate on

shoaling has similarly been observed by Garner (1997) and Sneddon et al. (2006) who observed a decrease in shoaling and social hierarchy in minnows and three-spined sticklebacks respectively with increasing flow rate. However, the opposite was observed by Allouche and Gaudin (2001) where chub increased aggregation in pools at high flow.

Infection with *G. turnbulli* ectoparasites caused a reduction in guppy shoal cohesion. However, the magnitude of this effect differed between the two flow conditions and between the group and individual level. At the group level, the reduction in shoal cohesion between infected and uninfected groups was more apparent in the static condition (Figure 2.2A). This may be because in the absence of flow, the infected individual was able to keep up with the shoal and so the group respond by increasing the distance to their nearest neighbours to avoid transmission of the parasite. Additionally, in flow there is a reduced ability of the shoal members to detect the chemical cues of parasitism in flowing water (Archard et al. 2008; James et al. 2008). However, at the individual level, there was an increased nearest neighbour distance between the infected individuals and the rest of the shoal members, which was more apparent under flow action (Figure 2.2B). This may be because the infection is preventing the infected fish from keeping up with the rest of the shoal, as suggested by van Oosterhout et al. (2007) where heavily infected individuals were more likely to be washed downstream during flooding events.

Parasitism was not an important predictor of the time spent shoaling and shoal size, suggesting that the antipredator and foraging benefits of shoaling still outweigh the potential costs of shoaling with infected individuals. This result is similar to that found by Richards et al. (2010) who demonstrated a decrease in shoal cohesion with increasing parasite intensity of focal guppies infected with *G. turnbulli* in a static flow tanks, but no significant difference in the overall time spent shoaling.

The current study found that larger guppies shoaled more by forming larger groups as size of the members increased. This is consistent with the findings of Pitcher et al. (1983) who observed larger group sizes of minnow (*Phoxinus phoxinus*) and dace (*Leuciscus leuciscus*) with increasing body size. Similarly, Paxton (1996) observed that larger guppies spent more time shoaling. Large fish may be able to benefit from the safety of group living, with the cost of competition for food within the group being lower (at least in the short term) as they are better competitors than smaller individuals (Hoare et al.

2000b). Rodgers et al. (2011) found that only large guppies showed preference for size-matched shoal mates, indicating that shoaling is more important for larger fish as an antipredator response because they are more conspicuous and have a higher calorific value than smaller individuals. The smaller, therefore younger, fish may have not yet developed discriminatory shoaling tendencies towards larger groups as demonstrated in Ledesma and McRobert (2008).

It is clear from the current study that flow condition plays an important role in shoaling decisions of fish. Many past laboratory studies have taken place in static tanks where the water remains stationary except for the minimal disturbance to the water by filter systems as opposed to strong, variable and unidirectional flow action encountered in natural rivers and streams. It is unlikely that in the wild guppies will experience completely static conditions, with a small amount of water movement in deeper pools in the stream systems. It would therefore be interesting for further study to compare a range of flow conditions likely experienced by guppies in the wild to determine optimal flow conditions for parasite avoidance and predator detection. Climate induced changes to hydrology will result in more frequent drought and flooding events (Floury et al. 2013), therefore it is important for future study into the behaviour of riverine fish to consider water velocity. Flooding events may also physically wash fish and their parasites (van Oosterhout et al. 2007) into low flow areas of a river, therefore generating spatial variation in disease prevalence. Flow condition may also play an important role in disease transmission. With an increased shoaling tendency in the static condition, the higher aggregation could lead to more host-host contacts and therefore increased parasite transmission. Although this is yet to be tested in this system, if this were to be the case, there could be important outcomes for disease management in both the aquarium trade and wildlife conservation by habitat manipulation.

References

Allouche S, Gaudin P (2001) Effects of avian predation threat, water flow and cover on growth and habitat use by chub, *Leuciscus cephalus*, in an experimental stream. Oikos 94:481–492

Archard GA, Cuthill IC, Partridge JC, van Oosterhout C (2008) Female guppies (*Poecilia reticulata*) show no preference for conspecific chemosensory cues in the field or an artificial flow chamber. Behaviour 145:1329–1346

Barber I, Hoare D, Krause J (2000) Effects of parasites on fish behaviour: a review and evolutionary perspective. Rev Fish Biol Fish 10:131–165

Barker DE, Cone DK (2000) Occurrence of *Ergasilus celestis* (Copepoda) and *Pseudodactylogryrus anguillae* (Monogenea) among wild eels (*Anguilla rostrata*) in relation to stream flow, pH and temperature and recommendations for controlling their transmission among captive eels. Aquaculture 187:261–274

Barton K (2013) MuMIn: Multi-model inference. R package version 1.9.13. http://CRAN.R-project.org/package=MuMIn

Bates D, Maechler M, Bolker B, Walker S (2013) lme4: Linear mixed-effects models using Eigen and S4. R package version 1.0-5. http://cran.r-project.org/package=lme4

Bodensteiner LR, Sheehan RJ, Wills PS, Brandenburg AM, Lewis WM (2000) Flowing water: an effective treatment for Ichthyophthiriasis. J Aquat Anim Health 12:209–219

Burnham K, Anderson DR (2002) Model Selection and Multimodel Inference. A Practical Information-Theoretic Approach, 2nd Edition. Springer, New York

Chicoli A, Butail S, Lun Y, Bak-Coleman J, Coombs S, Paley DA (2014) The effects of flow on schooling *Devario aequipinnatus*: school structure, startle response and information transmission. J Fish Biol 84:1401–1421

Croft DP, Edenbrow M, Darden SK, Ramnarine IW, van Oosterhout C, Cable J (2011) Effect of gyrodactylid ectoparasites on host behaviour and social network structure in guppies *Poecilia reticulata*. Behav Ecol Sociobiol 65:2219–2227

Dugatkin LA, FitzGerald GJ, Lavoie J (1994) Juvenile three-spined sticklebacks avoid parasitized conspecifics. Environ Biol Fish 39:215–218

Floury M, Usseglio-Polatera P, Ferreol M, Delattre C, Souchon Y (2013) Global climate change in large European rivers: long-term effects on macroinvertebrate communities and potential local confounding factors. Glob Change Biol 19:1085–99

Fraser BA, Ramnarine IW, Neff BD (2010) Temporal variation at the MHC class IIb in wild populations of the guppy (*Poecilia reticulata*). Evolution 64:2086–2096

Garner P (1997) Effects of variable discharge on the velocity use and shoaling behaviour of *Phoxinus phoxinus*. J Fish Biol 50:1214–1220

Gelman A, Su Y-S (2013) arm: Data analysis using regression and multilevel/hierarchical models. R package version 1.6-10. http://cran.r-project.org/package=arm

Griffiths SW, Magurran AE (1997) Familiarity in schooling fish: how long does it take to acquire? Anim Behav 53:945–949

Griffiths SW, Magurran AE (1998) Sex and schooling behaviour in the Trinidadian guppy. Anim Behav 56:689–693

Grueber CE, Nakagawa S, Laws RJ, Jamieson IG (2011) Multimodel inference in ecology and evolution: challenges and solutions. J Evol Biol 24:699–711

Hallett SL, Bartholomew JL (2008) Effects of water flow on the infection dynamics of *Myxobolus cerebralis*. Parasitology 135:371–384

Harris PD, Lyles AM (1992) Infections of *Gyrodactylus bullatarudis* and *Gyrodactylus turnbulli* on guppies (*Poecilia reticula*ta) in Trinidad. J Parasitol 78:912–914

Hoare DJ, Krause J, Peuhkuri N, Godin J-GJ (2000a) Body size and shoaling in fish. J Fish Biol 57:1351–1366

Hoare DJ, Ruxton GD, Godin J-GJ, Krause J (2000b) The social organization of free-ranging fish shoals. Oikos 89:546–554

Hockley FA, Wilson CAME, Brew A, Cable J (2014) Fish responses to flow velocity and turbulence in relation to size, sex and parasite load. J R Soc Interface 11:20130814

James CT, Noyes KJ, Stumbo AD, Wisenden BD, Goater CP (2008) Cost of exposure to trematode cercariae and learned recognition and avoidance of parasitism risk by fathead minnows *Pimephales promelas*. J Fish Biol 73:2238–2248

Krause J, Godin JG (1996) Influence of parasitism on shoal choice in the banded killifish (*Fundulus diaphanus*, Teleostei, Cyprinodontidae). Ethology 102:40–49

Ledesma JM, McRobert SP (2008) Shoaling in juvenile guppies: the effects of body size and shoal size. Behav Process 77:384–388

Leniham HS, Micheli F, Shelton SW, Peterson CH (1999) The influence of multiple environmental stressors on susceptibility to parasites: An experimental determination with oysters. Limnol Oceanogr 44:910–924

Magurran AE (2005) Evolutionary Ecology. The Trinidadian Guppy. Oxford Series in Ecology and Evolution. Oxford University Press, Oxford

Mikheev VN (2009) Combined effects of predators and parasites on shoaling behavior of fishes. J Ichthyol 49:1032–1041

Paxton CG. (1996) Isolation and the development of shoaling in two populations of the guppy. J Fish Biol 49:514–520

Pitcher TJ, Magurran AE, Allan JR (1983) Shifts of behaviour with shoal size in cyprinids. Proc Br Freshw Fish Conf 3:220–228

Pitcher TJ, Parrish JK (1993) Functions of shoaling behaviour in teleosts. In: Pitcher TJ (ed) Behaviour of Teleost Fishes. Fish Fisheries Series 7, 2nd Edition. Chapman & Hall, London, pp 363–440

Poulin R, Fitzgerald GJ (1989) Shoaling as an anti-ectoparasite mechanism in juvenile sticklebacks (*Gasterosteus* spp.). Behav Ecol 24:251–255

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Reznick D, Butler MJ, Rodd H (2001) Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. Am Nat 157:126–140

Richards EL, van Oosterhout C, Cable J (2010) Sex-specific differences in shoaling affect parasite transmission in guppies. PLoS ONE 5:e13285

Richards EL, van Oosterhout C, Cable J (2012) Interactions between males guppies facilitates the transmission of the monogenean ectoparasite *Gyrodactylus turnbulli*. Exp Parasitol 132:483–486

Rodgers GM, Ward JR, Askwith B, Morrell LJ (2011) Balancing the dilution and oddity effects: Decisions depend on body size. PLoS ONE 6:e14819

Ruhl N, McRobert SP (2005) The effect of sex and shoal size on shoaling behaviour in *Danio rerio*. J Fish Biol 67:1318–1326

Sneddon LU, Hawkesworth S, Braithwaite VA, Yerbury J (2006) Impact of environmental disturbance on the stability and benefits of individual status within dominance hierarchies. Ethology 112:437–447

Song Z, Boenke MC, Rodd FH (2011) Interpopulation differences in shoaling behaviour in guppies (*Poecilia reticulata*): roles of social environment and population origin. Ethology 117:1009–1018

Sousa WP, Grosholz ED (1991) The influence of habitat structure on the transmission of parasites. In: Bell SS, McCoy ED, Mushinsky HR (eds) Habitat Structure: The Physical Arrangement of Objects in Space. Chapman and Hall, London, pp 300–324

Stumbo AD, James CT, Goater CP, Wisenden BD (2012) Shoaling as an antiparasite defence in minnows (*Pimephales promelas*) exposed to trematode cercariae. J Anim Ecol 81:1319–1326

van Oosterhout C, Mohammed RS, Hansen H, Archard GA, McMullan M, Weese DJ, Cable J (2007) Selection by parasites in spate conditions in wild Trinidadian guppies (*Poecilia reticulata*). Int J Parasitol 37:805–812

Weetman D, Atkinson D, Chubb JC (1999) Water temperature influences the shoaling decisions of guppies, *Poecilia reticulata*, under predation threat. Anim Behav 58:735–741

Chapter 3. Effects of flow rate on the transmission of *Gyrodactylus turnbulli* infecting the Trinidadian guppy *Poecilia reticulata*

Hockley FA, Smallbone Z, Wilson CAME, Cable J

The ultimate aim is to submit this chapter for publication if further replicate shoals reveal similar results. Project design and implementation by FH and ZS, with supervision and comments on the manuscript by JC and CW. ZS was funded by the CUROP summer programme, with grant application written and submitted by FH.

Abstract

Shoaling can be beneficial to fish as it can reduce predation risk and lead to more efficient foraging. Conversely, shoal members may be at increased risk of infection from directly transmitted parasites. There is considerable temporal and spatial variability in the size, structure and cohesiveness of fish shoals, which can be heavily influenced by environmental conditions. Previous work has demonstrated that flow condition can influence whether fish shoal and subsequently how they react to a member being infected with parasites. This study investigated the transmission of the ectoparasite *Gyrodactylus turnbulli* in shoals of 5 female guppies (*Poecilia reticulata*) over a 7 day infection period in a flow and static flow condition. Initially, with only one individual infected, shoals in the flow condition were less cohesive compared to the static flow condition. Rate of parasite transmission did not vary between the flow and static conditions; however, as the parasite spread between shoal mates, the shoals became less cohesive with increasing prevalence. This effect was more evident in the static condition, with very little change in shoal cohesion with increasing prevalence in the flow condition. At low prevalence, the shoals in the static condition were more cohesive. However, once >3 fish in a shoal were infected shoals in the static condition became less cohesive than those in the flow condition. As a result, *G. turnbulli* reached a higher peak prevalence in the flow compared to the static condition. Shoals of larger sized fish were also observed to have reduced transmission rate, likely because the parasites have less competition for space on their hosts. Although further replicates are needed to increase the sample size in this experiment, this finding may have important consequences for setting appropriate flow conditions in both the aquaculture and aquarium trade.

Introduction

Fish are known to form shoals primarily to enhance predator detection and avoidance, but also to improve foraging success, provide energetic benefit when swimming and increase mating opportunities. However, due the close proximity of the fish to each other, transmission of infectious disease is a major cost of shoaling (Barber et al. 2000). Fish may avoid shoaling with infected conspecifics (for example Dugatkin et al. 1994; Krause and Godin 1996; Hockley et al. 2014) in order to reduce the chance of being infected. The extent at which parasites affect the shoaling behaviour of their fish hosts largely depends on whether the costs of parasitism outweigh the benefits of group living (Barber et al. 2000).

A crucial element of any mathematical model aiming to understand the transmission of infectious disease is the 'transmission rate'. This describes the rate at which susceptible hosts become infected per unit time (dI/dN), where I is the number of infected individuals at time t (Begon et al. 2002). The transmission rate is dependent on the number of susceptible hosts available, the rate of contact between individuals, the probability that contact is with an infected individual and that the contact leads to successful transmission (Begon et al. 2002). There are two recognised modes of transmission. Density-dependent transmission assumes that the rate of contact between infected and susceptible hosts increases linearly with increasing host density. For frequency-dependent transmission the rate of contact is not affected by the density of hosts in the population (Begon et al. 2002; Johnson et al. 2011). For social species, such as shoaling fish, frequency-dependent transmission may play a part as there is an increased rate of contact between individuals irrespective of density (Johnson et al. 2011). Frequency-dependent transmission increases the likelihood of a parasite persisting even at low host densities (Ryder et al. 2007; Johnson et al. 2011).

Understanding how paraite transmission in shoaling fish is affected by changing environmental conditions is important in both wild, aquaculture and aquarium systems. Changes to river flow caused by climate change and increasing pressures on watercourses due to human population increase will result in changes to fish population dynamics, which in turn will affect the prevalence of parasites. The effect of temperature on disease risk is widely studied (Graham and Harrod 2009; Karvonen et al. 2010) however flow condition is largely overlooked. Climate induced changes to river flow will see a higher

frequency of flooding and draught events (Graham and Harrod 2009). Increasing human population will result in increased pressures on watercourses for abstraction, agriculture and energy generation (Ormerod 2009). As a result, fish will experience different and more variable flow regimes. A change in flow rate will additionally have further interacting impacts on river ecosystems with a change in flow-related discharge of pollutants, sediment run-off and warming from the earth atmosphere (Ormerod 2009). Mitigation methods put in place to allow fish to survive in the changing hydrodynamic conditions will also need to consider the implications for disease spread. Similarly, fish farmers and aquaculturists will be concerned with selecting the most appropriate flow conditions to minimise disease epidemics whilst providing optimum conditions for fish growth and survival.

Of the few studies which have investigated the interaction between flow condition and parasitism in fish, the trend has been towards a higher prevalence and intensity of infection in reduced flows (Sousa and Grosholz 1991; Leniham et al. 1999; Barker and Cone 2000; Bodensteiner et al. 2000; Hallett and Bartholomew 2008; Hockley et al. 2014). However, it is often difficult to disentangle whether this occurs as a result of poor quality habitat (high turbidity, hypoxia and lower food availability) leading to increased stress and therefore higher susceptibility to disease (Leniham et al. 1999) or whether the infection compromises the host by debilitating energy reserves and prompting them to select energetically favourable regions.

A small number of studies have observed differences in shoaling behaviour with regard to flow, with most observing reduced shoaling with higher flow rates (Garner 1997; Sneddon et al. 2006; Hockley et al. 2014). Allouche and Gaudin (2001) observed the opposite effect, with increased aggregation of European chub (*Leuciscus cephalus*) in high flow but only with higher predation risk and in the presence of cover. In the absence of overhead cover, aggregation was lower in the high flow condition. Hockley et al. (2014) observed a reduction in guppy (*Poecilia reticulata*) shoaling when one member became infected with *Gyrodactylus turnbulli* but the magnitude of this effect was dependent on flow condition. However, in this previous study utilising a large flume, it was only possible to assess the immediate short term effect of parasitism on shoaling within a 30 min time frame and not transmission dynamics over several days (Hockley et al. 2014). The aim of this study was to assess whether parasite transmission rates were influenced by flow conditions using the same guppy-*Gyrodactylus* model system. *G.*

turnbulli is a well-studied ectoparasite both in laboratory studies and in the field and is a common parasite of the Trinidadian guppy, particularly in downstream populations (Cable 2011). The parasite is directly transmitted through host contact (Johnson et al. 2011) although can also survive for up to 24 hours off the host and be transmitted to new hosts by attaching to the surface water film and transferring to the guppy when it feeds at the surface (Cable et al. 2002). The parasite has no specific transmission stage during its lifecycle and combined with its short generation time (<24 hours at 25°C) and extreme progenesis, the parasite has exponential population growth which makes its reproductive strategy more akin to a micro- rather than a macroparasite (Bakke et al. 2007). The ecology of the guppy-*Gyrodactylus* system is well understood and therefore provides a reliable and straight-forward model to investigate patterns in evolution and epidemiology and can potentially model microparasites of other animals.

Methods

Study system

Experiments were conducted using laboratory reared Trinidadian guppies (*Poecilia reticulata*) which were offspring of fish caught from the Lower Aripo River, Trinidad in 2003 and maintained at Exeter University, UK before being transferred to Cardiff University, UK in 2011 and 2012. Female guppies (standard length 14.0-25.6 mm) were sized matched to within ± 4.5 mm in groups of five individuals. Each group was housed in a 6L aquaria to allow the fish to familiarise and form shoals for a minimum of 7 days. The shoals were maintained under a 12 h light: 12 h dark regime at 22-24°C with regular water changes and fed daily on diet of live *Artemia*, *Daphnia* and fish flakes (Aquarian®). An isogenic strain of *Gyrodactylus turnbulli* (Gt3) isolated from ornamental guppies in 1997 was used for experimental infections.

Experimental Design

The transmission experiments were conducted between June and July 2014 at the School of Biosciences, Cardiff University. A plastic recirculating open channel flume (Figure 3.1) with channel length 150 cm, 16 cm depth and 20 cm height was filled with dechlorinated water to 15 cm depth and maintained at 21-23°C. A 10 cm diameter impeller was attached to a 1 horse power three phase 4-pole motor with shaft speed maximum 1500 rpm (Machine Mart) wired to a 1.1 kW inverter (RS Components) to

control motor speed. At either end of the flume was a 20 mm thick aluminium honeycomb flow straightener with 6.4 mm cell diameter. The flow straightener was covered with netting to prevent fish from entering the impeller region therefore restricting them to a 100 cm length section (Test Area in Figure 3.2B). Two flow conditions were chosen for the experiment how parasite transmission and shoaling behaviour are affected by flow. A 'static' condition was used as a control with the motor switched off and water aerated with an air stone. For the flow condition, a flow speed of mean velocity 2.6 (SE 0.05) cm s^{-1} generated by setting the motor speed to 7 Hz was chosen which evoked rheotaxis without tiring or stressing the fish over a 7 day period. This flow velocity is akin to flows recorded in both medium and high predation guppy habitats the wild in Trinidad (Kodric-Brown and Nicoletto 2005). The flowrate was measured by measuring the mean time for a neutrally buoyant ball to drift 1 m downstream for 10 replicates.

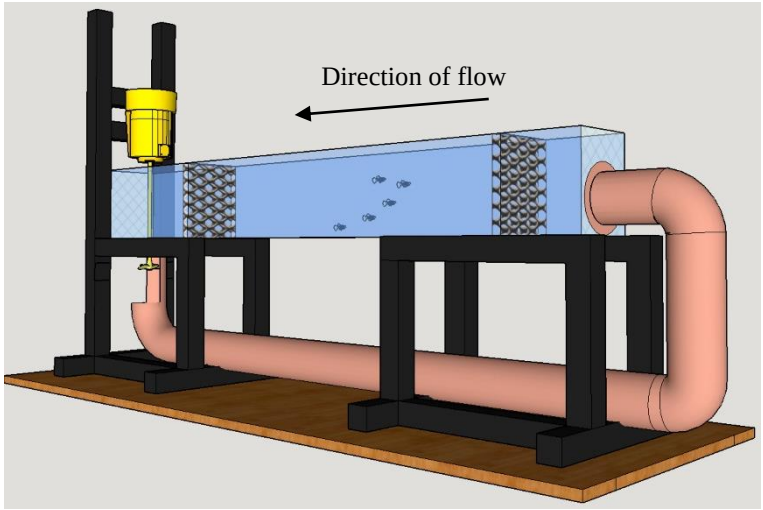

Figure 3.1. 3D schematic of open channel flume in which *Poecilia reticulata* fish shoals were placed at two different flow conditions to examine transmission dynamics of *Gyrodactylus turnbulli*.

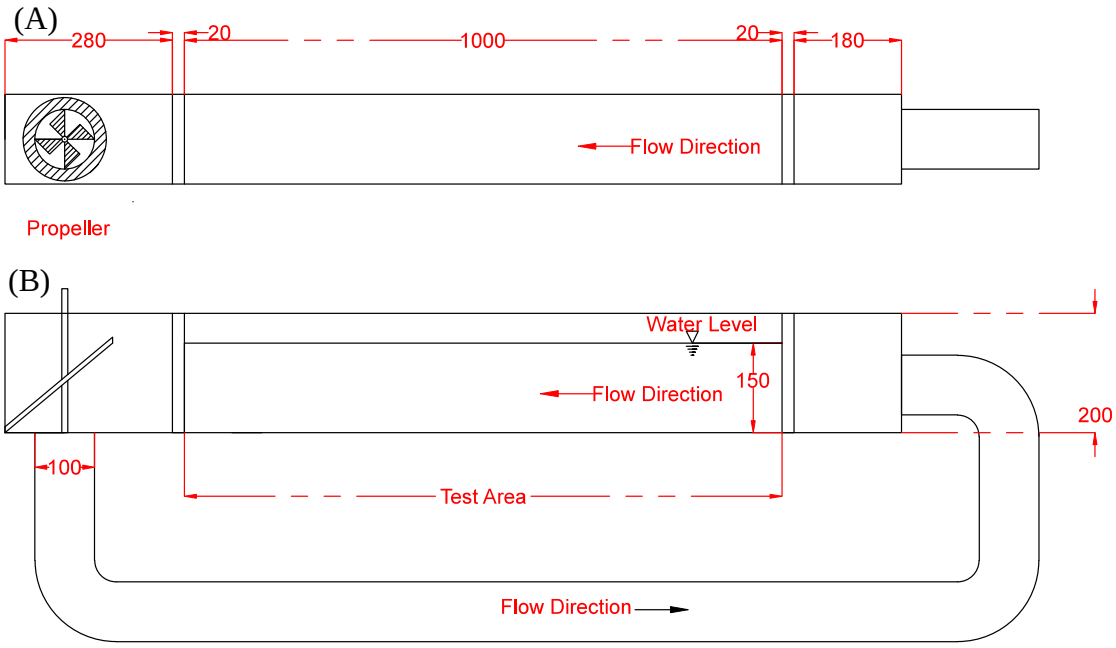

Figure 3.2. 2D schematic of open channel flume to assess parasite transmission in guppies at two different flow conditions. (A) plan view and (B) side view. Fish were retained within the 1 m test area with the use of netting covering honeycomb flow straighteners. Flow depth was maintained at 150 mm. Dimensions are given in millimetres.

Each shoal of five guppies (n=12) were placed in the flume to habituate for 24 hours before being briefly removed and infected with *Gyrodactylus turnbulli*. Six shoals were trailed in the static condition and six in the flow condition. On Day 0, one individual was randomly selected from each shoal and infected with approximately 30 *G. turnbulli* individuals by anaesthetising with 0.02% tricaine methane sulfonate (MS222) and bringing it into contact with a heavily infected donor fish until all worms had transferred from donor to recipient (taking <5 min). Transfer of the parasites was observed continuously under a stereo-microscope. The remaining four uninfected shoal members were sham infected by anaesthetising and manipulating under the microscope without transfer of parasites. A photograph and sketch of the markings on every fish was recorded to allow individual identification using a stereo-microscope. The fish were then placed in individual 1 L pots for an hour to recover. During this time the fish remained in visual contact and were fed with three individuals of *Daphnia*, before being returned to the flume. Infection was confirmed the following day (Day 1), then each fish was screened every two days on Day 3, 5 and 7. For each screening, all fish were removed from the flume for ca. 1 hour, individually anaesthetised and screened under a stereo-microscope

to record prevalence and mean intensity of infection. Transmission rate was calculated as the number of new host infections per day.

Observations of shoaling behaviour (number of fish shoaling, maximum shoal size, shoal size and nearest neighbour distance) were measured every minute for five min, every day at 10:00 by an observer. Maximum shoal size was defined by the number of fish in the largest shoal, with shoaling defined as being within 4 body lengths (Pitcher et al. 1983; Hockley et al. 2014). A 2 cm^2 grid adhered to the side and base of the flume was used to visually estimate distances between neighbouring fish when shoaling. Individuals were not distinguished during shoaling observations due to their similarity in appearance.

Statistical analysis

All analyses were conducted using R statistical interface (R Core Team 2013).

Generalised Linear Models (GLMs) were used to investigate the effect of flow condition and fish size on the transmission dynamics of *G. turnbulli* on the guppies. The dependent terms in the models were (1) mean transmission rate, (2) peak intensity, (3) time to reach peak intensity, (4) peak prevalence and (5) time to reach peak prevalence. The fixed terms in the models were flow condition (flow or static), mean standard length (SL), mean fork length (FL) and intensity of *G. turnbulli* on the donor fish on Day 1. The GLMs were fitted with Gaussian or Gamma error structures and identity or log link functions. Peak intensity and peak prevalence was $\log_{10}$ transformed to normalise the data.

Generalised Linear Mixed Models (GLMMs) were used to investigate the effects of flow condition, fish size and parasitism on the shoaling behaviour of guppies using the lme4 library (Bates et al. 2013b). The dependent terms in the models were (6) number of fish shoaling, (7) size of largest shoal and (8) mean nearest neighbour distance to give an indication of shoal cohesion. The fixed terms in the models were flow condition (flow or static), *G. turnbulli* intensity, *G. turnbulli* prevalence, mean fish size and intensity of *G. turnbulli* on the donor fish on Day 1. Shoal number was included as a random term to account for autocorrelation. The GLMMs were fitted with a Gaussian error structures and identity or log link functions. The number of fish shoaling was $\log_e$ transformed to normalise the data.

For the GLMMs and GLMs all fixed and random effects were included in global models and standardised to a mean 0 and standard deviation 0.5 using the arm library (Gelman

and Su 2013). The most important explanatory variables were determined by selecting the top most plausible models which fell within 2.5 corrected Akaike Information Criterion (AICc) of the best model and then model averaging the top models using the MuMIn library (Barton 2013). The output then gives the relative importance of each explanatory variable which is the sum of the Akaike weights for each variable for the models in which it appears across the top models, with the higher the value (closest to 1) giving a higher relative importance compared to the other variables (Burnham and Anderson 2002). The variables were considered significant if the 95% confidence intervals did not bound zero.

Results

Throughout the infection period, prevalence of *Gyrodactylus turnbulli* gradually increased in both flow and static conditions (Figure 3.3). Mean transmission rate (number of new infections per day) ranged from -0.5 to 1.5 worms day^{-1}. By Day 3, transmission had occurred from the artificially infected donor to at least one other shoal member in 10 shoals and had occurred in the remaining 6 shoals by Day 5. Intensity of infection on the infected donor on Day 1 ranged between 20-60 worms. The higher the initial intensity, the higher the transmission rate throughout the shoals. Initial intensity had no effect on the peak intensity or prevalence within the shoal, or the time to reach peak intensity and prevalence. By Day 7, prevalence ranged from 60 to 100% with mean intensity ranging from 3.8 to 99.0 worms. In two shoals, an individual fish lost infection, causing a reduction in prevalence between Day 5 and Day 7.

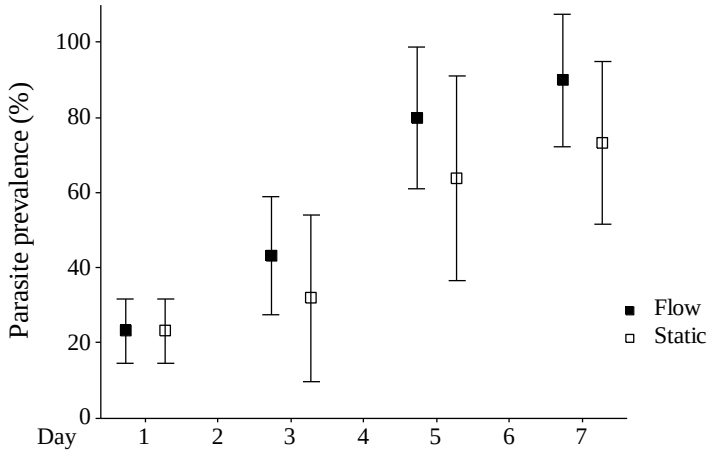

Figure 3.3. Increase in mean *Gyrodactylus turnbulli* prevalence over time in guppy *Poecilia reticulata* shoals in flow (black squares) and static (open squares) conditions. Error bars show 95% confidence intervals.

Transmission was higher in the flow condition, with peak prevalence of *G. turnbulli* reaching mean 93.3% compared to mean 76.7% in the static condition (Table 3.1, Figure 3.4). Larger fish had a lower transmission rate (Figure 3.5A) and reached lower peak prevalence (Figure 3.5B) compared to smaller individuals (Table 3.1).

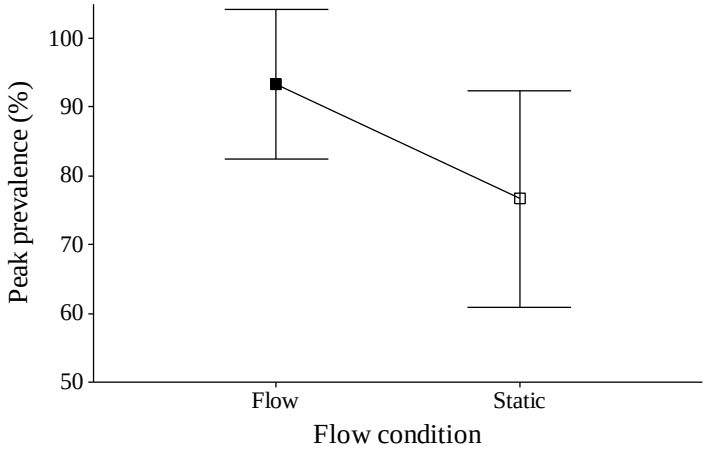

Figure 3.4. Relationship between the peak prevalence of *Gyrodactylus turnbulli* infecting *Poecilia reticulata* shoals and flow condition. Squares show mean values and error bars show 95% confidence intervals.

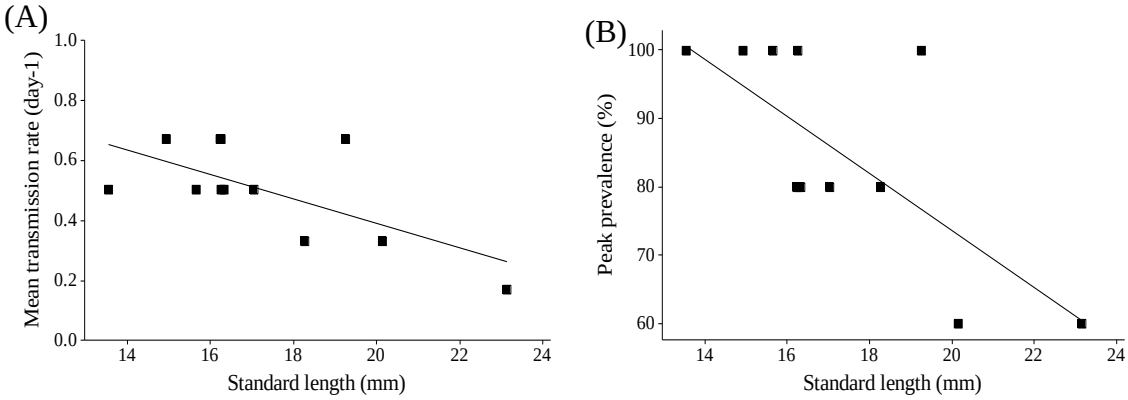

Figure 3.5. Decrease in (A) transmission rate and (B) peak prevalence of *Gyrodactylus turnbulli* with increasing guppy *Poecilia reticulata* host standard length.

Infection with *G. turnbulli* had a significant effect on the shoaling behaviour of guppies *P. reticulata*. With increasing parasite prevalence, the nearest neighbour distance between individuals of a shoal increased (reduction in shoal cohesion); however, this was dependent on flow condition. Initially, nearest neighbour distances in the flow condition were higher than those in the static condition when parasite prevalence was low. As parasite prevalence increased the nearest neighbour distances increased at a greater rate

in the static condition compared to the flow condition, indicating that in the absence of flow, parasitism has a greater effect on shoaling behaviour (Table 3.1, Figure 3.6).

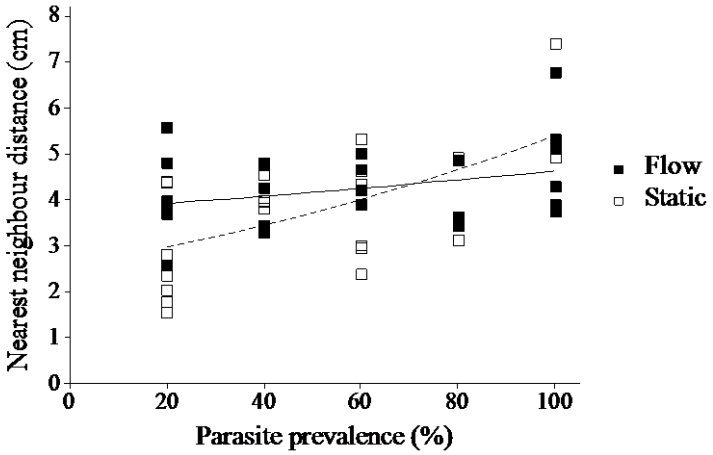

Figure 3.6. Reduction in shoal cohesion (increased nearest neighbour distance) with increasing *Gyrodactylus turnbulli* prevalence in flow (open squares) and static (black squares) condition. Lines show predictions from top model for flow (dashed line) and static (solid line).

Table 3.1. Summary of the standardised averaged model predictors in shoaling behaviour of female guppies *Poecilia reticulata* in flow and static conditions. Highlighted rows show predictors which confidence intervals do not bound zero and therefore are considered significant. CI=confidence intervals, SE = standard error, SL=Standard length, FL=fork length. * Approaching significance

Model	Dependent variable (transformation)	Predictor	Estimate	Adjusted SE	95% CI	Relative importance
(1)	Mean transmission rate	Intercept	0.49	0.03	0.42 – 0.55	
		SL	-0.20	0.07	-0.34 - -0.07	1.00
		Start intensity	0.16	0.06	0.03 – 0.28	0.83
(2)	Peak intensity (log_{10})	Intercept	3.87	0.18	3.51 – 4.24	
		Flow	-0.51	0.30	-1.09 – 0.07*	0.63
		SL	0.43	0.27	-0.09 – 0.96	0.26
(3)	Time to reach peak intensity	Intercept	1.46	0.20	1.08 – 1.84	
		FL	0.34	0.27	-0.19 – 0.88	0.27
(4)	Peak prevalence (log_{10})	Intercept	4.43	0.04	4.35 – 4.50	
		Flow	-0.17	0.07	-0.30 - -0.03	1.00
		SL	-0.26	0.08	-0.41 - -0.10	0.77
(5)	Time to reach peak prevalence	Intercept	0.31	0.69	-1.05 – 1.67	
		FL	-2.08	2.63	-7.24 – 3.08	0.35
(6)	Number of fish shoaling (log)	Intercept	1.19	0.05	1.09 – 1.30	
		Flow	0.16	0.10	-0.-4 – 0.36	0.24
		Day	-0.07	0.03	-0.13 – 0.00*	0.16
		Mean SL	-0.05	0.11	-0.25 – 0.16	0.00
(7)	Largest shoal size	Intercept	3.10	0.18	2.75 – 3.45	
		Flow	0.61	0.36	-0.09 – 1.31*	0.59
		Prevalence	-0.07	0.16	-0.38 – 0.24	0.06
		Day	-0.07	0.15	-0.36 – 0.22	0.05
(8)	Nearest neighbour distance	Intercept	1.39	0.06	1.28 – 1.50	
		Prevalence	0.29	0.10	0.32 – 0.13	1.00
		Flow	-0.09	0.11	0.10 – 0.47	0.77
		Flow: Prevalence	0.32	0.13	0.07 – 0.58	0.77
		Mean SL	0.10	.11	-0.13 – 0.32	0.16
		Day	-0.11	0.14	-0.38 – 0.15	0.16

The above results are supported by two non-significant findings (possibly due to low samples size). Firstly, the guppies formed larger shoals in the static condition (Figure 3.7B) and secondly infection was higher in the flow condition, with a higher peak mean intensity compared to the static condition (Figure 3.7B). There was no effect of flow condition on the number of days it took for *G. turnbulli* to reach peak prevalence and intensity.

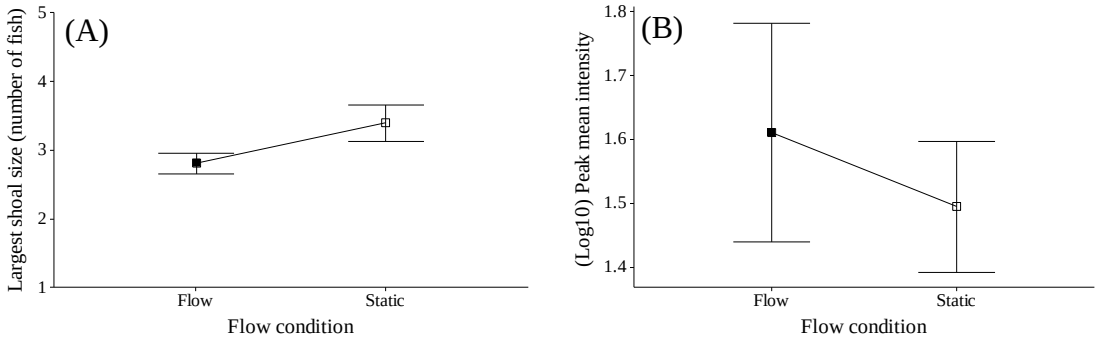

Figure 3.7. Non-significant effects of flow condition on (A) maximum shoal size and (B) peak mean intensity of guppies *Poecilia reticulata* infected with *Gyrodactylus turnbulli*. Squares show mean values and error bars 95% confidence intervals.

Discussion

The transmission dynamics of the ectoparasite *Gyrodactylus turnbulli* on guppy (*Poecilia reticulata*) shoals were investigated under flow and static conditions using a laboratory flume. A randomly selected individual from the shoal was artificially infected with a mean of 30.4 *G. turnbulli* worms and the infection was allowed to progress over 7 days. The rate of transmission was dependent on the number of parasites on the artificially infected fish on Day 1, with a higher transmission rate if the starting intensity was higher. At the initial stage of infection observations of shoaling behaviour were comparable to those previously observed by Hockley et al. (2014; Chapter 3). Under flow conditions, when the guppies were uninfected or infection prevalence was low, shoaling behaviour was reduced with smaller shoal size (Hockley et al. 2014) and reduced shoal cohesion (current study). In both studies, introduction of a parasitised individual (Hockley et al. 2014) or an increase in prevalence (current study) led to a reduction in shoal cohesion; but this effect was more pronounced in the static condition. Under flow conditions, the increase in *G. turnbulli* number had little effect on the shoaling behaviour of the fish.

With the onset of *G. turnbulli* transmission (which occurred by Day 3 in the majority of shoals) and the gradual increase in prevalence, shoal cohesion reduced. However, this trend was only observed in the static condition. Shoals in the flow condition did not appear to change their shoaling tendency with the increase in parasitism. Once >3 fish were infected with *G. turnbulli* in both conditions, the shoals in the static condition had become less cohesive than those in the flow condition. A reduction in shoaling tendency with parasitism has been observed in several previous studies (Dugatkin et al. 1994; Krause and Godin 1996; Hockley et al. 2014) which is not surprising as the shoal members are avoiding physical contact to prevent infection whilst still benefiting from being in a shoal. Overall, *G. turnbulli* infecting guppies in the static condition reached a lower peak prevalence (mean 76.7%) compared to those infecting shoals in the flow condition (mean 93.3%). *G. turnbulli* infecting shoals in the static condition had a lower mean intensity, although this result was not statistically significant. This lower level of parasitism may be a result of the lower shoaling tendency once prevalence was high (>3 individuals). Additionally, fish in the static condition formed larger shoals compared to the flow condition (although again this was not statistically significant). This could have resulted in lower infection levels due to the encounter-dilution effect where animals form large groups in order to dilute the effect of attacking parasites or predators (Mooring and Hart 1992).

A further explanation for the increased prevalence of *G. turnbulli* in the flow condition could be increased chance of the parasites being dislodged from the host, either through increased water current across the surface of the fish, increased tail beat frequency due to increased swimming or loss during transmission attempts. In static flow conditions Scott and Anderson (1984) estimated that only 35% of *G. turnbulli* successfully transmitted from infected to recipient hosts. Due to the recirculatory nature of the laboratory flumes, any parasites dislodged into the water column or attached to the water surface might have the opportunity to re-infect a new host as they circulate through the system. This transmission opportunity could be higher in the flow compared to the static condition where water movement is limited and there is no cycling of water through the pipe back into the test area (Figure 3.2). Additionally, the fish host may have higher stress levels due to the continuous swimming in the flow condition, initiating the parasite to transmit to a new host due to the development of a hostile microenvironment on the energetically compromised host (Bakke et al. 2007).

Although there was no observed difference in shoaling behaviour of different sized fish (compared to Hockley et al. 2014; Chapter 3 where larger fish were found to shoal more), *G. turnbulli* infecting larger fish had a lower transmission rate and lower peak prevalence. *Gyrodactylus* species are known to transmit to a new host once the infrapopulation on the host becomes too dense (Bakke et al. 2007). On larger hosts, there will be less competition for space, resulting in a reduced pressure to leave the host.

To our knowledge, this is the first investigation into the transmission dynamics of a parasite under different flow conditions. The only similar study by Chicoli et al. (2014) investigated (without considering parasites) the effect of schooling by *Devario aequipinnatus* in response to a startle stimuli in flow and static conditions. Chicoli et al. (2014) demonstrated that there was a higher probability of information transfer about the stimulus in schools in the static condition compared to the flow condition. This echoes findings of the current study where there was a reduction in shoal cohesion (but not shoal size) of the guppy shoals with increasing prevalence of the parasite but only in the static condition. It is possible that the transfer of information between shoal members is enhanced in the absence of flow.

This study has demonstrated that parasite transmission dynamics differ between hosts inhabiting different flow conditions. A higher peak prevalence of infection was reached in guppy shoals in the flow conditions. Transfer of information about parasite infection appeared to be higher in the static condition. This led to a reduction in shoal cohesion as prevalence increased as a host adaptation to avoid further transmission. In the flow condition there was very little behavioural response to the parasite infection which may have partly contributed to the higher peak infection. In the wild, it is known that parasite infection can affect population dynamics, with individuals harbouring higher infections being more likely to be washed downstream following spate conditions (van Oosterhout et al. 2007). With changes to water flow in rivers as a result of climate and anthropogenic change, along with fish farmers and aquaculturalists relying on low disease transmission for survival of their fish stock; this information could be the start of further research into how flow rate affects diseased fish.

References

Allouche S, Gaudin P (2001) Effects of avian predation threat, water flow and cover on growth and habitat use by chub, *Leuciscus cephalus*, in an experimental stream. Oikos 94:481–492

Bakke TA, Cable J, Harris PD (2007) The biology of gyrodactylid monogeneans: the "Russian-doll killers". Adv Parasitol 64:161–376

Barber I, Hoare D, Krause J (2000) Effects of parasites on fish behaviour: a review and evolutionary perspective. Rev Fish Biol Fish 10:131–165

Barker DE, Cone DK (2000) Occurrence of *Ergasilus celestis* (Copepoda) and *Pseudodactylogryrus anguillae* (Monogenea) among wild eels (*Anguilla rostrata*) in relation to stream flow, pH and temperature and recommendations for controlling their transmission among captive eels. Aquaculture 187:261–274

Barton K (2013) MuMIn: Multi-model inference. R package version 1.9.13. http://CRAN.R-project.org/package=MuMIn

Bates D, Maechler M, Bolker B, Walker S (2013) lme4: Linear mixed-effects models using Eigen and S4. R package version 1.0-5. http://cran.r-project.org/package=lme4

Begon M, Bennett M, Bowers RG, French NP Hazel SM (2002) A clarification of transmission terms in host-microparasite models: numbers, densities and areas. Epidemiol Infect 129:147–53

Bodensteiner LR, Sheehan RJ, Wills PS, Brandenburg AM, Lewis WM (2000) Flowing water: an effective treatment for Ichthyophthiriasis. J Aquat Anim Health 12:209–219

Burnham K, Anderson DR (2002) Model Selection and Multimodel Inference. A Practical Information-Theoretic Approach, 2nd Edition. Springer, New York

Cable J, Scott ECG, Tinsley RC, Harris PD (2002) Behavior favoring transmission in the viviparous monogenean *Gyrodactylus turnbulli*. J Parasitol 88:183–184

Cable J (2011) Poeciliid Parasites. In: Evans JP, Pilastro A, Schlupp I (eds) Ecology and Evolution of Poeciliid Fishes. Chicago University Press, pp 82–94

Chicoli A, Butail S, Lun Y, Bak-Coleman J, Coombs S, Paley DA (2014) The effects of flow on schooling *Devario aequipinnatus*: school structure, startle response and information transmission. J Fish Biol 84:1401–1421

Dugatkin LA, FitzGerald GJ, Lavoie J (1994) Juvenile three-spined sticklebacks avoid parasitized conspecifics. Environ Biol Fish 39:215–218

Garner P (1997) Effects of variable discharge on the velocity use and shoaling behaviour of *Phoxinus phoxinus*. J Fish Biol 50:1214–1220

Gelman A, Su Y-S (2013) arm: Data analysis using regression and multilevel/hierarchical models. R package version 1.6-10. http://cran.r-project.org/package=arm

Graham CT, Harrod C (2009) Implications of climate change for the fishes of the British Isles. Journal of Fish Biology 74, 1143-1205

Hallett SL, Bartholomew JL (2008) Effects of water flow on the infection dynamics of *Myxobolus cerebralis*. Parasitology 135:371–384

Hockley FA, Wilson CAME, Graham N, Cable J (2014) Combined effects of flow condition and parasitism on shoaling behaviour of female guppies *Poecilia reticulata*. Behav Ecol Sociobiol 68:1513-1520

Johnson MB, Lafferty KD, van Oosterhout C, Cable J (2011) Parasite transmission in social interacting hosts: monogenean epidemics in guppies. PLoS ONE 6:e22634

Karvonen A, Rintamäki P, Jokela J, Valtonen ET (2010) Increasing water temperature and disease risks in aquatic systems: climate change increases the risk of some, but not all, diseases. Int J Parasitol 40:1483–1488

Kodric-Brown A, Nicoletto PF (2005) Courtship behavior, swimming performance, and microhabitat use of Trinidadian guppies. Environ Biol Fishes 73:299–307

Krause J, Godin JG (1996) Influence of parasitism on shoal choice in the banded killifish (*Fundulus diaphanus*, Teleostei, Cyprinodontidae). Ethology 102:40–49

Leniham HS, Micheli F, Shelton SW, Peterson CH (1999) The influence of multiple environmental stressors on susceptibility to parasites: An experimental determination with oysters. Limnol Oceanogr 44:910–924

Mooring MS, Hart BL (1992) Animal grouping for protection from parasites: selfish herd and encounter-dilution effects. Behaviour 123:173–193

Pitcher TJ, Magurran AE, Allan JR (1983) Shifts of behaviour with shoal size in cyprinids. Proc Br Freshw Fish Conf 3:220–228

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Ryder JJ, Miller MR, White A, Knell RJ, Boots M (2007) Host-parasite population dynamics under combined frequency- and density-dependent transmission. Oikos 116:2017–2026

Scott ME, Anderson RM (1984) The population dynamics of *Gyrodactylus bullatarudis* (Monogenea) within laboratory populations of the fish host *Poecilia reticulata*. Parasitol 89: 159-194

Sneddon LU, Hawkesworth S, Braithwaite VA, Yerbury J (2006) Impact of environmental disturbance on the stability and benefits of individual status within dominance hierarchies. Ethology 112:437–447

Sousa WP, Grosholz ED (1991) The influence of habitat structure on the transmission of parasites. In: Bell SS, McCoy ED, Mushinsky HR (eds) Habitat Structure: The Physical Arrangement of Objects in Space. Chapman and Hall, London, pp 300–324

van Oosterhout C, Mohammed RS, Hansen H, Archard GA, McMullan M, Weese DJ, Cable J (2007) Selection by parasites in spate conditions in wild Trinidadian guppies (*Poecilia reticulata*). Int J Parasitol 37:805–812

Chapter 4. Burst swimming performance of the three-spined stickleback (*Gasterosteus aculeatus*)

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The experiment was designed and implemented in the laboratory by FH and CS. Data analysis and manuscript preparation was by FH. Supervision and comments on the manuscript was provided by JC and CW.

Abstract

Anaerobic swimming in fish is essential for successful escape responses, prey capture and tolerance of high flow velocities. However, few studies have investigated how variation in anaerobic swimming is affected by natural parasite infections, position in the water column or host factors. This experiment assessed the anaerobic swimming performance of the three-spined stickleback (*Gasterosteus aculeatus*) both from rest and during swimming using an open channel flume. As the flow rate was increased, fish became increasingly reliant on burst-and-coast swimming, but those which spent more time near the flume bed initiated fewer burst swims, suggesting that the sticklebacks use the bed boundary layer as a flow refuge. Sticklebacks infected with adult *Phyllodistomum folium* and larval digeneans displayed faster and more frequent burst swims, suggesting that fitter hosts are more exposed to the free-living transmission stages of these parasites in fast flowing water. No other parasite taxa affected the burst swimming performance of the stickleback. Following administration of a general purpose antihelminthic drug praziquantel (PZQ), *P. folium* prevalence and intensity was significantly reduced but the other five helminth taxa were not affected. Surprisingly, the treated group had a higher velocity of burst swim and spent less time swimming near the bed boundary layer compared to controls. A possible side effect of PZQ treatment therefore might be improved swimming performance with a tolerance to higher flow velocities.

Introduction

Locomotor performance can be essential to an animal's survival and is widely assumed to be an indicator of fitness (Marras et al. 2013). In fish, swimming behaviour can be loosely classified as either anaerobic burst swimming or aerobic endurance swimming. Anaerobic swimming uses glycolytic 'white' muscle used in short burst swimming at high velocity, such as in escape responses, prey capture (Blake et al. 2006a), or during

burst and coast swimming. The high energy burst swim response lasts for less than a second (Domenici and Blake 1997) and is performed at the maximum swimming speed (*Umax*) the fish can achieve (Tudorache et al. 2008). The burst swim is initiated either from rest or gentle swimming (Jayne and Lauder 1993) and is categorised into either S- or C-starts, named according to the body shape made during the response (Domenici and Blake 1997). Typically, S-starts are employed by predators to capture prey, whereas C-starts are used by prey escaping predators (Domenici and Blake 1997). The higher the velocity of the response the increased probability of escape from a predator (Walker et al. 2005; Blake et al. 2006a). Alternating active burst with passive coast movements may be energetically advantageous over continuous swimming to prolong swimming at high flow velocities (Dutil et al. 2007) and is commonly used by fish to pass through barriers such as culverts (Powers 1997).

Anaerobic swimming performance is typically measured in the laboratory using either aquaria with no water movement to initiate burst responses from rest (e.g. Blake et al. 2006a) or by increasing the flow velocity in a flow chamber (e.g. McDonald 1998; Farrell 2008). However, such swimming tests rarely take into consideration vertical position in the water column and fish may take advantage of the lower flow velocities in the bed boundary layer (Plaut 2001). This has been demonstrated in benthic fish by Hoover et al. (2011) who showed that adult shovelnose sturgeon (*Scaphirhynchus platorynchus*) achieved higher swimming speeds when swimming near the bed boundary compared to when swimming in the free stream. Similarly, juvenile coho salmon used the low velocity boundary layer to pass upstream in an open channel flume simulating a culvert fish passage and achieved the highest maximum swimming velocities (*Umax*) when swimming near the smooth pipe compared to a roughened turbulent bed (Powers 1997). Fish passage design often relies on measured *Umax* values for migrating fish species (Tudorache et al. 2008; Russon and Kemp 2011). If fish associate with the lower velocities in the bed boundary *Umax* may be overestimated which would have significant consequences for the suitability of fish passage structures. Indeed, previous studies have demonstrated that swimming speeds measured in the laboratory cannot be directly extrapolated to fish swimming ability in the wild. In confined spaces, such as swim tunnels, fish alter their behaviour and demonstrate different swimming speeds compared to the wild (Plaut 2001; Peake 2004; Tudorache et al. 2008).

Parasite infections can influence the anaerobic swimming performance of fish. *Schistocephalus solidus* infecting the three-spined stickleback (*Gasterosteus aculeatus*) can reduce the velocity, maximum acceleration (Blake et al. 2006a) and number of burst swim responses, while also reducing the number of directional responses and time to reach cover (Barber et al. 2004). Swimming endurance at high flow rates was significantly reduced by isopod infection in five lined cardinal fish (*Cheilodipterus quinquelineatus*) and bridled monocle bream *Scolopsis bilineatus* due to the parasite's hydraulic drag effect (Östlund-Nilsson et al. 2005; Binning et al. 2013). Unpublished observations summarised by Longshaw et al. (2003) also indicated that three cyprinid species infected with *Myxobolus buckei* had compromised burst swimming ability, with infected fish unable to sustain long periods of burst activity or escape behaviour. However, in natural populations it is rare for hosts to be infected with a single parasite species (Kalbe et al. 2002; Wegner et al. 2003; Jolles et al. 2008) and infections tend to be highly aggregated (Shaw and Dobson 1995; Shaw et al. 1998; Wilson et al. 2001) with only a small number of hosts having high infections. In terms of behaviour, it is frequently only those few individuals harbouring heavy infections which exhibit a behavioural change to infection (Barber et al. 2000) and are therefore most at risk from being eliminated from the population following high disturbance events (e.g. van Oosterhout et al. 2007). Even with these hosts, it may be difficult to determine whether the variation in host fitness is caused by parasite infection (due to parasite manipulation or pathological effects) or whether the parasite infection is a result of host fitness (for example due to increased exposure or tolerance levels (Roy and Kirchner 2000; Miller et al. 2007).

Previous studies have investigated how parasites and removal of parasites by antihelminthic drug treatment have influenced disease transmission, behaviour and social status in both domestic (e.g. Gauly et al. 2007; Forbes et al. 2007) and wild animals (e.g. Ferrari et al. 2003). However, to our knowledge, there have been no studies investigating whether fish swimming performance is enhanced by the removal of parasites through antihelminthic treatment. Praziquantel (PZQ), a broad spectrum antihelminthic drug commonly used to treat parasites of fish and mammals, is the drug of choice for treating human schistosomiasis. In aquaculture, PZQ is effective against a range of parasites, including the tapeworm *Bothriocephalus achelognathii* in grass carp (*Ctenopharyngodon idella*) (see Mitchell and Darwish 2009), red shiners (*Cyprinella lutrensis*) (see Iles et al. 2012) and bonytail chub (*Gila elegans*) (see Ward 2007). The drug is also effective

against various monogeneans (e.g. Schmahl and Taraschewski 1987; Chisholm and Whittington 2002; Hirazawa et al. 2004; Sitjà-Bobadilla et al. 2006; Williams et al. 2007) and digeneans (Voutilainen et al. 2009). The effect of PZQ on the host is thought to be mild and non-toxic (Doenhoff et al. 2008). Carp treated with PZQ (n=147) showed no visible effects of treatment with the exception of one fish which swam upside down and exhibited increased gill ventilation until replaced back into freshwater (Schmahl and Mehlhorn 1985). A similar effect was observed in a later study in sticklebacks (n=30) when using a high concentration of PZQ (50 μg ml^{-1} for 60 and 90 min and 20 μg ml^{-1} for 120 min), which again showed a fast recovery when replaced into freshwater (Schmahl and Taraschewski 1987). No studies have assessed the effect of PZQ on specific fish behaviours such as swimming performance.

The aim of the current study was to assess the relationship between the natural parasite fauna and burst swimming performance of three-spined sticklebacks. Burst swimming performance was assessed both during swimming using an open channel flume and from rest in aquaria with no flow rate. The time spent swimming in the lower third of the water column in the open channel flume was recorded to measure whether flow refuging near to the bed boundary layer improved burst swimming performance. The antihelminthic drug PZQ was used to treat naturally acquired parasite infections to assess whether the subsequent reduction in parasite load improved swimming performance.

Methods

The burst swimming performance of three-spined sticklebacks (*G. aculeatus,* hereafter referred to as stickleback) naturally infected with parasites was assessed under laboratory conditions in active flow and static flow conditions. The effect of antihelminthic treatment was then assessed to determine whether subsequent parasite removal affected burst swimming performance.

Study system

Sticklebacks (n=64) with natural parasite infections were caught using dip nets in Roath Brook, South Wales, Cardiff (NGR ST 18833 78526) in January 2013. Sticklebacks had a mean standard length 42.46 mm (range 35.40-53.10 mm) and mean weight of 1.01 g (range 0.59-2.34 g). Fish were housed in 80 L aquaria of dechlorinated water in the School of Biosciences, Cardiff University, UK at 15 ± 1^{o}C with a 16 h light: 8 h dark regime at

maximum density of 16 fish and fed daily on blood worm. Experiments took place between January and April 2013 between 09:00-18:00. All work was conducted under Home Office approved project PPL 30/2357.

Burst swimming performance – active flow

An Armfield C4 Multi-purpose Flume in the School of Engineering, Cardiff University, was used to assess the burst swimming response of sticklebacks in flowing water. The flume had a smooth bed and clear acrylic sides and was 4 m long, 76 mm wide and 25 mm deep. The flume was set to a negative gradient of 1 in 1000. The flume was driven by a submersible pump drawing water from a sump tank and flow rate controlled by a valve. Chlorides were removed from the water by the addition of Haloex at 0.02 ml L^{-1} and temperature was maintained between 13-16°C. On Day 1 the sticklebacks were placed in the flume in groups of 4 and acclimatized for 30 min at 0 L s^{-1}. The fish were then restricted to the downstream 1.5 m of the flume and burst responses were initiated by sudden increases in flow rate in stepwise increments of 0.2 L s^{-1} every min for a total of 10 min (Farrell 2008). Flow depth increased with increasing flow rate from 31.6 to 89.5 mm. Only two individuals fatigued before completion of the flume trial and so were excluded from the dataset. An establishment of *Umax* (assumed from the maximum flow rate the fish could station hold before fatigue) was therefore not possible in this study due to the limitations in maximum flow rate which the flume could achieve. Each flume trial was video recorded using an Apple iPad at 30 fps and videos later analysed using JWatcher 1.0 to record the total number of burst responses and the time spent in bottom third of the water column for each fish. A burst response was defined as when the fish formed a C-shape followed by a rapid increase in swimming velocity. The velocity of the burst swims was not measured due to the large arena area and depth of water making it unfeasible to measure accurate swimming distances.

Burst swimming performance – static condition

Following a two day recovery period, the burst swimming response in sticklebacks was tested in the absence of water movement on Day 4. The fish were individually placed in aquaria of dechlorinated water measuring 60 x 30 cm and depth of 3 cm to restrict vertical movement, maintained at 12-15°C. After 30 min acclimatisation, burst responses were initiated by striking one side of the tank with a rubber ball attached to a 1m pole from 30

cm distance. Strikes were initiated in three series of three striking events separated by 4 min intervals with 20 min intervals between each series to prevent habituation to the stimulus (see similar method by Garenc et al. 1999). The responses were recorded using digital cameras (Fugifilm Finepix F50fd recording at 25 fps, Sony Cyber-shot DSC-S930 recording at 30 fps and two Fugifilm Finepix F100fd recording at 30 fps). The video sequences were analysed using Ethovision XT 8.0 to record the number of burst swim responses to the stimulus and the velocity (*Umax*) of each response. The start of the response was recorded when the fish formed the C-shape and ended either when the fish stopped moving or changed direction (i.e. the initial escape response). Video sequences where the fish was less than 2 body lengths from the sides of the tank were excluded. An average of the three highest velocity measurements for each fish was calculated and converted to relative swimming speed, standard length SL s^{-1} to account for differences in size of the fish. The total number of burst swims was also recorded.

Praziquantel treatment and parasite screening

Following the burst swimming trials, half of the test fish (n=32) were treated with 50% Praziquantel (Fluke-solve, Fish Treatment Ltd) in individual pots at 4mg L^{-1} for 24 hours. The remaining fish (n=32) were sham treated with an equivalent volume of dechlorinated water. Fish were given a two day recovery period before repeating the burst swimming performance in the active flow (Day 7) and static (Day 10) condition as described above.

Upon completion of all swimming trials the sticklebacks were dissected, screened for parasites and weight (*W*) and standard length (*SL*) measured. Fulton's condition factor *K* was calculated as $K=100(W/SL^3)$. The sticklebacks were dispatched individually using an overdose of tricaine methane sulphonate (MS222) and pithed. Dissection took place under a low power dissection microscope with fibre optic light source. The skin and fins were screened externally for ectoparasites, then the body cavity opened and all organs (gills, intestinal tract, stomach, gall bladder, swim bladder, liver, kidneys, urinary bladder, heart, gonads, eyes and body cavity) removed and dissected on separated petri dishes to screen for endoparasites. No attempt was made to identify the digenean metacercariae and for the purpose of this study all such larvae were grouped together as one taxon. Prevalence and intensity of each taxon was recorded according to Bush et al. (1997). Species richness (number of parasite taxa infecting the host), Simpsons Diversity Index and Shannon's

Diversity Index was calculated to take in account of multiple species infections. The Simpson's Diversity Index was calculated as 1-*D*, where:

$$D = \frac{\sum n\,(n-1)}{N(N-1)}$$

n = total number of organisms of a species and *N* = total number of organisms of all species. The Shannon-Weiner Diversity Index (*H*) was calculated as:

$$H = -\sum_{i=1}^{n} pi \ln pi$$

where *pi* is the proportion of individuals of species *i*.

Data analysis

All data analysis was conducted using R statistical interface (R Core Team 2013).

To test the repeatability of the performance tests within the swimming trials, the correlation between the individual burst swimming responses in the active and static flow conditions for individual fish was assessed using linear regression analysis. The relationship between the number of burst swims and flow rate in the open channel flume was analysed using a General Linear Mixed Model (GLMM).

Factors influencing the burst swimming performance of sticklebacks were assessed using GLMMs with Gaussian family and identity or log link functions. For each behavioural response (velocity of burst swim, number of burst swims and time near bed boundary) three sub-models were run. The three sub-models included either parasite intensity, prevalence or parasite diversity (*D-1*, *H* and species richness). Additional fixed terms were morphological characters (*W* or *K*) and time spent near the flume bed. Interaction terms were included to account for any interactions between host weight or condition factor and parasite infection. Fish number was included as a random term to account for repeated measures where the same fish were used for different tests. Model selection and model averaging based were based on corrected Akaike Information Criterion (AICc) using the lme4 library (Bates et al. 2013). The fixed and random effects were included in global sub model GLMMs with all plausible explanatory variables and interactions. The variables were then standardised using the arm R library (Gelman and Su 2013) then the

most important explanatory variables determined using model averaging of the top models with delta AICc<2.5 using the MuMIn library (Barton 2013) as described by Grueber et al. (2011).

The efficiency of parasite removal by praziquantel was assessed using analysis of variance (ANOVA) to compare mean intensity and prevalence of infection of each parasite taxon between the treated and untreated stickleback host groups.

Results

In the active flow trials in the open channel flume, sticklebacks initiated burst swim responses an average of 14.56 (SE 0.74) times during the 10 min period. Generally, as the flow rate was increased the stickleback initiated more frequent burst swims, suggesting a transition from aerobic to burst-and-coast swimming (GLMM, X^2=258.60, P<0.001) (Figure 4.1).

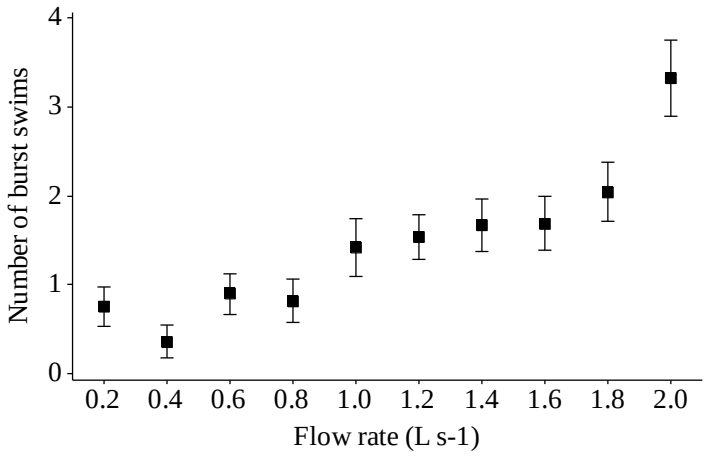

Figure 4.1. Increase in number of burst swims by sticklebacks with increasing flow rate in open channel flume. Squares indicate means, error bars indicate 95% confidence intervals.

The time spent near the bed boundary layer of the open channel flume was an important and significant explanatory variable in the frequency of burst responses. The more time spent near the bed, the fewer burst swims were initiated (Figure 4.2, Table 4.1.)

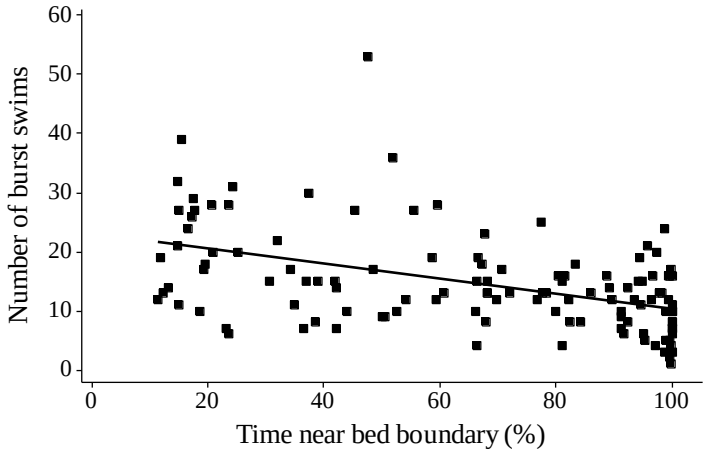

Figure 4.2. Reduction in number of burst swim responses initiated with increasing time spent near the bed boundary layer in an open channel flume. Line shows predictions from the top model.

Table 4.1. Summary of the averaged model predictors. Only explanatory variables with relative importance >0.90 are included. Semicolons indicate interactions, shaded rows indicate confidence intervals which do not bound zero and therefore are considered significant. SE = unconditional standard error, CI = confidence intervals. PZQ = praziquantel, K = condition factor.

Dependent Variable	Model	Predictor	Estimate	SE	95% CI	Relative importance
Velocity of response *Umax* (SL s^{-1})	Intensity	Intercept	249.27	18.22	213.57 – 284.98	
		PZQ treatment	-75.88	39.08	-152.48 – 0.71	1.00
		P. folium intensity	122.15	44.60	34.73 – 209.56	1.00
		G. gasterostei intensity	-87.47	46.85	-179.30 – 4.35	1.00
	Prevalence	Intercept	5.97	0.46	5.08 – 6.87	
		Weight	-2.48	0.92	-4.28 – -0.68	1.00
		P. folium prevalence	2.46	1.45	-0.39 – 5.31	0.97
		PZQ treatment	-1.94	0.98	-3.86 – -0.02	0.95
	Diversity	Intercept	6.11	0.45	5.20 – 6.99	
		Weight	-2.75	0.91	-4.52 – -0.98	1.00
Number of burst swims in static condition	Intensity	No predictors with relative importance >0.90				
	Prevalence	No predictors with relative importance >0.90				
	Diversity	No predictors with relative importance >0.90				
Number of burst swims in active flow condition	Intensity	Intercept	16.88	2.77	11.45 – 22.31	
		Percent time near bed	-7.46	1.73	-10.85 – -4.06	1.00
		K	-6.36	4.59	-15.36 – 2.65	0.91
	Prevalence	Intercept	15.04	0.87	13.34 – 16.76	
		Metacercariae prevalence	4.94	2.03	0.97 – 8.91	1.00
		Percent time near bed	-7.18	1.65	-10.41 – -3.95	1.00
	Diversity	No predictors with relative importance >0.90				
Time near bed boundary (%)	Intensity	Intercept	4.05	0.08	3.89 – 4.20	
		PZQ treatment	0.46	0.16	0.14 – 0.78	1.00
	Prevalence	Intercept	4.05	0.08	3.89 – 4.20	
		PZQ treatment	0.45	0.16	0.13 – 0.78	1.00
	Diversity	Intercept	3.96	0.06	3.85 – 4.07	
		PZQ treatment	0.54	0.11	0.32 – 0.76	1.00
		K	-0.09	0.12	-0.33 – 0.14	1.00
		PZQ treatment: K	0.59	0.22	0.15 – 1.03	1.00

There was a significant correlation between number of burst swims initiated in the active flow flume trials and static flow trials (F=4.713, DF=1, 122, P=0.032), indicating that fish which performed more frequent burst swims in the active flow trial also performed more frequent burst swims in the static flow condition. In the static flow condition, sticklebacks reacted on average 3.17 (SE 0.19) times. The mean velocity of response in the static condition was 24.64 (SE 14.02) cm s^{-1} or 5.87 (SE 0.34) SL s^{-1}. There was a negative correlation with fish weight and velocity of burst swim, indicating that heavier fish initiate slower responses (Table 4.1).

The sticklebacks were naturally infected with 6 parasite taxa (Table 4.2), with the highest prevalence (93.8%) of *Diplostomum* spp. and lowest prevalence (3.1% just one infected fish) of *Anguillicoloides crassus*. Digenean metacercariae were found in various host tissues including the intestinal tract, stomach, liver, kidneys and gills.

Table 4.2. Prevalence, mean intensity and range of parasite taxa infecting three-spined sticklebacks. ANOVA results compare the difference in parasite prevalence, mean intensity and diversity between untreated (n=32) and praziquantel (PZQ) treated (n=32) stickleback. Shaded rows indicate significance at P<0.05.

	Location	**Untreated**	**Treated**	**F**	**P**
Monogenea					
Gyrodactylus gasterostei prevalence	Skin	8 (25.0%)	4 (12.5%)	1.09	0.30
Gyrodactylus gasterostei intensity (range)		72.0 (1-500)	3.0 (1-4)	1.28	0.26
Digenea					
Diplostomum spp. prevalence	Eye	30 (93.8%)	29 (90.6%)	0.21	0.65
Diplostomum spp. intensity (range)		3.77 (1-17)	2.69 (1-7)	2.46	0.12
Phyllodistomum folium prevalence	Urinary	8 (25.0%)	0 (0.0%)	10.33	<0.01
Phyllodistomum folium intensity (range)	bladder	1.13 (1-2)	0.00	9.27	<0.01
Metacercariae prevalence	Various	24 (75.0%)	27 (84.4%)	0.85	0.36
Metacercariae intensity (range)		2.71 (1-6)	3.59 (1-9)	3.85	0.05
Nematoda					
Anguillicoloides crassus prevalence	Swim	0 (0.0%)	1 (3.1%)	1.00	0.32
Anguillicoloides crassus intensity (range)	bladder	1.00 (1-4)	0.00	1.00	0.32
Acanthocephala					
Ancanthocephalus lucii prevalence	Intestine	8 (25.0%)	4 (12.5%)	1.63	0.21
Ancanthocephalus lucii intensity (range)		1.50 (1-4)	1.50 (1-2)	1.15	0.29
Simpsons Diversity Index 1-D (range)		0.46 (0.00- 0.8)	0.48 (0.00-1.00)	0.16	0.70
Shannon-Wiener Index H (range)		0.60 (0.00-1.09)	0.53 (0.00-1.08)	0.87	0.36

In the static condition, sticklebacks infected with ***P. folium*** had a higher velocity of burst swim (*Umax*) than uninfected individuals (Table 4.2). There was also a positive relationship between the number of burst swims in the flow condition and digenean metacercariae prevalence (Table 4.1). Parasite intensity, diversity or host condition factor did not affect the number of burst swims in static or active flow or the time spent near the bed boundary layer.

Treatment with praziquantel caused a significant reduction in the prevalence and intensity of *Phyllodistomum folium* but did not affect the prevalence, intensity or diversity of any other parasite taxa (Table 4.2). Sticklebacks treated with PZQ significantly improved their anaerobic swimming performance by displaying a higher *Umax* compared to untreated counterparts (Figure 4.3A, Table 4.1). However, PZQ treatment did not affect the number of burst swims in either static or active flow conditions. Untreated fish spent significantly more time near the bed boundary layer (Figure 4.3B).

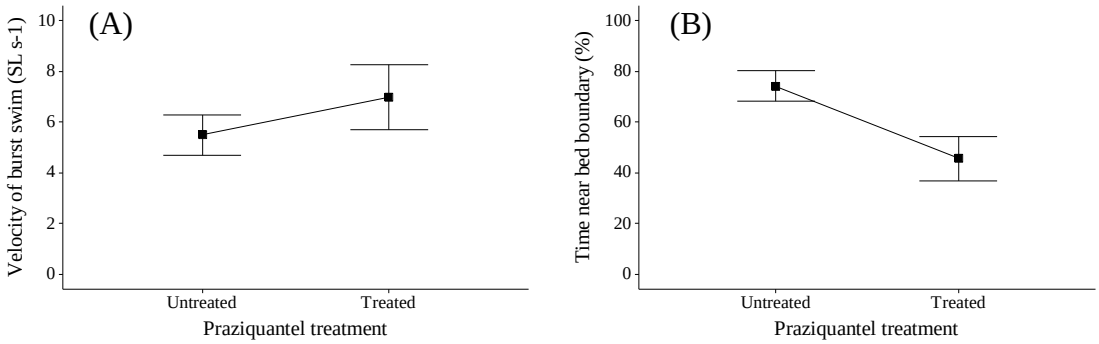

Figure 4.3. (A) Increase in velocity (*Umax*) of the burst response in the static condition and (B) reduction in time spent near the bed boundary layer in open channel flume of three-spined sticklebacks when treated with the antihelminthic praziquantel. Squares show means and bars 95% confidence intervals.

Discussion

This study assessed the anaerobic burst swimming ability of three-spined stickleback in terms of their vertical position in the water column, natural parasite infection and morphological characteristics in both static flow conditions and an open channel flume. As the flow rate was increased, the stickleback initiated significantly more burst swims indicating an increased reliance on burst-and-coast swimming. However, burst-and-coast swimming was reduced if the fish spent more time associating with the bottom third of the water column closest to the bed, using it as a flow refuge. The fish with a faster velocity of burst swim response had higher the intensities of *Phyllodistomum folium*. More frequent burst swims were initiated when the fish were infected with metacercariae. No other parasite taxa affected the burst swimming performance of the stickleback. The antihelminthic drug, praziquantel (PZQ) reduced *P. folium* infection but did not affect the other five parasite taxa. Surprisingly, fish treated with PZQ had a higher velocity of burst response and spent less time swimming near the bed boundary layer compared to controls.

In streams and rivers, fish survival may depend on their ability to avoid areas of high flow velocity, instead selecting areas in the wake of obstacles or in the bed boundary layer where water velocity is reduced in order to conserve energy (Liao 2007; Carlson and Lauder 2011; Hoover et al. 2011; Hockley et al. 2014). In the current study, as the flow velocity was rapidly increased, stickleback swimming shifted from aerobic to burst-and-coast swimming in order to station hold against the current. However, fewer burst swims were initiated if the fish spent more time in the lower section of the water column where flow velocities are lower adjacent to the bed boundary layer. It is likely then that the fish associating with the bed boundary layer are able to reach higher *Umax* values as they are not yet reaching a stage where they are resorting to anaerobic burst-and-coast swimming to maintain position (Dutil et al. 2007).

Natural variation in anaerobic swimming performance could be explained by multiple factors including parasite infection, fish size, temperature, water quality and habitat type (Domenici and Blake 1997; Dutil et al. 2007; Tierney 2011). In the current study, there was a negative relationship between fish weight and *Umax* while accounting for fish standard length. This may simply be because heavier fish are not able to gain as much propulsion compared to lighter individuals when initiating a burst swim. To date, studies investigating the parasite effects on the burst swimming performance of fish have only focussed on the effect of one or two parasite species (e.g. Barber et al. 2004; Blake et al. 2006) rather than the natural burden of multiple parasite taxa. The natural parasite fauna of the sticklebacks in the current study had no detrimental effect on recorded fish swimming performance. In fact, intensity of *Phyllodistomum folium* and presence of digenean metacercariae was correlated with greater swimming performance. Residing in the urinary bladder, it is unlikely that *P. folium* directly manipulates the host to improve swimming ability. It may be that differences in host fitness led to a higher exposure to the parasite larval stages. Freshwater mussels, the intermediate host of *P. folium*, inhabit fast flowing water. Therefore sticklebacks which perform higher velocity burst responses are more likely to be able to tolerate the higher flow rates thereby coming into contact with more infective stages of the parasite. Previous studies demonstrating the effects of parasites on fish swimming performance have involved artificially infecting the fish with high burdens of a single parasite species. In reality, parasite infections tend to be highly aggregated, with only a small number of hosts harbouring heavy parasite burdens. Therefore, any population level effects of the parasite are likely to be marginal, with any

high disturbance events only affecting the heavily infected few. Any poor-performing fish are likely to be removed from the population at an early stage following a disturbance events, such as a river in spate (van Oosterhout et al. 2007).

A 24 hour bath treatment of the general purpose antihelminthic drug praziquantel (PZQ, Fluke Solve, Fish Treatment Ltd) significantly reduced the prevalence and intensity of *P. folium*, but had no effect on any other macroparasites. Surprisingly, the sticklebacks treated with PZQ demonstrated an increased burst swimming performance, with a higher velocity of burst swim when treated compared to their untreated counterparts. Additionally, the sticklebacks treated with PZQ spent less time near the bed boundary layer than untreated fish. The mode of action of PZQ is generally unknown (You et al. 2013) but it is thought that the drug causes activation of voltage-gated calcium Ca^{2+} channels in the parasite which results in an influx of extracellular calcium and causes the contraction and paralysis of the parasite's muscle (Doenhoff et al. 2008; You et al. 2013). In addition PZQ causes the formation of lesions in the tegument of many parasites, including cestodes and trematodes (Chan et al. 2013). Depending on the initial dosage, PZQ can be detected in fish muscle and skin several days post treatment, with a decline in concentration over time (Kim et al. 2003). Without further study, we cautiously speculate that the drug may also cause calcium flux in the host muscle tissues and therefore increase muscle contraction and neural excitement resulting in higher velocity burst responses and increased ability to tolerate swimming in the water column. The PZQ treated sticklebacks did not perform more burst swims despite spending more time higher in the water column, suggesting that they were able to maintain aerobic swimming at this higher velocity compared to the untreated fish. However, this finding requires further investigation to determine whether PZQ could affect host muscular activity in addition to its use as a general purpose antihelminthic.

In summary, this study has highlighted the importance of considering vertical position within an open channel when measuring fish swimming performance. Fish are able to utilise the heterogeneous variation in flow velocity in the water column to refuge from undesirable flows. Although the natural parasite burden of sticklebacks had limited effect on their burst swimming performance, there is a potentially important effect of the antihelminthic drug PZQ to enhance the stickleback anaerobic swimming performance both in active flow and static flow conditions.

References

Barber I, Hoare D, Krause J (2000) Effects of parasites on fish behaviour: a review and evolutionary perspective. Rev Fish Biol Fish 10:131–165

Barber I, Walker P, Svensson PA (2004) Behavioural responses to simulated avian predation in female three-spined sticklebacks: the effect of experimental *Schistocaphalus solidus* infections. Behaviour 141:1425–1440

Barton K (2013) MuMIn: Multi-model inference. R package version 1.9.13. http://CRAN.R-project.org/package=MuMIn

Bates D, Maechler M, Bolker B, Walker S (2013) lme4: Linear mixed effects models using Eigen and S4. R package version 1.0-5. http://cran.r-project.org/package=lme4

Binning SA, Roche DG, Layton C (2013) Ectoparasites increase swimming costs in a coral reef fish. Biol Lett 9:20120927

Blake RW, Kwok PYL, Chan KHS (2006) Effects of two parasites, *Schistocephalus solidus* (Cestoda) and *Bunodera* spp. (Trematoda), on the escape fast-start performance of three-spined sticklebacks. J Fish Biol 69:1345–1355

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al revisited. J Parasitol 83:575–583

Carlson RL, Lauder GV (2011) Escaping the flow: boundary layer use by the darter *Etheostoma tetrazonum* (Percidae) during benthic station holding. J Exp Biol 214:1181-1193

Chan JD, Zarowiecki M, Marchant JS (2013) Ca^{2+} channels and praziquantel: a view from the free world. Parasitol Int 62:619–628

Chisholm LA, Whittington ID (2002) Efficacy of praziquantel bath treatments for monogenean infections of the *Rhinobatos typus*. J Aquat Anim Health 14:230–234

Doenhoff MJ, Cioli D, Utzinger J (2008) Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. Curr Opin Infect Dis 21:659–667

Domenici P, Blake RW (1997) The kinematics and performance of fish fast-start swimming. J Exp Biol 200:1165–1178

Dutil J-D, Sylvestre E-L, Gamache L, Larocque R, Guderley H (2007) Burst and coast use, swimming performance and metabolism of Atlantic cod *Gadus morhua* in sub-lethal hypoxic conditions. J Fish Biol 71:363-375

Farrell AP (2008) Comparisons of swimming performance in rainbow trout using constant acceleration and critical swimming speed tests. J Fish Biol 72:693–710

Ferrari N, Cattadori IM, Nespereira J, Rizzoli A, Hudson PJ (2003) The role of host sex in parasite dynamics: field experiments on the yellow-necked mouse *Apodemus flavicollis*. Ecol Lett 7:88–94

Forbes AB, Huckle CA, Gibb MJ (2007) Evaluation of the effect of eprinomectin in young dairy heifers sub-clinically infected with gastrointestinal nematodes on grazing behaviour and diet selection. Vet Parasitol 150:321–332

Garenc C, Couture P, Laflamme M-A, Guderley H (1999) Metabolic correlates of burst swimming capacity of juvenile and adult threespine stickleback (*Gasterosteus aculeatus*). J Comp Physiol B 169:113–122

Gauly M, Duss C, Erhardt G (2007) Influence of *Ascaridia galli* infections and anthelmintic treatments on the behaviour and social ranks of laying hens (*Gallus gallus domesticus*). Vet Parasitol 146:271–280

Gelman A, Su Y-S (2013) arm: Data analysis using regression and multilevel/hierarchical models. R package version 1.6-10. http://cran.r-project.org/package=arm

Grueber CE, Nakagawa S, Laws RJ, Jamieson IG (2011) Multimodel inference in ecology and evolution: challenges and solutions. J Evol Biol 24:699–711

Hirazawa N, Mitsuboshi T, Hirata T, Shirasu K (2004) Susceptibility of spotted halibut *Verasper variegatus* (Pleuronectidae) to infection by the monogenean *Neobenedenia girellae* (Capsalidae) and oral therapy trials using praziquantel. Aquaculture 238:83–95

Hockley FA, Wilson CAME, Brew A, Cable J (2013) Fish responses to flow velocity and turbulence in relation to size, sex and parasite load. J R Soc Interface 11:20130814

Hoover JJ, Collins J, Boysen KA, Katzenmeyer AW, Killgore KJ (2011) Critical swimming speeds of adult shovelnose sturgeon in rectilinear and boundary-layer flow. J Appl Ichthyol 27:226–230

Iles AC, Archdeacon TP, Bonar SA (2012) Novel praziquantel treatment regime for controlling Asian tapeworm infections in pond-reared fish. N Am J Aquac 74:113–117

Jolles AE, Ezenwa VO, Etienne RS, Turner WC, Olff H (2008) Interactions between macroparasites and microparasites drive infection patterns in free-ranging African buffalo. Ecology 89:2239–2250

Kalbe M, Wegner KM, Reusch TBH (2002) Dispersion patterns of parasites in 0+ year three-spined sticklebacks: a cross population comparison. J Fish Biol 60:1529–1542

Kim CS, Cho JB, Ahn KJ, Lee JI, Kim KH (2003) Depletion of praziquantel in muscle tissue and skin of cultured rockfish (*Sebastes schlegeli*) under the commercial culture conditions. Aquaculture 219:1–7

Liao J (2007) A review of fish swimming mechanics and behaviour in altered flows. Phil Trans Soc B 362:1973–1993

Longshaw M, Frear P, Feist SW (2003) *Myxobolus buckei* sp. n. (Myxozoa), a new pathogenic parasite from the spinal column of three cyprinid fishes from the United Kingdom. Folia Parasitol 50:251–262

Marras S, Killen SS, Domenici P, Claireaux G, McKenzie DJ (2013) Relationships among traits of aerobic and anaerobic swimming performance in individual European sea bass *Dicentrarchus labrax*. PLoS One 8:e72815

McDonald DG, McFarlane WJ, Milligan CL (1998) Anaerobic capacity and swim performance of juvenile salmonids. Can J Fish Aquat Sci 55:1198-1207

Miller MR, White A, Boots M (2007) Host life span and the evolution of resistance characteristics. Evolution 61:2–14

Mitchell A, Darwish A (2009) Efficacy of 6-, 12-, and 24-h praziquantel bath treatments against Asian tapeworms *Bothriocephalus acheilognathi* in grass carp. N Am J Aquac 71:30–34

Östlund-Nilsson S, Curtis L, Nilsson GE, Grutter AS (2005) Parasitic isopod *Anilocra apogonae*, a drag for the cardinal fish *Cheilodipterus quinquelineatus*. Mar Ecol Prog Ser 287:209–216

Peak S (2004) An evaluation of the use of critical swimming speed for determination of culvert water velocity criteria for smallmouth bass. Trans Am Fish Soc 133:1472–1479

Plaut S (2004) Critical swimming speed: its ecological relevance. Comp Biochem Physiol Part A 131:41–50

Powers PD (1997) Culvert hydraulics related to upstream juvenile salmon passage. Lands and Restoration Services Program. Washington Department of Fish and Wildlfe, Project No. 982740

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Roy BA, Kirchner JW (2000) Evolutionary dynamics of pathogen resistance and tolerance. Evolution 54:51–63

Russon IJ, Kemp PS (2011) Experimental quantification of the swimming performance and behaviour of spawning run river lamprey *Lampetra fluviatilis* and European eel *Anguilla anguilla*. J Fish Biol 78: 1965–1975

Schmahl G, Mehlhorn H (1985) Treatment of fish parasites. 1. Praziquantel effective against Monogenea (*Dactylogyrus vastator, Dactylogyrus extensus, Diplozoon paradoxum*). Z Parasitenkd 71:727–737

Schmahl G, Taraschewski H (1987) Treatment of fish parasites. 2. Effects of praziquantel, niclosamide, levamisole-HCl, and metrifonate on Monogenea (*Gyrodactylus aculeati*, *Diplozoon paradoxum*). Parasitol Res 73:341–351

Shaw DJ, Dobson AP (1995) Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. Parasitology 111:S111–S133

Shaw DJ, Grenfell BT, Dobson AP (1998) Patterns of macroparasite aggregation in wildlife host populations. Parasitology 117:597–610

Sitjà-Bobadilla A, de Felipe MC, Alvarez-Pellitero P (2006) *In vivo* and *in vitro* treatments against *Sparicotyle chrysophrii* (Monogenea: Microcotylidae) parasitizing the gills of gilthead sea bream (*Sparus aurata* L.). Aquaculture 261:856–864

Tierney KB (2011) Swimming performance assessment in fishes. J Vis Exp 51:e2572

Tudorache C, Viaene P, Blust R, Vereecken H, De Boeck G (2008) A comparison of swimming capacity and energy use in seven European freshwater fish species. Ecol Freshw Fish 17:284–291

van Oosterhout C, Mohammed RS, Hansen H, Archard GA, McMullan M, Weese DJ, Cable J (2007) Selection by parasites in spate conditions in wild Trinidadian guppies (*Poecilia reticulata*). Int J Parasitol 37:805–812

Voutilainen A, Saarinen M, Suonpää A, Taskinen J (2009) *In vitro* efficacy of praziquantel against the cercariae of *Diplostomum* sp., *Rhipidocotyle fennica* and *R. campanula*. J Fish Dis 32:907–909

Walker JA, Ghalambor CK, Griset OL, McKenney D, Reznicks DN (2005) Do faster starts increase the probability of evading predators? Funct Ecol 19:808–815

Ward DL (2007) Removal and quantification of Asian tapeworm from bonytail chub using praziquantel. N Am J Aquac 69:207–210

Wegner KM, Reusch TBH, Kalbe M (2003) Multiple parasites are driving major histocompatibility complex polymorphism in the wild. J Evol Biol 16:224–232

Williams RE, Ernst I, Chambers CB, Whittington ID (2007) Efficacy of orally administered praziquantel against *Zeuxapta seriolae* and *Benedenia seriolae* (Monogenea) in yellowtail kingfish *Seriola lalandi*. Dis Aquat Organ 77:199–205

Wilson K, Bjørnstad ON, Dobson AP, Merler S, Poglayen G, Randolph SE, Read AF, Skorping A (2001) Chapter 2: Heterogeneities in macroparasite infections: patterns and processes. In: Hudson PJ, Rizzoli A, Grenfell BT, Heesterbeek H, Dobson AP (eds) The Ecology of Wildlife Diseases. OUP Oxford: 6–44

You H, McManus DP, Hu W, Smout MJ, Brindley PJ, Gobert GN (2013) Transcriptional responses of *in vivo* praziquantel exposure in schistosomes identifies a functional role for calcium signalling pathway member CamKII. PLoS Pathog 9:e1003254

Chapter 5. Use of the bed boundary layer to enhance critical swimming performance

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This chapter is in preparation for submission to Hydrobiologia. The experiment was designed and implemented in the laboratory by FH and CS. ADV data analysis was by ZS. Statistical data analysis and manuscript preparation was by FH. Supervision and comments on the manuscript was provided by JC and CW.

Abstract

Fish passage design is often supported by data obtained from aerobic swimming performance tests implemented in the laboratory. The critical swimming performance test is one such method which measures the sustained swimming performance of fish by incrementally increasing the flow velocity until the fish fatigues. These methods have often been criticised in their use of flow chambers which produce artificial and unrealistic flow conditions compared to those found in the wild or through passage facilities. One key omission of these methods is the consideration of bed substrate types and the velocity shear and associated turbulence produced by the bed. This study measured the critical swimming performance of a model species the three-spined stickleback (*Gasterosteus aculeatus*) using three different bed types – smooth, coarse sand and gravel. The fish that spent more time swimming near the bed achieved higher critical swimming speeds, indicating that the fish were able to use the boundary layer effects as a flow refuge. Less time was spent near the gravel and sand beds compared to the smooth bed due to the higher velocity, turbulence intensity, turbulent kinetic energy and Reynolds stresses associated with the gravel and sand. As the flow velocity was incrementally increased during the swimming performance trial, fish spent less time swimming near the bed. This was probably because of the reduction in thickness of the bed boundary layer, increase in turbulent shear stress and increase in total turbulent energy production within this zone with increasing velocity. Bed conditions may therefore be an important consideration for laboratory swimming performance methods and have subsequent implications for fish passage design.

Introduction

The water demands on rivers and other freshwater ecosystems by human activity have increased on a global scale and have led to substantial watercourse modifications and alterations of the natural flow regime (Tharme 2003; Enders et al. 2009b). In combination with climate induced changes in rainfall, the frequency of drought and flood events are predicted to increase (Floury et al. 2013), which are major regulators of aquatic communities (Jowett et al. 2005). In many watercourses fish bypasses, fishways and culverts have been installed to assist the migration of fish past obstacles such as barrages, dams, weirs and sluice gates. The design of passage facilities are often verified by data on fish aerobic swimming performance obtained in the laboratory (Peake 2004), which generally target commercially important fish such as salmonids (Knaepkens et al. 2006).

Aerobic swimming refers to sustained or prolonged swimming which is employed during migration or when station holding against a current (Marras et al. 2013). Biologists and aquaculturalists utilise fish swimming performance to identify differences in fitness and survival. This information can then feed into recommendations about fish passage and mitigation methods for engineering design to successfully allow fish to move throughout modified watercourses. The critical swimming speed *Ucrit* (presented either as the absolute swimming speed, cm s^{-1}, or relative swimming speed, standard length SL s^{-1}) is a measure of constant swimming performance and is often used in laboratory swim chambers to study muscle energetics, swimming mechanics, gas exchange, cardiac physiology, disease, pollution, hypoxia and temperature effects (Tierney 2011). The test involves increasing the flow speed in the swimming chamber in constant increments until the fish fatigues (Brett 1964; Plaut 2001). A 'ramped' critical swimming test may be used to shorten the duration of the test by reducing the time interval of the first few steps. The test is generally accepted as a rough estimate of swimming performance capability, which reflects aerobic capacity, provides comparable data on the swimming ability between fish and is individually repeatable (Plaut 2001). Laboratory swimming performance tests using swimming chambers similar to those used first by Brett (1964) have been criticised in their design in which they force fish to swim in highly artificial conditions with restricted volitional movement (Russon and Kemp 2011). It has been demonstrated that swimming performance measured in open channel flumes and the wild is higher

compared to when fish movement is restricted in enclosed swimming chambers (Peake 2004; Haro et al. 2004; Russon and Kemp 2011).

When water flows over a solid object, such as a river bed, the layer of fluid particles in contact with the bed has a velocity of zero due to frictional forces known as the 'no-slip' condition (Massey and Ward-Smith 2006). With increasing height above the bed, the fluid particles will increase in velocity until they reach the same velocity as the free stream. This zone between the bed and the free stream is known as the boundary layer (Massey and Ward-Smith 2006). Laboratory swim tests commonly assume the fish are swimming in an unbounded fluid (Webb 1993), whereas in nature or in an open channel flume a boundary layer is formed near the walls and bed with a lower velocity and higher turbulence levels than in the free stream (Plaut 2001). Fish may therefore find locations with slower velocities in the boundary layer to rest, resulting in a higher velocity tolerance than that demonstrated by *Ucrit* tests in swim chambers.

The ability of fish to take advantage of boundary layer flows has been previously documented in laboratory experiments. For example Carlson and Lauder (2011) demonstrated that the darter (*Etheostoma tetrazonum*) is small enough to use boundary layer flow to escape the stream flow in order to station hold. Barbin and Krueger (1994) studied the upstream migration ability of American eel (*Anguilla rostrata*) elvers in an experimental flume with and without a substrate representative of the bed type where the fish were sampled. Upstream migration was greatly enhanced by the presence of the substrate as the elvers were able to use the boundary layer to avoid free stream velocities to rest between burst swims in the water column (Barbin and Krueger 1994). The time elvers spent in the substrate increased with increasing flow rates (Barbin and Krueger 1994). Atlantic cod (*Gadus morhua*) used ripples to refuge from flow velocities of 49-109 cm s^{-1} (Gerstner 1998). Similarly Hoover et al. (2011) demonstrated that adult shovelnose sturgeon (*Scaphirhynchus platorynchus*) reached higher critical swimming speeds when swimming in boundary layer flow on a flat bottomed bed compared to swimming in free stream flow in a tube chamber. However, none of these experiments explicitly measured the time the fish spent associating with the flume bed and the critical swimming speed achieved.

The pelagic three-spined stickleback (*Gasterosteus aculeatus*) is an ideal model and sentinel species due to its ubiquity, ease in which it can be housed in a laboratory, and its

well documented life history traits (Katsiadaki 2007). Three-spined sticklebacks occur throughout the water column in a range of habitats, from small potholes to oceans (Bell and Foster 1994). The anaerobic swimming performance of stickleback is fairly well reported (Tierney et al. 1993; Garenc et al. 1999; Barber et al. 2004; Blake et al. 2006b), however aerobic swimming ability is less well studied (Taylor and McPhail 1986; Blake et al. 2005; Tudorache et al. 2007).

The current study assessed the critical swimming speed of three-spined sticklebacks in an open channel flume. Taking into account fish morphological characters and parasite infection (as demonstrated in Chapter 4), three bed substrate types were used to study the effect of bed boundary layer conditions (turbulence and velocity) on fish swimming performance.

Methods

Study system

Three-spined sticklebacks with natural parasite infections were caught using dip nets in Roath Brook, South Wales, Cardiff (NGR ST 18833 78526) in June 2013 (n=21). Sticklebacks had a mean standard length of 50.68 mm (range 46.80-54.40 mm) and mean weight 1.39 g (range 0.94-1.78 g). The fish were housed in 80 L aquaria of dechlorinated water in the School of Biosciences, Cardiff University, UK at 15 ± 1^{o}C with a 16 h light: 8 h dark regime at maximum density of 16 fish and fed daily on *Tubifex* blood worm. Experiments took place between June and July 2013 between 09:00-18:00. All work was conducted under Home Office approved project PPL 30/2357.

Critical swimming speed

Critical swimming speed was measured using a 10 m length x 0.29 m width glass walled unidirectional recirculating open channel flume in the Hydro-environmental Research Centre (HRC), Cardiff University, at 14-17^{o}C. The channel was set at a negative gradient of 1 in 1000 and a tailgate weir at the downstream end of the flume was used to maintain the flow depth at mean 0.15 m. Aluminium honeycomb flow straighteners were used to smoothen the inflow and outflow conditions at both ends of the flume. Discharge was adjusted by controlling the power provided to the pump through a control box. Three different bed types, on removable 15 mm thick PVC sheets, were used to assess critical swimming speed. The substrate types were classified using British Standard 1377-2:1990

for grain size distribution. The d_{50} values were used to represent the mean roughness height of the bed (k) (Nikuradse 1950). The first substrate was a smooth bed using PVC sheeting only, the second was coarse sand (uniformly graded with a d_{50} of 1.5 mm, d_{30} of 1.4 mm and d_{90} of 3.3 mm) adhered to the sheeting using waterproof PVA glue and the third was medium gravel (uniformly graded with a d_{50} of 12 mm, d_{30} of 11 mm and d_{90} of 17 mm) adhered to the PVC sheeting using clear silicon adhesive sealant. Horizontal lines were drawn on the sides of the flume at 20 mm spacing to allow the observer to estimate the vertical distance of the fish from the bed.

The fish were restricted to a 1 m downstream section of the flume through the use of an additional flow straightener to prevent movement downstream and a hand net to prevent the fish swimming upstream. Fish were acclimatised at the lowest flow velocity of 10 cm s^{-1} for 30 min. Critical swimming speed was measured using a ramped method with an increase in area mean velocity (defined as $U = Q/A$ where u is area mean velocity, Q is discharge and A is cross sectional area) of 5 cm s^{-1} every 20 min with the first three steps ramped with 10 min intervals. The test was terminated when the stickleback could no longer swim against the current and became impinged on the flow straightener. Critical swimming speed was then calculated using the equation:

$$Ucrit = U_i + U_{ii}\,(t_i/t_{ii})$$

Where U_i is the area mean velocity at the last completed step, U_{ii} is the step height (5 cm s^{-1} in this study), t_i is the duration of final incomplete step and t_{ii} is the step duration (10 or 20 min in this study) (Tierney 2011). The time spent near the bed boundary was recorded when the fish were within 30 mm from the bed mean roughness height. The time was then converted as a percentage of the total time that the fish was swimming at each velocity. Each test was then repeated for each substrate in a randomised order with a 24 hour recovery period between each test.

Hydraulic measurements

The velocity and turbulence field near the bed surface for the 20 cm s^{-1} flow velocity was characterised using a Nortek Vectrino II downwards-looking acoustic Doppler velocimeter (ADV) profiler with a sampling frequency of 100 Hz. The water was seeded using Q-Cel hollow microspheres to increase the signal to noise ratio (SNR) above 30 and a minimum correlation of 70%. A horizontal measurement grid spanning 360 mm in

the longitudinal direction by 110 mm in the lateral direction starting at 40 mm from the flow straightener and 35 mm from the side walls was used for measurements at a spatial resolution of 20 mm (resulting in a total of 114 velocity profiles). Flow symmetry across the width of the flume was assumed so only half the flume width was profiled. The 35 mm vertical profile from the top of the bed roughness was measured for a sampling period of 3 min every 2 mm for the smooth and sand bed, and for 5 min every 1 mm for the gravel bed. The ADV profile data was then exported and processed using Velocity Signal Analyser v1.5.38 (Jesson et al. 2013a, b) to filter the data with a minimum correlation of 70% and minimum SNR of 30. The velocity statistics were time- and spatially-averaged for each distance from the bed roughness and velocity and turbulence metrics were calculated (Table 5.1) and plotted to allow visual comparison between the three bed types (Figure 5.1). The time-averaged point velocities in the longitudinal, transverse and vertical directions are denoted as $\bar{u}$, $\bar{v}$ and $\bar{w}$ respectively. The instantaneous turbulent fluctuation of the longitudinal velocity from the time mean velocity is:

$$u'(t) = u(t) - \bar{u}$$

The turbulence strength is defined as:

$$u_{rms} = \sqrt{\overline{u'(t)^2}}$$

where 'rms' is the root-mean-square. Similar definitions apply to the transverse and vertical velocities, *v(t)* and *w(t)*. The turbulent kinetic energy *(k)* per unit mass which is a measure of the total turbulent energy production associated with the turbulent structures such as eddies and vortices and hence a bulk measure of the turbulence was calculated as:

$$k = 0.5(u_{rms}^2 + v_{rms}^2 + w_{rms}^2)$$

The normalised turbulent kinetic energy is defined by $\sqrt{k}.u^{-1}$ and is used in this study to normalise the turbulence intensity from the velocity magnitude. The turbulent shear stress (Reynolds stress), across the vertical plane was calculated as:

$$\tau_{uw} = \left|\rho\overline{u'w'}\right|$$

Where ρ is the density of water and, $u'w'$ is the covariance of the instantaneous velocity fluctuations. Reynolds stress is caused by the irregular movement of fluid particles and their continuous exchange of momentum from one portion of the fluid to another. The volume-averaged velocity and turbulence parameters are denoted using the square brackets i.e. <$\bar{u}$>, <$\bar{v}$>, <$\bar{w}$> and <k> etc.

Table 5.1. Volume-averaged velocity and turbulence parameters for the 35 mm profiles for the three bed types at nominal area mean velocity of 20 cm s^{-1}. Ranges of the minimum and maximum time-averaged velocity for the longitudinal ($\bar{u}$), transverse ($\bar{v}$) and vertical ($\bar{w}$) velocity components together with the turbulence intensities (u', v' and w'), turbulent kinetic energy (k) and turbulent shear stress (τ_{uw}) are given. The standard deviation (σ) for each volume-averaged parameter is given in brackets. Reynolds number is based on the hydraulic radius and area mean velocity where *Re = (4UR)/ν*.

Variable		**Smooth bed**	**Sand bed**	**Gravel bed**
Discharge Q	(L s^{-1})	8.7	8.7	8.7
Mean flow depth	(mm)	140	143	160
Bed height d_{50}	(mm)	12.0	1.5	0.0
Area mean velocity	(cm s^{-1})	20.7	20.2	18.1
Reynolds number		52,300	51,800	48,900
$<\bar{u}>$ (σ)	(cm s^{-1})	14.03 (4.45)	17.78 (2.21)	17.34 (4.07)
Range $\bar{u}$		0.02 – 18.54	0.07 – 22.16	0.00 – 24.13
$<u'>$ (σ)		1.84 (0.00)	2.74 (0.41)	3.94 (0.75)
Range u'		0.09 – 2.95	0.13 – 5.50	0.00 – 7.88
$<\bar{v}>$ (σ)	(cm s^{-1})	-0.66 (0.26)	0.17 (0.24)	1.84 (1.51)
Range $\bar{v}$		-1.32 – 0.33	-0.96 – 1.26	-5.01 – 10.50
$<v'>$ (σ)		1.65 (0.73)	2.43 (0.54)	3.29 (0.72)
Range v'		0.08 – 15.70	0.11 – 5.42	0.00 – 10.36
$<\bar{w}>$ (σ)	(cm s^{-1})	0.18 (0.20)	0.14 (0.23)	-0.60 (0.57)
Range $\bar{w}$		-0.66 – 0.20	-0.81 – 0.71	-5.05 – 2.70
$<w'>$ (σ)		0.68 (0.24)	0.99 (0.22)	1.45 (0.41)
Range w'		0.68 – 0.24	0.04 – 1.35	0.00 – 2.32
$<k>$ (σ)	(cm^2 s^{-2})	3.59 (1.71)	7.45 (2.19)	14.86 (4.23)
Range k		0.01 – 17.41	0.02 – 19.61	0.00 – 68.72
$<\tau_{uw}>$ (σ)	(Nm^{-2})	0.04 (0.02)	-0.09 (0.03)	0.21 (0.10)
Range τ_{uw}		-0.07 - 0.05	-0.14 – 0.04	-0.44 – 0.46

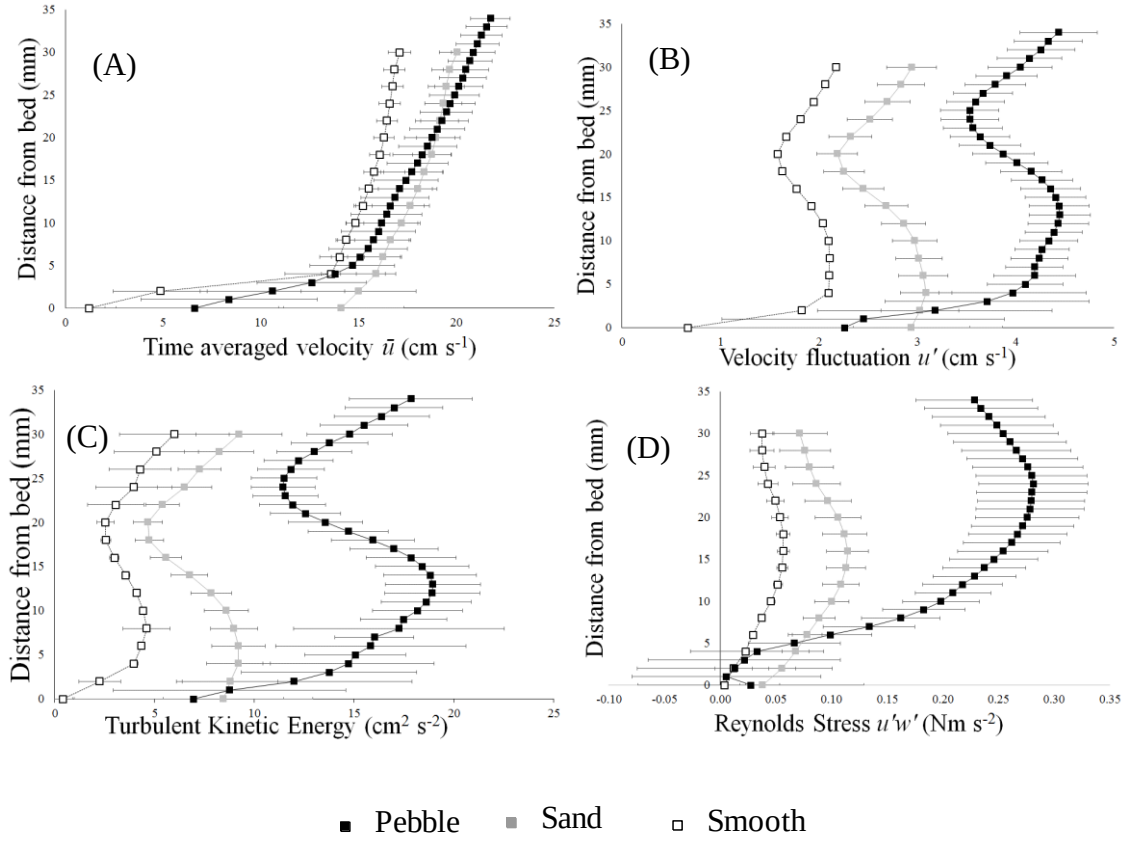

Figure 5.1. Time and spatially averaged (A) velocity, (B) velocity fluctuation, (C) turbulent kinetic energy and (D) Reynolds stress with respect to distance from the roughness top in the stream wise direction for the smooth, sand and gravel bed substrates for a notational area mean velocity 20 cm s$^{-1.}$ Error bars show the standard deviation of the spatial variation.

Parasite screening

Upon completion of all behavioural trials the sticklebacks were dissected and screened for parasites. The sticklebacks were dispatched individually using an overdose of tricaine methane sulphonate (MS222) and pithed. Dissection took place under a low power dissection microscope with a fibre optic light source. The skin and fins were screened externally for ectoparasites, then the body cavity opened and all organs (gills, intestinal tract, stomach, gall bladder, swim bladder, liver, kidneys, urinary bladder, heart, gonads, eyes and body cavity) removed and dissected in separated petri dishes filled with dechlorinated water to screen for endoparasites. Additionally the stickleback weight (W) and standard length (SL) were measured to calculate Fulton's condition factor, *K*, calculated as $100(W/SL^3)$. Sex of the fish was also determined from observation of the gonads.

Data analysis

All data analysis was conducted using R statistical interface (R Core Team 2013).

To test the repeatability of the performance tests, the correlation between the individual repeat swimming performances were assessed. The correlation between the critical swimming speed for the individuals' aerobic swimming performance with the smooth, coarse sand and gravel bed types were assessed using linear regression analysis. This tested whether fish achieving a high critical swimming speed with the smooth bed also performed well on the coarse sand and gravel bed substrates.

Factors influencing the critical swimming speed and time spent near the bed boundary layer in the stickleback were assessed using a General Linear Mixed Models (GLMM) with Gaussian family and identity link functions. Fish number was included as a random term to account for repeated measures where the same fish were used for different tests. The fixed terms for the critical swimming speed GLMM included substrate type, parasite species intensity, host condition, host sex, trial number and time spent near the flume bed. Interaction terms included were *K* and parasite species intensities; and substrate type and time near the flume bed. The fixed terms in the time spent near the bed GLMM included trial number, substrate type, *K*, host sex, parasite species intensities and interactions between *K* and parasite species intensities. Model selection and model averaging were based on corrected Akaike Information Criterion (AICc) using the lme4 library (Bates et al. 2013a). The fixed and random effects were included in global GLMMs with all plausible explanatory variables and interactions. The variables were then standardised using the arm R library (Gelman and Su 2013) and the most important explanatory variables determined using model averaging of the top models with delta AICc<2.5 using the MuMIn library (Barton 2013) as described by Grueber et al. (2011). The relevel function was used to select different substrate types as the 'baseline' level to compare against the other levels.

Results

Sticklebacks reached a mean critical swimming speed of 21.52 cm s^{-1} (SE 0.91) or 4.24 (SE 0.17) SL s^{-1}. There were significant correlations between the critical swimming speed of the sticklebacks on the three different substrate types (gravel versus sand F=8.452, DF=1, 17, p=0.010; gravel versus smooth F=7.982, DF=1, 17, p=0.012, sand versus smooth F=6.15, DF=1,17, p=0.024) indicating that this method is repeatable and those fish which reached higher critical swimming speeds, did so on all substrate types. The order of trials had no effect on the critical swimming speed of the fish, with no change in swimming speed between the first, second and third swim performance test. Fish condition factor and sex also had no effect on the aerobic swimming performance.

The total time spent within the 30 mm above the bed mean roughness height of the open channel flume for each swimming performance test was an important and significant explanatory variable in explaining the variation in critical swimming speed. For each swimming performance test the more time spent near the bed, the higher the critical swimming speed achieved for all bed substrate types (Table 5.2, Figure 5.2). There was no difference in *Ucrit* between the three bed types.

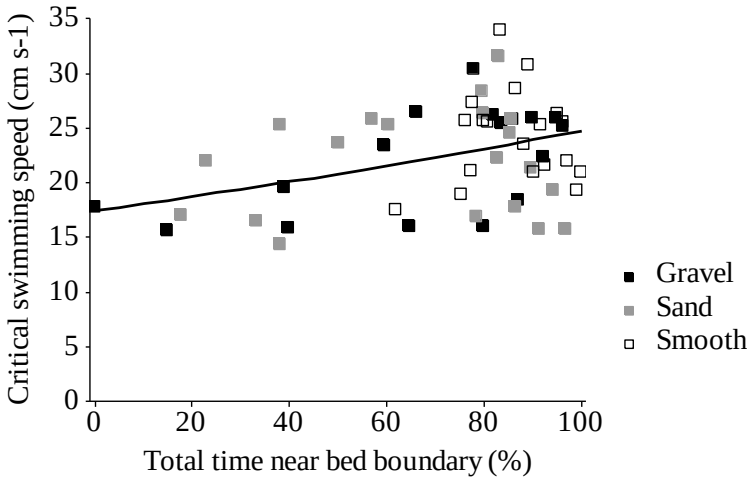

Figure 5.2. Increase in critical swimming speed with increasing total time spent within 30 mm of bed roughness top in an open channel flume for all three substrate types. Line shows predictions from top model.

Fish spent significantly more time near the bed boundary layer with the smooth substrate compared to the sand and gravel (Table 5.2, Figure 5.3). There was no difference in the time spent near the gravel and sand beds. As the flow velocity increased, fish spent less time near the bed for the smooth (GLM, t=-10.98, p<0.01), sand (GLM, t=-8.036, p<0.01) and gravel (GLM, t=-6.012, p<0.01) bed substrate types (Figure 5.3).

Table 5.2. Summary of averaged model predictors. Variables with relative importance of 0.0 not included. Semicolons indicate interactions. Variables shaded have confidence intervals which do not bound zero and therefore considered significant. SE = standard error, K= condition factor.

Dependent Variable	Predictor	Estimate	SE	95% Confidence intervals	Relative importance
Critical swimming speed (cm s^{-1})	Intercept	3.162	0.031	3.088 – 3.237	
	Time near bed	0.157	0.046	0.067 – 0.246	1.00
	Ichthyophthirius multifiliis cysts	-0.112	0.074	-0.258 – 0.0322	0.15
Time near bed (%)	Intercept	73.690	10.089	53.137 – 94.243	
	Sex	0.363	14.347	-27.756 – 28.482	1.00
	Substrate – Sand	-17.761	6.894	-31.274 - -4.248	1.00
	Substrate - Gravel	-16.044	7.884	-31.497 - -0.591	1.00
	Diplostomum sp.	30.397	22.069	-12.857 – 73.650	1.00
	Glugea spp. cysts	-9.091	17.626	-43.637 – 25.456	1.00
	K	-19.389	21.584	-61.962 – 22.915	1.00
	Trial	-10.424	6.344	-22.858 – 2.009	0.89
	I. multifiliis cysts	-36.154	18.712	-72.830 – 0.521	1.00
	K: *Diplostomum* spp.	22.768	29.495	-35.041 – 80.576	0.93
	K: *Glugea* spp. cysts	-60.317	97.130	-250.688 – 130.054	1.00
	K: *I. multifiliis* cysts	-66.974	42.769	-150.800 – 16.851	1.00

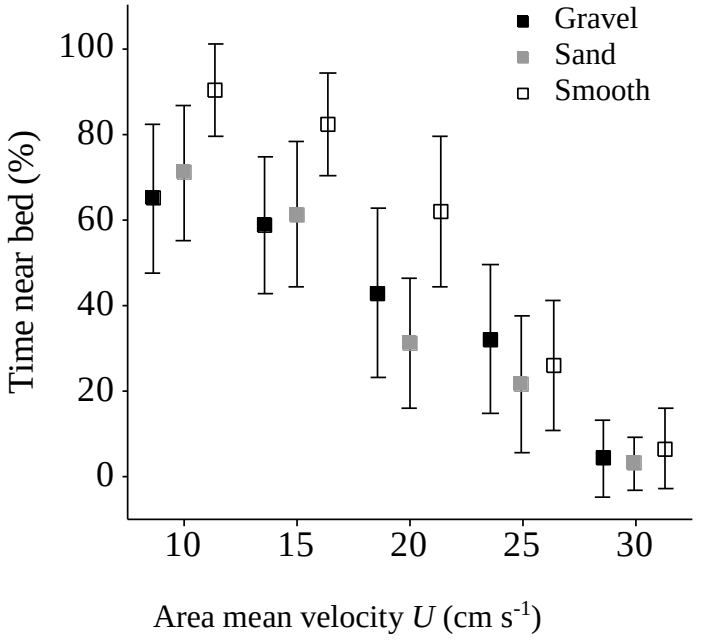

Figure 5.3. Decrease in time that three-spined sticklebacks spent in the 30 mm above the bed roughness as flow velocity increased for three different bed substrates; gravel (black squares), sand (grey squares) and smooth (open squares). Squares show mean values and bars show 95% confidence intervals.

The sticklebacks were naturally infected with 4 parasite taxa (Table 5.3). Individual parasite species intensity did not affect the critical swimming speed achieved by the sticklebacks nor the time spent near the bed for any of the 3 substrates (Table 5.2).

Table 5.3. Prevalence, mean intensity and range of macroparasites in three-spined sticklebacks *Gasterosteus aculeatus* (n=21) from Roath Brook, Cardiff in June 2013.

Parasite species	Location	Prevalence	Mean intensity	Range
Microsporidia: *Glugea* sp.	Skin	57.1%	1.8	1 - 4
Protozoa: *Ichthyophthirius multifiliis*	Skin	76.2%	90.5	1 - 343
Monogenea: *Gyrodactylus gasterostei*	Skin	4.8%	1.0	1
Digenea: *Diplostomum* spp.	Eye	85.7%	5.7	1 - 13

Discussion

An open channel flume with three bed types was used to measure the effects of varying bed boundary conditions on the critical swimming performance (*Ucrit*) of the three-spined stickleback. The stickleback had a mean *Ucrit* of 21.52 cm s^{-1} (4.24 SL s^{-1}), which is comparable to the value of 19.5 cm s^{-1} (3.5 SL s^{-1}) measured by Schaarschmidt and Jürss (2003) but lower than the 43.52 cm s^{-1} (8 SL s^{-1}) recorded by Tudorache et al. (2008). Higher critical swimming speeds were achieved if the fish spent more time swimming near the bed. The sticklebacks spent more time near the bed boundary layer above the smooth bed compared to the gravel and coarse sand beds, although there was no difference in *Ucrit* between the different bed types. As the flow velocity was increased, sticklebacks spent less time near the bed boundary for all substrate types.

Aerobic swimming performance tests commonly do not take into consideration bed boundary effects and are designed such that natural movement by the fish is restricted (Webb 1993; Peake 2004; Haro et al. 2004; Russon and Kemp 2011). In the current study, the fish which spent more time swimming in the 30 mm layer immediately above the bed roughness achieved higher critical swimming speeds. This indicates that the fish are able to utilise the boundary conditions in order to shelter from the free stream velocities and therefore tolerate higher area mean velocities. A similar effect was previously observed by Carlson and Lauder (2011) in darters, Hoover (2011) in sturgeon and by Gerstner (1998) in Atlantic cod.

The sticklebacks spent significantly more time near to the bed boundary with the smooth bed compared to the gravel bed substrate. From the ADV measurements at a notational area mean velocity of 20 cm s^{-1}, the gravel bed was the most turbulent, with the highest time-average velocity fluctuation *u'*, turbulent kinetic energy *k* and Reynolds stress

(Figure 5.1). The sand bed had intermediate turbulence levels, with similar vertical distribution patterns of turbulence as the smooth bed (Figure 5.1). The gravel bed has the thickest boundary layer. This is shown by the gradient of the velocity profile in Figure 5.1A, where the rate of change in velocity with distance from the bed is lower compared to the sand and smooth beds.

The bed boundary conditions would therefore be much more favourable for fish swimming compared to the turbulent and high velocity shear conditions generated by the gravel bed. As the flow velocity was increased in stepwise increments in the *Ucrit* test, the fish spent less time associating with the bed boundary layer for all bed types. It is likely that this is due to the bed boundary layer reducing in thickness and increasing further in turbulence intensity due to the increased shear layer between the bed roughness and free stream (Denny 1993).

There have been several studies describing how single species parasite infections affect the swimming performance of fish. Coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*) exposed to the trematode *Nanophyetus salmincola* had reduced swimming speeds compared to uninfected controls (Butler and Millemann 1971). Atlantic salmon (*Salmo salar*) had significantly lower critical swimming speeds when infected with high levels (0.13 g^{-1}) of sea lice *Lepeophtheirus salmonis* compared to low infections (0.02 g^{-1}) and uninfected individuals (Wagner et al. 2003). Natural parasite infections of *Myxobolus arcticus* reduced the critical swimming speed of sockeye salmon (*Oncorhynchus nerka*) from 4.37 body length s^{-1} when uninfected to 2.89 body length s^{-1} when infected (Moles and Heifetz 1998). However, in this current study, the four parasite species infecting the stickleback had no effect on the host critical swimming speed. This was similarly observed in Chapter 4 where there was little effect of parasites on the burst swimming performance of sticklebacks.

With global increase in population levels, the increased reliance on rivers for water abstraction has led to modifications of watercourses and fragmentation of fish habitat. Subsequent conservation measures have been put in place to allow migrant and resident fish species to move within the river systems through fish passes, fishways and culverts. These often rely on data obtained from laboratory studies which measure swimming performance of the target species (Peake 2004). The current study used one such swimming performance measure, the critical swimming test, to investigate how three-

spined sticklebacks interact with different bed substrates to enhance swimming performance. With increased time spent swimming near the bed, the fish were able to station hold against higher area mean flow velocities, suggesting that the bed boundary is an important consideration in fish passage design. The size of the fish in relation to the boundary layer thickness might also be relevant for design purposes. The fish spent less time associating with the gravel and coarse sand bed compared to the smooth bed, likely due to the increased turbulence generated in the near-bed boundary by these bed types. Again, this is important for the design of fish passage as gravel river beds are more akin to conditions encountered in the wild compared to the smooth bed of a laboratory swimming chamber. As flow velocity was increased, the fish spent less time near the bed substrate, likely due to the decrease in boundary layer height, increased shear and bed generated turbulence. It is therefore important that fish passage design considers the bed type and associated turbulence generated by the bed to provide optimum conditions for fish migration.

References

Barber I, Walker P, Svensson PA (2004) Behavioural responses to simulated avian predation in female three-spined sticklebacks: The effect of experimental *Schistocaphalus solidus* infections. Behaviour 141:1425–1440

Barbin GP, Krueger WH (1994) Behaviour and swimming performance of elvers of the American eel, *Anguilla rostrata*, in an experimental flume. J Fish Biol 45:111–121

Barton K (2013) MuMIn: Multi-model inference. R package version 1.9.13. http://CRAN.R-project.org/package=MuMIn

Bates D, Maechler M, Bolker B, Walker S (2013) lme4: Linear mixed effects models using Eigen and S4. R package version 1.0-5. http://cran.r-project.org/package=lme4

Bell MA, Foster SA (1994) The Evolutionary Biology of the Threespine Stickleback. Oxford University Press, Oxford

Blake RW, Kwok PYL, Chan KHS (2006) Effects of two parasites, *Schistocephalus solidus* (Cestoda) and *Bunodera* spp. (Trematoda), on the escape fast-start performance of three-spined sticklebacks. J Fish Biol 69:1345–1355

Blake RW, Law TC, Chan KHS, Li JFZ (2005) Comparison of the prolonged swimming performances of closely related, morphologically distinct three-spined sticklebacks *Gasterosteus* spp. J Fish Biol 67:834–848

Brett JR (1964) The respiratory metabolism and swimming performance of young sockeye salmon. J Fish Res Board Canada 21:1183–1226

Butler JA, Millemann RE (1971) Effect of the "salmon poisoning" trematode, *Nanophyetus salmincola*, on the swimming ability of juvenile salmonid fishes. J Parasitol 57:860–865

Carlson RL, Lauder GV (2011) Escaping the flow: boundary layer use by the darter *Etheostoma tetrazonum* (Percidae) during benthic station holding. J Exp Biol 214:1181-1193

Denny MW (1993) Air and Water: The Biology and Physics of Life's Media. Princeton University Press, Princeton

Enders EC, Scruton DA, Clarke KD (2009) The 'Natural Flow Paradigm' and Atlantic salmon - moving from concept to practice. River Res Applic 25:2–15

Floury M, Usseglio-Polatera P, Ferreol M, DeLattre C, Souchon Y (2013) Global climate change in large European rivers: long-term effects on macroinvertebrate communities and potential local confounding factors. Glob Chang Biol 19:1085–1099

Garenc C, Couture P, Laflamme M-A, Guderley H (1999) Metabolic correlates of burst swimming capacity of juvenile and adult threespine stickleback (*Gasterosteus aculeatus*). J Comp Physiol B 169:113–122

Gelman A, Su Y-S (2013) arm: Data analysis using regression and multilevel/hierarchical models. R package version 1.6-10. http://cran.r-project.org/package=arm

Gerstner CL (1998) Use of substratum ripples for flow refuging by Atlantic cod, *Gadus morhua*. Environ Biol Fishes 51:455–460

Grueber CE, Nakagawa S, Laws RJ, Jamieson IG (2011) Multimodel inference in ecology and evolution: challenges and solutions. J Evol Biol 24:699–711

Haro A, Castro-Santos T, Noreika J, Odeh M (2004) Swimming performance of upstream migrant fishes in open-channel flow: a new approach to predicting passage through velocity barriers. Can J Fish Aquat Sci 61:1590–1601

Hoover JJ, Collins J, Boysen KA, Katzenmeyer AW, Kilgore KJ (2011) Critical swimming speeds of adult shovelnose sturgeon in rectilinear and boundary-layer flow. J Appl Ichthyol 27:226–230

Jesson M, Bridgeman J, Sterling M (2013a) An experimental study of turbulence in a heterogeneous channel. Water Manag 166:16–26

Jesson M, Sterling M, Bridgeman J (2013b) Despiking velocity time-series - Optimisation through the combination of spike detection and replacement methods. Flow Meas Instrum 30:45–51

Jowett IG, Richardon J, Bonnett ML (2005) Relationship between flow regime and fish abundances in a gravel-bed river, New Zealand. J Fish Biol 66:1419–1436

Katsiadaki I (2007) The use of the stickleback as a sentinel and model species in exotoxicology. In: Ostlund-Nilsson S, Mayer I, Huntingford FA (eds) Biology of the Three-Spined Stickleback. CRC Press, Taylor & Francis Group, 353–372

Knaepkens G, Baekelandt K, Eens M (2006) Fish pass effectiveness for bullhead (*Cottus gobio*), perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) in a regulated lowland river. Ecol Freshw Fish 15:20–29

Marras S, Killen SS, Domenici P, Claireaux G, McKenzie DJ (2013) Relationships among traits of aerobic and anaerobic swimming performance in individual European sea bass *Dicentrarchus labrax*. PLoS One 8:e72815

Massey B, Ward-Smith J (2006) Boundary layers, wakes and other shear layers. Mechanics of Fluids. Taylor & Francis, pp 298–360

Moles A, Heifetz J (1998) Effects of the brain parasite *Myxobolus arcticus* on sockeye salmon. J Fish Biol 52:146–151

Nikora VI, Aberle J, Biggs BJF, Jowett IG, Sykes JRE (2003) Effects of fish size, time-to-fatigue and turbulence on swimming performance: a case study of *Galaxias maculatus*. J Fish Biol 63:1365–1382

Nikuradse J (1950) Laws of flow in rough pipes. National Advisory Committee for Aeronautics. Technical memorandum 1292. Washington

Peake S (2004) An evaluation of the use of critical swimming speed for determination of culvert water velocity criteria for smallmouth bass. Trans Am Fish Soc 133:1472–1479

Plaut I (2001) Critical swimming speed: its ecological relevance. Comp Biochem Physiol Part A 131:41–50

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Russon IJ, Kemp PS (2011) Experimental quantification of the swimming performance and behaviour of spawning run river lamprey *Lampetra fluviatilis* and European eel *Anguilla anguilla*. J Fish Biol 78:1965–1975

Schaarschmidt TH, Jürss K (2003) Locomotory capacity of Baltic Sea and freshwater populations of the threespine stickleback (*Gasterosteus aculeatus*). Comp Biochem Physiol Part A 135:411-424

Taylor EB, McPhail JD (1986) Prolonged and burst swimming in anadromous and freshwater threespine stickleback, *Gasterosteus aculeatus*. Can J Zool 64:416–420

Tharme RE (2003) A global perspective on environmental flow assessment: emerging trends in the development and application of environmental flow methodologies for rivers. River Res Applic 19:397–441

Tierney JF, Huntingford FA, Crompton DWT (1993) The relationship between infectivity of *Schistocephalus solidus* (Cestoda) and anti-predator behaviour of its intermediate host, the three-spined stickleback, *Gasterosteus aculeatus*. Anim Behav 46:603–605

Tierney KB (2011) Swimming performance assessment in fishes. J Vis Exp 51:e2572

Tudorache C, Blust R, De Boeck G (2007) Swimming capacity and energetics of migrating and non-migrating morphs of three-spined stickleback *Gasterosteus aculeatus* L. and their ecological implications. J Fish Biol 71:1448–1456

Wagner GN, McKinley RS, Bjørn PA, Finstad B (2003) Physiological impact of sea lice on swimming performance of Atlantic salmon. J Fish Biol 62:1000–1009

Webb PW (1993) The effect of solid and porous channel walls on steady swimming of steelhead trout *Oncorhynchus mykiss*. J Exp Biol 178:97–108

Chapter 6. Fish responses to flow velocity and turbulence in relation to size, sex and parasite load

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This chapter is adapted from the published article in the Journal of the Royal Society Interface (Hockley FA, Wilson CAME, Brew A, Cable J (2014) Fish responses to flow velocity and turbulence in relation to size, sex and parasite load. J R Soc Interface 11:20130814).

Abstract

Riverine fish are subject to heterogeneous flow velocities and turbulence and may use this to their advantage by selecting regions that balance energy expenditure for station holding whilst maximising energy gain through feeding opportunities. This study investigated microhabitat selection by guppies (*Poecilia reticulata*) in terms of flow characteristics generated by hemisphere boulders in an open channel flume. Velocity and turbulence influenced the variation in swimming behaviour with respect to size, sex and parasite intensity. With increasing body length, fish swam further and more frequently between boulder regions. Larger guppies spent more time in the areas of high velocity and low turbulence beside the boulders, whereas smaller guppies frequented the low velocity and high turbulence regions directly behind the boulders. Male guppies selected the regions of low velocity, indicating possible reduced swimming ability due to hydrodynamic drag imposed by their fins. With increasing *Gyrodactylus turnbulli* burden, fish spent more time in regions with moderate velocity and lowest turbulent kinetic energy which were the most spatially and temporally homogeneous in terms of velocity and turbulence. These findings highlight the importance of heterogeneous flow conditions in river channel design due to the behavioural variability within a species in response to velocity and turbulence.

Introduction

Rivers differ from oceanic and estuarine habitats in that flow is primarily in the longitudinal direction and particularly subject to disturbance events such as flooding as a result of heavy rainfall. Natural and man-made structures such as boulders, woody debris and bridge piers create physical obstructions to the water flow and generate localised regions where velocity magnitudes and turbulence levels are spatially heterogeneous.

Ecological theory suggests that habitat heterogeneity is positively related to fish species diversity, with flow regime strongly influencing patterns of global-scale species richness (Guégan et al. 1998). Placement of in-stream structures such as boulders and woody debris to improve connectivity and complexity has been increasingly employed in river rehabilitation programmes (Branco et al. 2013). Improvement in fish species richness and abundance as a result of restoration projects is variable, with increased densities reported for some species, but not for others, depending on species habitat requirements (Roni et al. 2006). For example, the placement of boulders in watersheds in southwest Oregon caused an increase in the number of pools, providing more suitable habitat for coho salmon (*Oncorhynchus kisutch*) and trout (*O. mykiss*), but saw a decrease in dace (*Rhinichthys* spp.) and no change in abundance of young-of-year trout (Roni et al. 2006). Thus, successful restoration projects need to be both species- and site specific (Branco et al. 2013).

Stationary obstacles such as a boulders or coarse river beds generate turbulence which can lead to the development of vortices. Turbulence and vortices can be either beneficial or detrimental to fish, depending on the directionality and strength of each velocity component, their temporal fluctuation, the turbulent stresses imposed and the ability of the fish to maintain stability (Liao 2007). The capability of fish to maintain position in a current relative to the substratum (station holding) (Gerstner 1998) is an essential survival strategy to avoid being washed downstream and may limit individuals or species to specific areas of the river (Garner 1997). When station holding, stream dwelling fish orientate themselves upstream (positive rheotaxia) in order to minimise energy expenditure, maximise food capture and intercept chemical cues (Hughes and Dill 1990; Northcutt 1997) by detecting water currents with neuromasts on their lateral line (Northcutt 1997; Voigt et al. 2000). The size of the vortices (turbulence length scale) in relation to fish length is critical when considering the effect of perturbed flow on swimming performance (Lupandin 2005; Lacey et al. 2011). If a vortex generated from a stationary body is small relative to the body size of the fish, then the moments of force are evenly distributed across its body and balance is not affected. If the size of the vortex is similar to the size of the fish, then the rotating vortex will introduce a torque and the fish may overturn (Nikora et al. 2003; Lupandin 2005). Lupandin (2005) concluded that perch (*Perca fluviatilis*) swimming performance decreased when the turbulence length

scale exceeded two thirds of the fish length, but this has never been tested on other fish species.

Fish will avoid an environment where there are large fluctuations in temporal velocity (an indication of the turbulence level). In turbulent flows water particles move irregularly causing a continuous exchange of momentum from one portion of the water to another and this momentum exchange can cause turbulent shear stress across the fish's body. This stress can have a negative effect on fish, for example individuals of the Iberian barbel (*Luciobarbus bocagei*) avoided areas of high horizontal shear stress (Silva et al. 2011). In extreme cases, such as in hydroelectric dams, the rotating turbine can generate turbulent shear stresses which can cause injury or mortality (Nietzel et al. 2000; Čada 2001) which will vary between species and size categories (Neitzel and Dauble 2004). Additionally, turbulent shear stress, velocity shear and velocity magnitude are negatively correlated with abundance, taxa richness and community composition of macroinvertebrates (Brooks et al. 2005), potential prey for fish. On the other hand, fish may use turbulence to their advantage with some species capable of capturing energy from vortices to propel themselves upstream or station hold (Hinch and Rand 2000). For example, at intermediate area mean velocities of 25-50 cm s^{-1} chub (*Nocomis micropogon*) harnessed the energy from vortices generated by vertical cylinders to maintain position within the water column (Hinch and Rand 2000). However at lower area mean velocities (<25 cm s^{-1}), the chub avoided swimming behind the cylinders and at higher area mean velocities (50-75 cm s^{-1}) they were displaced from their positions on the cylinder and forced to swim in the adjacent free stream (Webb 1998). Numerous studies have used experimental flumes in the laboratory to examine the critical swimming speeds and optimal longitudinal velocities ($\bar{u}$) of fish both in the laboratory and field (Everest and Chapman 1972; Nicoletto 1991; Aadland 1993; Heggenes et al. 1996; Davey et al. 2011). However, very few studies have investigated the effects of turbulence parameters (vortices, turbulent kinetic energy and turbulent shear stress) in relation to fish behaviour on a microhabitat scale (Silva et al. 2011). Differences in behaviour within a species in response to these turbulence parameters might explain why some restoration processes using boulders have been a success whilst others have seen little benefit.

Biotic factors also influence fish swimming ability, for instance fin size. Wild-type zebrafish (*Danio rerio*) had significantly higher critical swimming speed than long-tailed

varieties, due to the drag effect of the larger fins (Plaut 2000). Large and colourful fins in male fish is common in nature and the evolution of this exaggerated ornamentation is driven by sexual, rather than natural selection (Meyer 1997). In guppies (*Poecilia reticulata*), females select males with larger tails, as an indicator of reproductive fitness (Bischoff et al. 1985), yet males with longer tails exhibit poorer swimming performance (Karino et al. 2006), indicating a trade-off between swimming performance (natural selection) and courtship success (sexual selection). Additionally, infectious disease may impair swimming ability by causing symptoms such as atrophy of musculature, nervous system pathology, obstruction of blood flow and physiological interference from parasite waste products (Barber et al. 2000). Heavy infections may also reduce a host's physical agility or modify the shape and size of the fish, affecting its profile in the water and thus generating increased hydrodynamic drag (Barber et al. 2000). Few studies have attempted to examine the impact of infection on fish behaviour within a flow environment, with the majority of experiments being conducted in tanks of static water (Lopez 1998; Kolluru et al. 2008; Richards et al. 2010). To our knowledge no previous study has quantified how turbulence levels impact on infected fish. Generally little is known about the potential implications of habitat heterogeneity on host-parasite interactions in aquatic systems. On one hand, differences in swimming ability, different life stages, or sexual segregation may cause spatial repartition of individuals within a population, thus affecting parasite transmission. On the other hand, parasite infection may cause a change in fish behaviour or reduction in swimming ability thus affecting microhabitat use within the river system.

Guppies and their natural parasites *Gyrodactylus* spp. are popular host-parasite model organisms for use in ecological, genetic and behavioural studies (Cable 2011; Fraser et al. 2011). This is partly due to the fact that guppies are highly sexually dimorphic, with males being smaller in size with ornate dorsal and caudal fins. These larger caudal fins have been associated with a reduced swimming ability (Plaut 2000; Karino et al. 2006; Karino et al. 2011) and compared to females, males exhibit lower critical swimming speeds and have an affiliation for slower water velocities in the wild (Croft et al. 2004; Karino et al. 2006). *Gyrodactylus turnbulli* is an ectoparasitic monogenean which naturally infects guppies in Trinidad and Tobago (Cable 2011). The parasite causes behavioural changes, for example by inducing erratic swimming behaviour (Cable et al. 2002) and in the later stages of infection the host fins become contracted and the fin rays fuse together (Cable et al. 2002).

The aim of this study was to assess how swimming behaviour of fish is affected by velocity and turbulence characteristics in a heterogeneous flow field generated by hemispherical boulders. Using open channel flume experiments, we explored intra-species variation in swimming behaviour of guppies *P. reticulata* between different flow regions with respect to size, sex and parasite load. The flow regions around the boulders were characterised in terms of their spatial variation in velocity, turbulence and turbulent shear stress through relatively high frequency measurements of velocity. The interaction of the fish within the habitat was examined in terms of the frequency of movement both in the near field locality of the boulder and further afield between upstream and downstream boulders. We hypothesised that turbulence and velocities generated by the boulders act as niche habitats for the fish to enhance station holding and that large, female and unparasitised fish would have stronger swimming abilities and therefore tolerate regions of higher velocity and turbulence and higher spatial variability, compared to their smaller, larger-finned and parasitised counterparts.

Methods

Open channel flume and velocity measurements

The study took place in a glass walled recirculating open channel flume in the Hydro-environmental Research Centre (HRC), Cardiff University, UK. The flume was 10 m long and 0.29 m wide and the surface water profile controlled by a downstream tailgate weir. The flume was set at a negative gradient of 1 in 1000 and concrete quasi-hemisphere boulders of diameter 150 mm and height 75 mm were positioned at 0.5 m intervals along the centreline of the flume bed (Figure 6.1). Uniform flow conditions were established at a discharge (Q) of 0.0049 $m^3 s^{-1}$ and flow depth of 135.7 mm, which gives an average pore velocity (taking into account the boulder area) of 12.7 cm s^{-1}. The selected flow velocity is typical of natural conditions in guppy streams in Trinidad (Reznick et al. 2001). The Reynolds number, based on the hydraulic radius, was 37,062, which relates to a turbulent flow regime (Douglas et al. 2005). Chlorides were removed from the water by the addition of Haloex at 0.02 ml L^{-1} and water was heated to 25 ± 1°C using an Electro Titanium Digital heater.

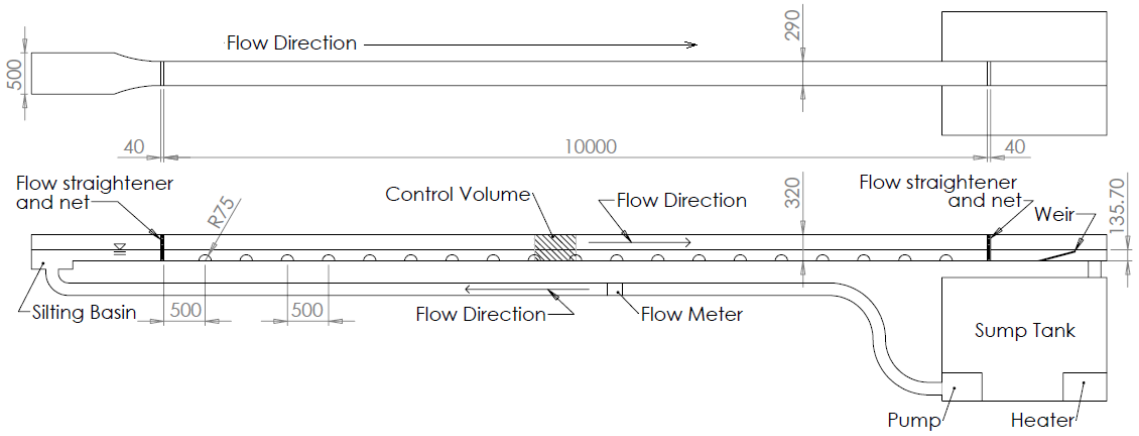

Figure 6.1. Plan and side view schematic showing positioning of boulders along length of open channel flume. The control volume where velocity and turbulence were measured is shown by the diagonal hatched area. All dimensions are in mm.

The velocity and turbulence field around the hemispheres was characterised using a Nortek Vectrino I downwards-looking acoustic Doppler velocimeter (ADV). The water was seeded using Q-Cel® hollow microspheres to increase the signal to noise ratio (SNR) in order to produce sufficient sound scatter (Nortek 2009). The velocity measurement grid was generated with point velocity measurements taken at 10-20 mm intervals within a representative control volume of 0.5 m length (longitudinal direction), 0.29 m width (transverse direction) and 0.06 m depth (vertical direction) located at the mid-length of the flume. The measurement grid was composed of 1112 point measurements and captured the wake immediately behind the boulder and the high pressure region immediately upstream of the boulder. Higher densities of velocity measurements were taken in the vicinity of the boulder where velocity gradients were steepest. All readings were taken at a sampling rate of 200 Hz for 4 min with a nominal velocity range of +/- 0.3 m s^{-1}, transmit length of 1.8 mm and sampling volume of 7 mm height and 6 mm diameter. The time-averaged velocity and turbulence statistics for each reading were calculated using the WinADV software (Wahl 2011) after filtering with a minimum correlation of 70% and minimum SNR of 20 as recommended by Rusello et al. (2006).

Velocity and Turbulence Definitions

The time-averaged point velocities in the longitudinal, transverse and vertical directions are denoted as $\bar{u}$, $\bar{v}$ and $\bar{w}$ respectively. The instantaneous turbulent fluctuation of the longitudinal velocity from the time mean velocity is:

$$u'(t) = u(t) - \bar{u}$$

The turbulence strength is defined as:

$$u_{rms} = \sqrt{\overline{u'(t)^2}}$$

where 'rms' is the root-mean-square. Similar definitions apply to the transverse and vertical velocities, $v(t)$ and $w(t)$. The turbulent kinetic energy (k) which is a measure of the total turbulent energy production per unit mass and hence a bulk measure of the turbulence intensity was calculated as:

$$k = 0.5(u_{rms}^2 + v_{rms}^2 + w_{rms}^2)$$

The normalised turbulent kinetic energy is defined by $\sqrt{k}.u^{-1}$ and is used in this study to normalise the turbulence intensity from the velocity magnitude. The turbulent shear stress (Reynolds stress) in each plane was calculated as:

$$\tau_{uv} = \left|\rho\overline{u'v'}\right|;\ \tau_{uw} = \left|\rho\overline{u'w'}\right|;\ \text{and}\ \tau_{vw} = \left|\rho\overline{v'w'}\right|$$

Where ρ is the density of water and $u'v'$, $u'w'$ and $v'w'$ are the covariance of the instantaneous velocity fluctuations. The volume-averaged velocity and turbulence parameters for each flow region are denoted using the square brackets i.e. $\langle\bar{u}\rangle$, $\langle\bar{v}\rangle$, $\langle\bar{w}\rangle$, <k> and $\langle\sqrt{k}\ \bar{u}^{-1}\rangle$ etc. The ratio of volume-averaged longitudinal, transverse and vertical turbulent length scale to fish standard length of an individual fish is given by $\langle l_u\rangle$/SL, $\langle l_v\rangle$/SL and $\langle l_w\rangle$/SL respectively. The turbulent length scale was calculated using the autocorrelation function (Pope 2000) where the longitudinal turbulent length scale l_u is given by:

$$l_u = \bar{u} \int_0^T R(t)dt$$

where T is the sampling time and R is the autocorrelation function defined as:

$$R(t) = \frac{\overline{u\prime(t).u\prime(t+s)}}{\overline{u\prime(t)^2}}$$

where s is the time lag in seconds. The transverse and vertical turbulent length scales (l_v and l_w) were calculated in a similar manner.

Region Characterisation

From the depth-averaged velocity field, each boulder control volume was divided into four regions (Figure 6.2). The areas where the flow accelerates around the sides of the boulder are referred to as the high velocity regions (region H) and the regions downstream where the flow decelerates and recovers are referred to as the 'moderate velocity region' (region M). A recirculation zone lies directly in the wake of the boulder (region R) and a region of low velocity in the boulder wake forms the velocity deficit region (region L). A summary of the volume-averaged data are given in Table 6.1.

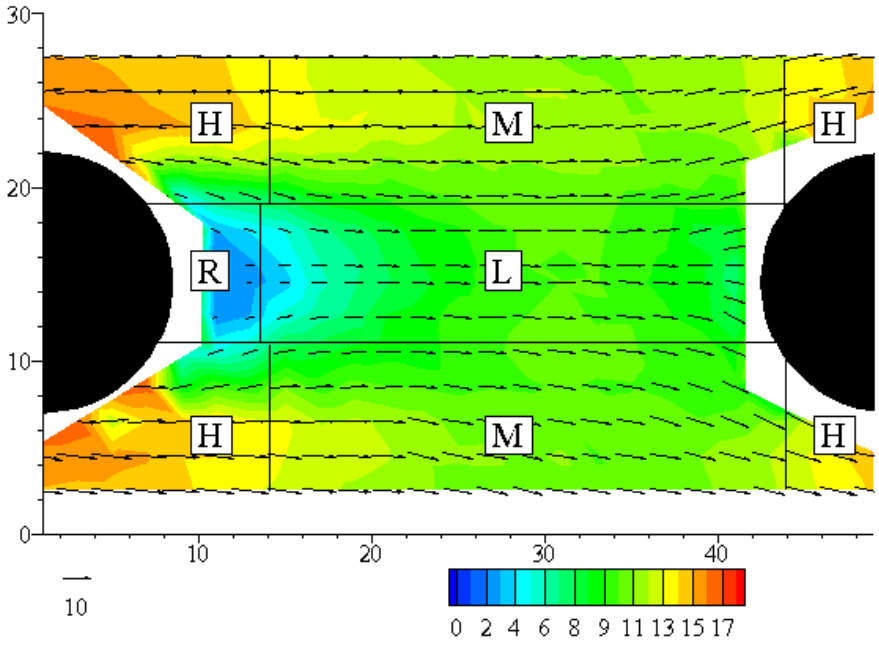

Figure 6.2. Flow regions around boulders defined by depth- and time-averaged longitudinal velocity $\bar{u}$. (H) High velocity region where there is an acceleration of flow between the side walls and the boulder, (M) moderate velocity region where the flow from region H decelerates, (R) the Recirculation zone and (L) the velocity deficit zone in the wake of the boulder (solid black areas). The white areas around the boulders are the limits of the ADV probe so measurements were not taken in these areas. Velocity units are in cm s^{-1} and length units are in cm.

Study System

Guppies (*Poecilia reticulata*) imported from the lower Aripo River, Northern Trinidad in 2003, were maintained in aquarium facilities at the School of Biosciences, Cardiff

University, UK. At the time and place of sampling, the lower Aripo River had width 481 cm, depth 17 cm and surface flow velocity approximately 8.5 cm s^{-1} (measured by the mean time for a plastic float to travel 100 cm). The lower Aripo River site is known to be a high predation site (Margurran and Seghers 1990). Holding tanks each had an air supply and filter and fish were maintained under a 12 h light: 12 h dark regime at 22 ± 1°C, fed on a diet of fish flakes (Aquarian®) and bloodworm. A total of 60 female (mean ± S.D. standard length 21.3 ± 3.5 mm) and 51 male guppies (mean ± S.D. standard length 16.2 ± 1.3 mm) were used in the behavioural experiments. The standard length (L_s) to fork length and L_s to dorsal fin length ratios were 25% and 36% larger in males than females respectively (t-test, t=-8.29 and -8.45, df=50 and 38, both P<0.001) from a subsample of 60 individuals.

An isogenic strain of the ectoparasitic worm *Gyrodactylus turnbulli* (Gt3) was used to infect 30 females and 27 males with four worms per individual following standard procedures e.g. (Richards et al. 2010). The remaining 30 female and 24 male guppies were sham-infected under anaesthetic without exposure to parasites. All fish were housed in individual 1 L pots and the infections developed for 8 days. Infection was confirmed by restraining each individual in a small amount of water in a crystallizing dish under stereo-microscope. All uninfected fish were sham-screened. After 8 days post-infection, the mean intensity of *G. turnbulli* was 24.8 (SE 2.76) worms. No individuals showed any symptoms of infection such as fin clamping or noticeable reduced mobility.

Experimental trials

The open channel flume behavioural experiments took place on 1st to 27th February 2012 between 8:00 and 19:00. Each fish was given a 30 min acclimatisation period and then observed for 10 min, recording position in relation to the boulders (Figure 6.2), starting with the position of the fish at the end of the acclimatisation period. Frequency of movement was recorded as the number of times the fish moved from one boulder region to another and the distance moved up- and downstream was recorded as the number of movements to a different boulder control volume (Figure 6.1) in each direction. If the fish entered an area within 0.5 m length of the upstream or downstream ends of the flume the timer was paused until the fish returned to the main working section, as these flow areas may be subject to disturbance from the flow straightening material at the upstream end of the flume and the weir at the downstream end. If the fish moved into the top 70 mm

elevation of the flow depth, data was discarded as velocity measurements could not be taken in this region due to the measurement limitations of the downward looking ADV. This resulted in a mean observation period of 4 min 36 s per fish. Parasite infection, host size or sex did not affect the time spent in the top 70 mm of the flow depth (GLM, $P>0.05$). Individual fish was tested only once so the total sample size was 30 infected and 30 uninfected females and 27 infected and 24 uninfected males.

Statistical analysis

All analyses were conducted using R v2.1.0 statistical software (R Core Team 2013).

The total time individual fish spent in each flow region was totalled and corrected the different volumes of each region by dividing time by the region volume (to give units of s cm^{-3}) producing a comparative value for time budget allocation in each different-sized region. Times were converted to percentage of total time occupying each velocity region and arcsine transformed for statistical analysis.

Differences in the time spent in each region for the pooled data was analysed using a linear mixed model (GLMM) with Gaussian distribution using the lmer function in the lme4 package (Bates et al. 2013) followed by Tukey's honestly significant difference (HSD) multiple comparisons using the ghlt function from the multcomp package (Hothorn et al. 2008). The term 'Fish ID' was included in the GLMM as a random effect to account for autocorrelation.

The effects of host standard length, sex, parasite prevalence and intensity on the distance moved up- and down-stream and the frequency of movement between boulder regions were analysed using a generalised linear model (GLM) with negative binomial error distribution and square root link function. The host effects on percent time spent in each flow region analysed using a GLM with inverse-gaussian error distributions and either identity or $1/mu^{\wedge 2}$ link functions. Fish sex and standard length were included in the models as an interaction term to account for size differences of male and female guppies. Parasite intensity and standard length were included as an interaction term to account for any differences in parasite infection between different sized fish. GLMs were refined using Akaike Information Criterion (AIC) values to select the best finishing model.

Results

Microhabitat hydrodynamics

An open channel flume was used to quantify guppy swimming behaviour according to fish size, sex and parasite load in relation to microhabitat variation around hemispherical boulders placed at 0.5 m intervals. Flow was ejected over the boulder crest and there was a strong downwards movement in the lee of the boulder where the vertical velocity reached as high as 3.29 cm s^{-1}, which is 26% of the longitudinal average pore velocity of 12.7 cm s^{-1} (Table 6.1). Whilst it was difficult to precisely interpret the type of coherent flow structure, it was clear that this recirculating region had the highest kinetic energy (Figure 6.3), highest relative turbulence intensity and the strongest shear stresses in the horizontal, longitudinal and vertical planes (Table 6.1). In the recirculating region the flow was highly three-dimensional and the magnitude of the shear stresses in both horizontal and vertical planes was fairly equal. Although the longitudinal velocity was lowest in this region, the spatial and temporal variability of the longitudinal velocity was the greatest. Furthermore, this region had the greatest turbulent shear stresses in all planes and the highest mean vertical turbulent length scale l_w which ranged between 0.01 and 2.77 cm and therefore exceeded the size of the fish in some instances.

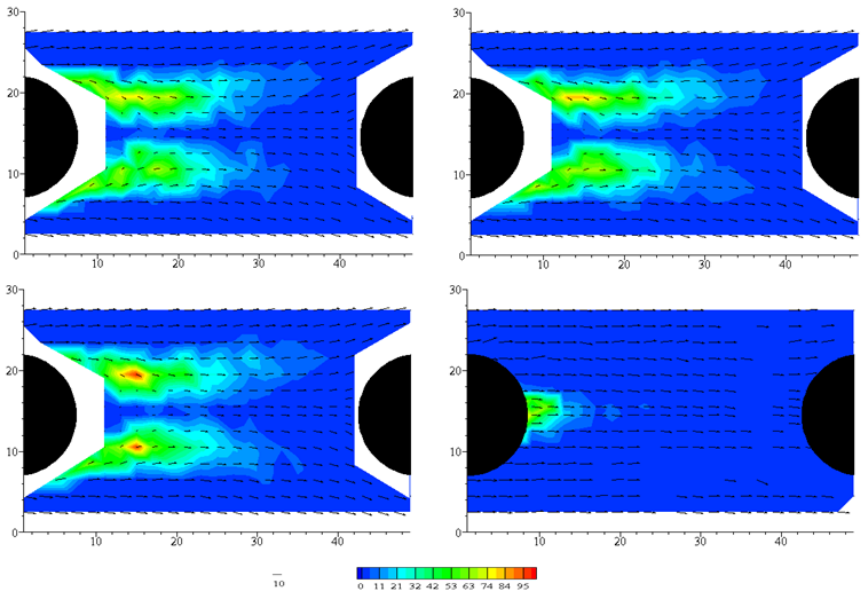

Figure 6.3. Turbulent kinetic energy (*k*) plots of flow field at normalised elevations (*z*/*H*) (a) 0.05, (b) 0.09, (c) 0.12 and (d) 0.85 around boulders in an open channel flume. Flow elevation (z) is normalised by the boulder height (H). Units are given in cm^2 s^{-2}, axis scales are in cm.

Table 6.1.Volume-averaged velocity and turbulence parameters for the four velocity regions depicted in Figure 2. Ranges of the minimum and maximum time-averaged velocity for the longitudinal, transverse and vertical velocity components with the turbulence intensities(u', v' and w'), turbulent kinetic energy (k) the relative turbulence intensity ($\sqrt{k}\bar{u}^{-1}$), turbulent shear stresses (τ_{uv}, τ_{uw} and τ_{vw}) and turbulent length scale (l_u, l_v, l_w) within each flow volume are given. Negative velocities for $\bar{u}$, $\bar{v}$ and $\bar{w}$ refer to upstream, towards the left hand side flume wall (looking in the downstream direction) and downwards movement respectively. The standard deviation (σ) for each volume-averaged parameter is given in brackets.

Parameter		Moderate Velocity Region	High Velocity Region	Velocity Deficit Region	Recirculation Region
<ū> (σ)	(cm s^{-1})	11.67 (1.28)	14.05 (2.26)	9.15 (3.18)	6.46 (6.97)
Range ū		8.87 – 15.07	2.85 – 17.36	0.62 – 17.42	-2.56 – 17.90
<u'> (σ)		2.91 (0.60)	2.74 (0.88)	3.49 (0.60)	4.04 (0.89)
Range u'		1.98 – 4.79	1.88 – 5.78	2.39 – 5.30	2.38 – 5.89
<$\bar{v}$> (σ)	(cm s^{-1})	-0.52 (1.90)	-0.26 (2.22)	-0.47 (1.29)	-0.38 (1.25)
Range $\bar{v}$		-5.90 – 5.34	-7.07 – 7.78	-4.36 – 5.98	-3.13 – 2.93
<v'>(σ)		2.98 (1.64)	2.96 (1.23)	3.90 (1.15)	4.11 (1.16)
Range v'		1.80 – 18.63	1.85 – 12.38	2.06 – 6.41	1.92 – 6.08
<$\bar{w}$> (σ)	(cm s^{-1})	0.12 (0.46)	-0.25 (0.82)	-0.50 (0.94)	-1.18 (1.07)
Range $\bar{w}$		-1.63 – 1.51	-2.18 – 1.32	-2.95 – 3.26	-3.29 – 0.38
<w'> (σ)		1.31 (0.26)	1.39 (0.33)	2.06 (0.56)	2.48 (0.58)
Range w'		0.92 – 2.34	0.57 – 2.78	0.82 – 3.46	0.82 – 3.52
<k> (σ)	(cm^2 s^{-2})	3.56 (6.09)	5.07 (13.73)	10.83 (17.60)	25.28 (27.92)
Range k		0.01 – 36.54	0.00 – 71.88	0.02 – 99.16	0.02 – 89.28
<$\sqrt{k}\bar{u}$-1> (σ)	na	0.13 (0.12)	0.12 (0.23)	0.42 (0.61)	0.74 (5.04)
Range $\sqrt{k}\bar{u}$-1		0.01 – 0.63	0.00 – 1.64	0.02 – 5.71	-12.68 – 27.50
<τ_{uv}> (σ)	(Nm^{-2})	0.17 (0.18)	0.14 (0.25)	0.29 (0.31)	0.36 (0.38)
Range τ_{uv}		0.00 – 0.83	0.00-1.14	0.00 – 1.30	0.00 – 1.22
<τ_{uw}> (σ)	(Nm^{-2})	0.09 (0.05)	0.09 (0.10)	0.16 (0.10)	0.33 (0.34)
Range τ_{uw}		0.00 – 0.36	0.00 – 0.62	0.00 – 0.62	0.01 – 1.33
<τ_{uw}> (σ)	(Nm^{-2})	0.02 (0.02)	0.03 (0.03)	0.04 (0.04)	0.08 (0.08)
Range τ_{uw}		0.00 – 0.18	0.00 – 0.18	0.00 – 0.31	0.00 – 0.51
<lu> (σ)	(cm)	10.46 (0.79)	12.23 (1.80)	8.56 (2.51)	6.36 (4.95)
Range lu		8.09 – 12.83	3.36-15.06	0.80 – 14.32	0.28 – 14.89
<lv> (σ)	(cm)	0.59 (0.08)	0.35 (1.01)	0.42 (0.75)	0.33 (0.74)
Range lv		0.008 – 4.82	0.01 – 6.30	0.01 – 3.97	0.03 – 3.02
<lw> (σ)	(cm)	0.08 (0.28)	0.28 (0.46)	0.51 (0.55)	1.09 (0.81)
Range lw		0.01 – 2.08	0.01 – 1.93	0.01 – 2.70	0.01 – 2.77

The proximity of the flume wall to the boulder sides created a region of accelerated flow around the boulders (H in Figure 6.2). The high velocity region had the highest longitudinal velocity, highest turbulent length scale in the horizontal plane, but lowest turbulent kinetic energy and shear stresses, relatively low turbulence intensities and low standard deviations of relative turbulence intensity (Figure 6.2, Figure 6.3, Table 6.1).

This indicates that while the velocity was at its highest, the temporal variability of the velocity and turbulence at a given point within this region was relatively low. The narrowing of the channel at this point resulted in enhanced shear layer development between the flow at the walls and in the wake of the boulder, which probably enhanced rotational strength of the vortices in the recirculation region (Figure 6.3).

The moderate velocity region had moderate longitudinal velocities (Figure 6.4 and Figure 6.5A), the lowest turbulent kinetic energy (Figure 6.4 and Figure 6.5B), low relative turbulence intensities (Figure 6.5C), low turbulent shear stresses in all planes and the lowest spatial variation for each parameter as indicated by the standard deviations in Figure 6.4 and Figure 6.5. These low values indicate that this region was the most spatially and temporally homogeneous region within the boulder control volume and therefore the most predictable and stable region (see comparison, Table 6.1). This region had the highest turbulent length scale in the horizontal plane l_v, but with the lowest standard deviation, again suggesting a relatively stable region.

A summary of the volume- and time-averaged parameters of each velocity region (Figure 6.2) around the boulders is presented in Table 6.1. For each velocity region, volume-averaged parameters and the standard deviation with respect to the time allocation of the fish in these regions are shown in Figure 6.4 and Figure 6.5. For all regions, the turbulent length scale exceeded the size of the fish in the longitudinal plane (l_u).

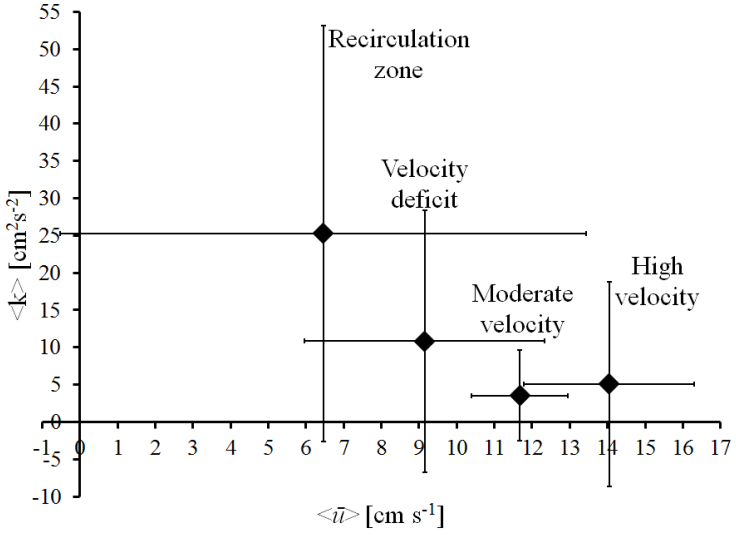

Figure 6.4. Volume-averaged turbulent kinetic energy (k) in relation to volume and time-averaged longitudinal velocity $\bar{u}$ for each velocity region. Horizontal and vertical error bars show the standard deviation of the volume-averaged value for each velocity region.

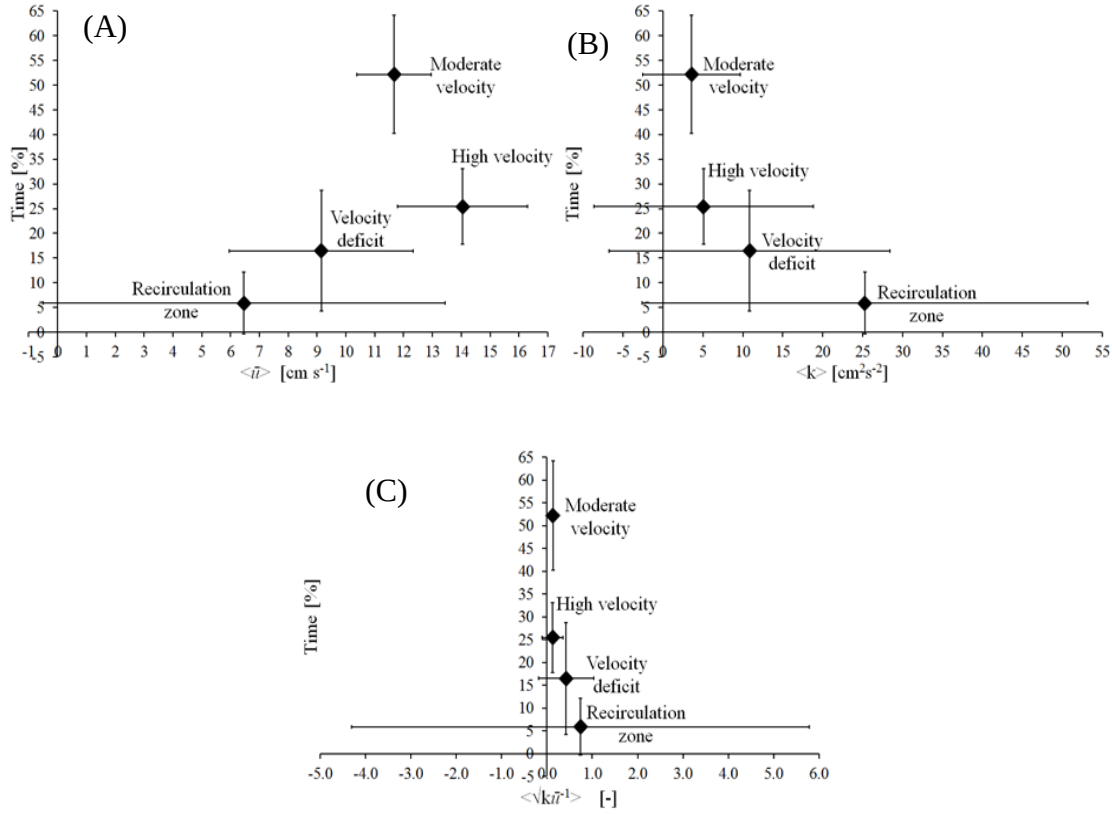

Figure 6.5. Time spent by guppies in the four velocity regions around a hemisphere boulder in relation to volume-averaged (A) longitudinal velocity (<$\bar{u}$>) (B) turbulent kinetic energy (<k>) and (C) relative turbulence intensity (<$\sqrt{k}\bar{u}^{-1}$>). The horizontal and vertical bars shown in (A) and (B) denote the standard deviation of the time spent in each region and the spatial variation of the longitudinal velocity and turbulent kinetic energy respectively.

Fish movement and position in relation to microhabitat hydrodynamics

Fish moved both upstream and downstream continuously throughout the 10 min observation period and spent only short periods station holding within different areas of the flume. With increasing standard length, guppies moved more frequently between boulder velocity regions (GLM, adjusted R-squared = 0.964, $Z_{1,109}$=3.40, P<0.001) and swam a greater distance in both the upstream and downstream directions (GLM, adjusted R-squared = 0.923 and 0.934, $Z_{1,109}$=3.443 and 3.33, P<0.001). There was no effect of host sex, parasitism (prevalence or intensity) or the interaction terms on the frequency of movement or distance moved.

In terms of flow microhabitat use around the boulders, guppies spent significantly more time in the region of moderate velocity magnitude compared to the high, velocity deficit and recirculation zones (Tukey's HSD multiple comparisons following GLMM F=5.508,

0.771 and 7.468 respectively, all P< 0.001). This region may therefore represent the best trade-off between reduced longitudinal velocity and stable turbulence levels. There was a significant interaction between parasite intensity and fish standard length (GLM, adjusted R-squared = 0.126, $t_{3,107}$=-2.496, P=0.014), with an increase in time spent in the moderate velocity region with increasing parasite intensity; however this relationship was stronger for smaller fish (Figure 6.6). There was no difference in the time spent in the moderate velocity region between male and female guppies.

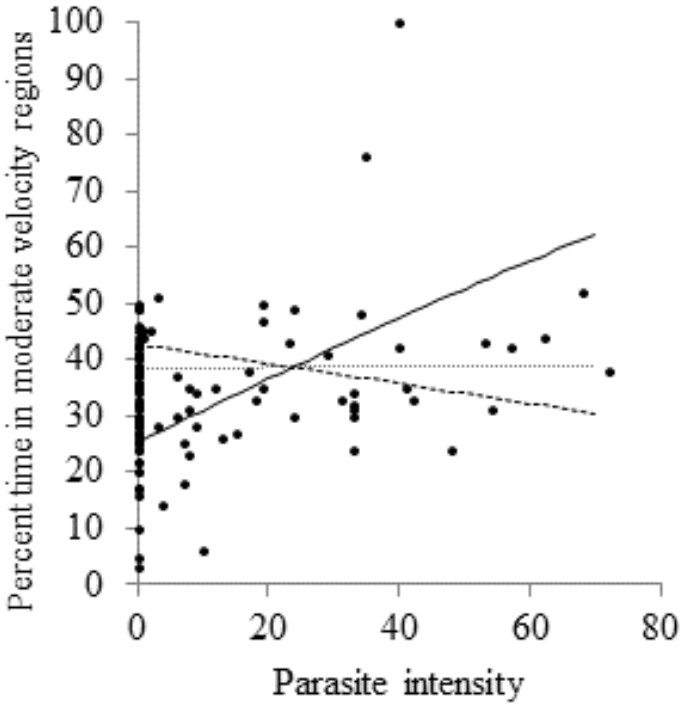

Figure 6.6. Interaction between parasite intensity and host standard length significantly affecting the percentage of time spent by guppies in the moderate velocity regions around boulders. Filled circles show raw data, lines show predictions from the Generalised Linear Models (GLMs) for small (13 mm - solid line), medium (25 mm - dotted line) and large (29 mm - dashed line) fish.

The second most frequented region was the high velocity region (H in Figure 6.2), although the time spent in this region was not significantly higher than the velocity deficit or recirculation zone. This region had the lowest shear stresses in all planes and was also a predictable environment for the fish to swim due to the low temporal variation in velocity. Time spent in the high velocity region was significantly related to fish standard length (GLM, adjusted R-squared = 0.102, $t_{2,108}$=3.634, P<0.001), with larger guppies spending increasingly more time in the high velocity region (Figure 6.7). Host sex, parasite infection (prevalence or intensity) and the interaction terms had no effect on the time spent in the area of high velocity.

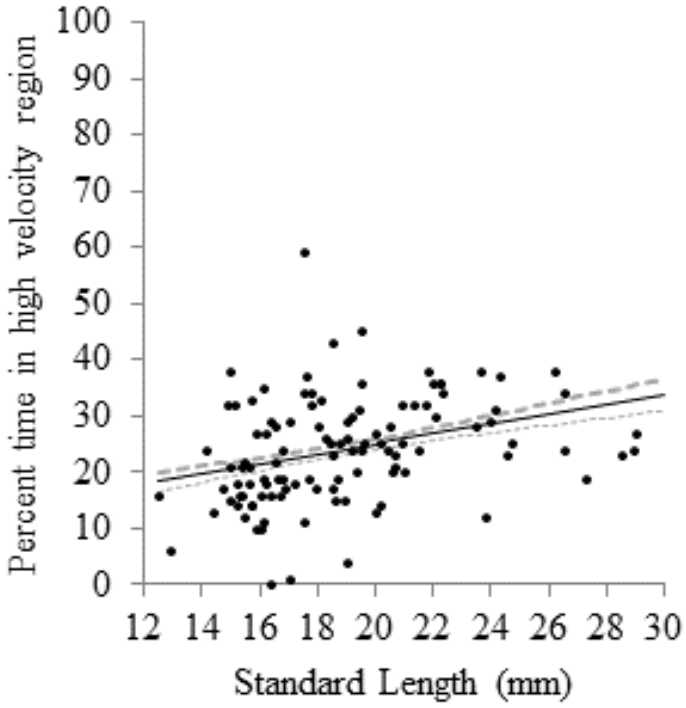

Figure 6.7. Host factors significantly affecting the percent time spent by guppies in the high velocity regions around boulders. Black lines represent the predicted relationship from the Generalised Linear Models and dotted grey lines indicated standard errors of the models.

The velocity deficit region (L in Figure 6.2) had intermediate values of longitudinal velocity, turbulent kinetic energy and relative turbulence intensity. Male guppies spent significantly more time in the velocity deficit region (mean 25.3%, SD 15.0) compared to females (mean 18.9%, SD 12.8), regardless of standard length (GLM, adjusted R-squared = 0.032, $t_{2,108}$=-2.253, P=0.026) . Parasite infection (prevalence or intensity) and the interaction terms had no effect on the time spent in the velocity deficit region.

Fish spent the least amount of time in the recirculation zone (R in Figure 6.2) where the relative turbulence intensity, turbulent shear stresses and ratio of vertical turbulent length scale to fish standard length were at their greatest (see Table 6.1). Time spent in the recirculation zone was negatively associated with fish standard length, with smaller fish spending more time in this region than larger counterparts (GLM, adjusted R-squared=0.039, $t_{2,108}$=2.906, P=0.004) (Figure 6.8). Fish sex and parasite infection (prevalence or intensity) had no effect on the time spent in the recirculation region.

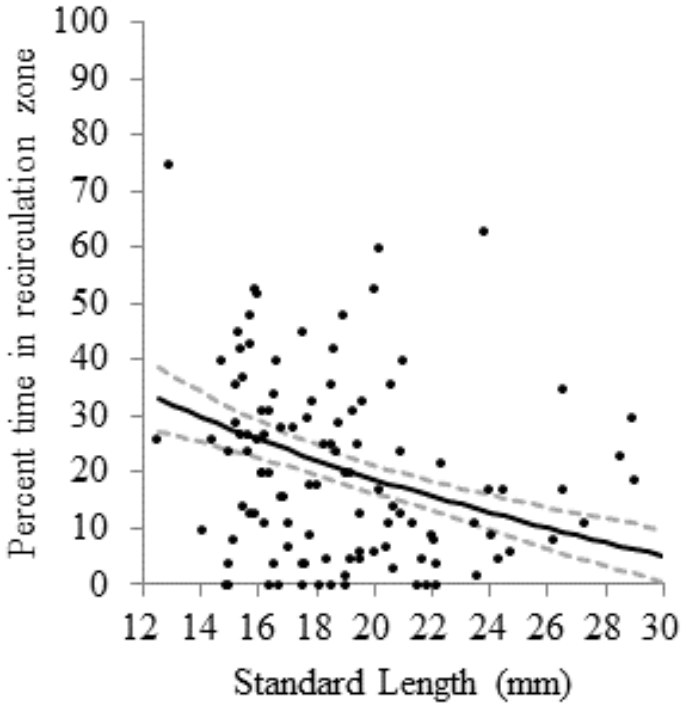

Figure 6.8. Host factors significantly affecting the percent time spent by guppies in the recirculation zones around boulders. Black lines represent the predicted relationship from the Generalised Linear Models and dotted grey lines indicated standard errors of the models.

Discussion

In this study, we identified intra-specific variation in fish swimming behaviour under different flow conditions in terms of longitudinal velocity and turbulence within a heterogeneous habitat and for the first time demonstrated that microhabitat use can be affected by parasite infection. With increasing standard length, fish were more active and spent more time in areas of high velocity and low turbulence. Males spent more time in the region of low velocity and moderate turbulence, indicating a trade-off between velocity reduction and turbulence level increase. When infected, smaller fish appear to opt for the most stable flow conditions. Although we are unable to determine whether spatial repartition of the fish is due to active habitat selection or are a constraint due to energetic depletion or insufficient physical ability to station hold, we discuss possible reasons for the spatial position of individuals.

Guppies spent most time swimming in the moderate and high velocity regions which were the least spatially and temporally variable providing a more stable and predictable environment. The least amount of time was spent in the recirculation region characterised by low velocity, high turbulence and high shear stress, where the spatial-variability of the

parameters was also the highest. This reiterates the conclusions by Silva *et al.* (2011) who demonstrated a significant negative correlation between horizontal shear stress and transit time by the Iberian barbel (*Luciobarbus bocagei*) in an experimental fishway. Thus, shear stress could be an important turbulence property to consider when observing fish behaviour. Additionally, the mean turbulent length scale in the vertical plane (l_w) was greater than two-thirds of the standard length for 28% of the fish in the recirculation region. As Lupandin (2005) proposed this threshold as being important in affecting fish swimming ability, this could be an additional reason as to why this region was avoided by the fish in the current study.

The time spent in the high velocity region increased with increasing standard length, indicating that larger fish were better able to tolerate the relatively higher flow velocities associated with this area compared to the highly three-dimensional flow field experienced in the recirculation zone immediately downstream of the boulder wake. Fish standard length was also associated with time spent in the recirculation zone which had low velocity, high turbulent and high shear stress, with smaller guppies spending more time in this region than their larger counterparts. The size effect of guppies on swimming behaviour is in line with previous studies that have shown smaller fish occupy slow moving water and move to faster moving water as they become larger (Everest and Chapman 1972). It has also been demonstrated that large juvenile rainbow trout select channels with high velocities and low turbulence over the low velocity, high turbulence channels (Smith et al. 2005). However, small juveniles had no preference for either channel until the area mean velocity reached 28 cm s^{-1} where they selected the low velocity and highly turbulent channel (Smith et al. 2005). As discussed by Plaut (2001), as fish grow their swimming ability improves, as there is a positive correlation between critical swimming speed and body size. The higher energy requirement of larger fish means they will be more able to move out into areas with higher velocity magnitude where the chances of food capture are higher (Hughes and Dill 1990).

The size of guppies also correlated with the amount of movement in the open channel. Large guppies displayed more frequent movement, between the velocity regions and swam further distances both up- and downstream. This increased movement may be due to the enhanced swimming ability of bigger fish to tolerate changes in velocity magnitude and turbulence intensity as they move around the open channel flume. Fish constantly

explore their surroundings to forage and seek shelter and this has previously been found to be associated with body size. For example, Kramer and Chapman (1999) found a positive relationship between home range size and body size in several coral reef fishes. In guppies, there is a significant positive relationship between fork length and the amount of movement between natural pools separated by riffles in a Trinidadian stream (Croft et al. 2003). This could be due to a higher energy requirement of larger fish, interaction between fish size and reproductive strategy, or benefits of dispersal for colonisation by larger individuals (Croft et al. 2003).

After taking into account standard length of the fish, male guppies were found to occupy the region of velocity deficit more frequently than females. Guppies are sexually dimorphic, with males being smaller in size, more colourful and have longer dorsal and caudal fins. Although longer fins serve as a secondary sex characteristics in guppies (Bischoff et al. 1985; Nicoletto and Kodric-Brown 1999), for a given velocity a fish with larger fins experience increased drag compared to smaller finned counterparts (Plaut 2000). Indeed several studies have attributed reduced guppy swimming performance and predator escape response (Karino et al. 2006) to the larger fin size (Plaut 2000; Karino et al. 2006; Karino et al. 2011). In a study where the surface area of three shapes of caudal fins of guppies did not differ, there was no observed difference in swimming ability (Nicoletto 1991). Therefore, it appears that the longer tails in guppies are a trade-off between sexual selection and natural selection. Differences in microhabitat selection between sexes have also been observed in wild guppies, for example with regard to shallow water usage (Noltie and Johansen 1986). In the wild, male guppies are found more commonly in shallower habitats (Croft et al. 2004) and slower moving water (Karino et al. 2006), with females occurring in deeper water (Croft et al. 2004). This leads us to question whether the longer tail fins in male guppies also cause a reduction in tolerance to turbulence and shear stresses in the wild.

Even after a relatively short infection period, *Gyrodactylus turnbulli* caused behavioural changes in guppies, with increasing time spent in the regions of moderate velocity magnitude with increasing parasite intensity, but this relationship was only apparent in small fish. The moderate velocity regions had the lowest turbulent kinetic energy, relative turbulence intensity, turbulent shear stresses and lowest spatial variability of these measures, making these regions the most stable and predictable. A small infected fish

may seek these stable areas in order to offset energetic costs associated with the parasite infection. Although the influence on habitat structure on the transmission of parasites is not a new concept (see review by Sousa and Grosholz 1991), few studies have focused on the aquatic environment (e.g. Upatham 1974). Extreme flow events during spate conditions are important in affecting guppy swimming ability when parasitised (van Oosterhout et al. 2007) and previous studies have reported fin clamping associated with late stages of gyrodactylid infection (Cable et al. 2002) which would inevitably result in decreased swimming performance. By affecting the swimming behaviour, therefore foraging ability of their hosts, parasites may exert strong selection pressures by population control. Host-parasite interactions may be affected by habitat heterogeneity in several ways: i) the habitat may cause spatial segregation of the hosts, thus affecting parasite transmission opportunities; ii) the habitat may cause spatial segregation of the parasites, whether free living or via intermediate hosts or vectors; or iii) the parasites themselves may affect host behaviour and thus affecting spatial positioning and further transmission opportunities for the parasite.

In summary, we demonstrate that fish of the same species but of varying size, sex and parasite intensity have different requirements in terms of microhabitat use around boulders in relation to velocity magnitude, turbulence and turbulent shear stress. Smaller and male fish (characterised by having larger fins than females) spent more time in the region of low velocity magnitudes, whereas larger fish more frequently swam in the region of increased velocity magnitude. Small guppies infected with an increasing number of *G. turnbulli* worms spent more time in the moderate velocity, low turbulence and low turbulent shear stress regions, where the spatial and temporal variability of the velocity field was the lowest. This demonstrates the importance of flow heterogeneity within a river system for fish species populations, to provide shelter for weaker or smaller individuals or those at different life stages. In the natural environment, guppies are further restricted in microhabitats not only due to velocity and turbulence tolerances, but also due to exclusion by predators and competitive exclusion from larger or more dominant individuals. Headwater stream habitats are devoid of predators and guppies are found to be more widely distributed compared to the downstream populations where they are restricted to shallower, slow moving waters due to the presence of predators in deeper water (Kodric-Brown and Nicoletto 2005). Although boulder placement is a commonly employed to encourage habitat diversity in river restoration schemes, the success in

improving fish populations has been variable (Roni et al. 2006; Branco et al. 2013). Relatively few studies have bridged the gap between field observations and the use of static flow tanks (e.g. Webb 1998; Branco et al. 2013 and current study), particularly with regard to intraspecific variation in fish behaviour.

References

Aadland L (1993) Stream habitat types: their fish assemblages and relationship to flow. North Am J Fish Manag 13:790–806

Barber I, Hoare D, Krause J (2000) Effects of parasites on fish behaviour: a review and evolutionary perspective. Rev Fish Biol Fish 10:131–165

Bates D, Maechler M, Bolker B, Walker S (2013) lme4: Linear mixed-effects models using Eigen and S4. R package version 1.0-5. http://cran.r-project.org/package=lme4

Bischoff RJ, Gould JL, Rubenstein DI (1985) Tail size and female choice in the guppy (*Poecilia reticulata*). Behav Ecol Sociobiol 17:253–255

Branco P, Boavida I, Santos JM, Pinheiro A, Ferreira MT (2012) Boulders as building blocks: improving habitat and river connectivity for stream fish. Ecohydrol 6:627–634

Brooks AJ, Haeusler T, Reinfelds I, Williams S (2005) Hydraulic microhabitats and the distribution of macroinvertebrate assemblages in riffles. Freshw Biol 50:331–344

Cable J (2011) Poeciliid Parasites. In: Evans JP, Pilastro A, Schlupp I (eds) Ecology and Evolution of Poeciliid Fishes. Chicago University Press, pp 82–94

Cable J, Scott ECG, Tinsley RC, Harris PD (2002) Behavior favoring transmission in the viviparous Monogenean *Gyrodactylus turnbulli*. J Parasitol 88:183–184

Čada GF (2001) The development of advanced hydroelectric turbines to improve fish passage survival. Fisheries 26:14–23

Croft DP, Albanese B, Arrowsmith BJ, Botham M, Webster M, Krause J (2003) Sex-biased movement in the guppy (*Poecilia reticulata*). Oecologia 137:62–8

Croft DP, Botham MS, Krause J (2004) Is sexual segregation in the guppy, *Poecilia reticulata*, consistent with the predation risk hypothesis? Environ Biol Fish 71:127–133

Davey AJH, Booker DJ, Kelly DJ (2011) Diel variation in stream fish habitat suitability criteria: implications for instream flow assessment. Aquat Conserv Mar Freshw Ecosyst 21:132–145

Douglas JF, Gasiorek JM, Swaffield JA, Jack LB (2005) Fluid Mechanics, 5th ed. Pearson Education Limited, Harlow, Essex

Everest FH, Chapman DW (1972) Habitat selection and spatial interaction by juvenile Chinook salmon and steelhead trout in two Idaho streams. J Fish Res Board Canada 29:91–100

Fraser BA, Weadick CJ, Janowitz I, Rodd FH, Hughes KA (2011) Sequencing and characterization of the guppy (*Poecilia reticulata*) transcriptome. BMC Genomics 12:202

Garner P (1997) Effects of variable discharge on the velocity use and shoaling behaviour of *Phoxinus phoxinus*. J Fish Biol 50:1214–1220

Murchie Guégan J-F, Lek S, Oberdorff T (1998) Energy availability and habitat heterogeneity predict global riverine fish diversity. Nature 391:382–384

Heggenes J, Saltveit J, Lingaas O (1996) Predicting fish habitat use to changes in water flow: Modelling critical minimum flows for Atlantic salmon, *Salmo salar*, and brown trout, *S. trutta*. Regul Rivers Res Manag 12:331–344

Hinch SG, Rand PS (2000) Optimal swimming speeds and forward-assisted propulsion: energy-conserving behaviours of upriver-migrating adult salmon. Can J Fish Aquat Sci 57:2470–2478

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biom J 50:346–63

Hughes NF, Dill LM (1990) Position choice by drift-feeding salmonids: model and test for Arctic Grayling (*Thymallus arcticus*) in subarctic mountain streams, interior Alaska. Can J Fish Aquat Sci 47:2039–2048

Karino K, Ishiwatari T, Kudo H, Sato A (2011) Female mate preference for a costly ornament in male guppies. Behav Ecol Sociobiol 65:1305–1315

Karino K, Orita K, Sato A (2006) Long tails affect swimming performance and habitat choice in the male guppy. Zoolog Sci 23:255–260

Kodric-Brown A, Nicoletto PF (2005) Courtship behavior, swimming performance, and microhabitat use of Trinidadian guppies. Environ Biol Fishes 73:299–307

Kolluru GR, Grether GF, Dunlop E, South SH (2007) Food availability and parasite infection influence mating tactics in guppies (*Poecilia reticulata*). Behav Ecol 20:131-137

Kramer DL, Chapman MR (1999) Implications of fish home range size and relocation for marine reserve function. Environ Biol Fishes 55:65–79

Lacey RWJ, Neary VS, Liao JC, Enders EC, Tritico HM (2011) The IPOS framework: linking fish swimming performance in altered flows from laboratory experiments to rivers. River Res Appl 28:429–443

Liao JC (2007) A review of fish swimming mechanics and behaviour in altered flows. Philos Trans R Soc Lond B 362:1973–1993
Lopez S (1998) Acquired resistance affects male sexual display and female choice in guppies. Proc R Soc B 265:717–723
Lupandin AI (2005) Effect of flow turbulence on swimming speed of fish. Biol Bull 32:461–466
Margurran AE, Seghers BH (1990) Risk sensitive courtship in the guppy (*Poecilia reticulata*). Behaviour 112:194–201
Meyer A (1997) The evolution of sexually selected traits in male swordtail fishes (*Xiphophorus*: Poeciliidae). Heredity 79:329–337
Neitzel DA, Dauble DD, Čada GF, Richmond MC, Guensch GR, Mueller RP, Abernethy CS, Amidan B (2004) Survival estimates for juvenile fish subject to a laboratory-generated shear environment. Trans Am Fish Soc 133:447–454
Nicoletto PF (1991) The relationship between male ornamentation and swimming performance in the guppy, *Poecilia reticulata*. Behav Ecol Sociobiol 28:365–370
Nicoletto PF, Kodric-brown A (1999) The relationship among swimming performance, courtship behavior, and carotenoid pigmentation of guppies in four rivers of Trinidad. Environ Biol Fishes 55:227–235
Nietzel DA, Richmond MC, Dauble DD, Mueller RP, Moursund RA, Abernethy CS, Guensch GR, Cada GF (2000) Laboratory Studies on the Effects of Shear on Fish. Report prepared for the US Department of Energy Idaho Operations Office
Nikora VI, Aberle J, Biggs BJ, Jowett IG, Sykes JRE (2003) Effects of fish size, time-to-fatigue and turbulence on swimming performance: a case study of *Galaxias maculatus*. J Fish Biol 63:1365–1382
Noltie DB, Johansen PH (1986) Laboratory studies of microhabitat selection by the guppy, *Poecilia reticulata* (Peters). J Freshw Ecol 3:299–307
Nortek AS (2009) Vectrino velocimeter user guide.Vangkroken, Norway. Nortek AS
Northcutt RG (1997) Swimming against the current. Nature 389:915–916
Plaut I (2000) Effects of fin size on swimming performance, swimming behaviour and routine activity of zebrafish *Danio rerio*. J Exp Biol 203:813–820
Plaut I (2001) Critical swimming speed: its ecological relevance. Comp Biochem Physiol Part A 131:41–50
Pope SB (2000) Turbulent Flows. Cambridge University Press
R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/
Reznick D, Butler MJ, Rodd H (2001) Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. Am Nat 157:126–140
Richards EL, van Oosterhout C, Cable J (2010) Sex-specific differences in shoaling affect parasite transmission in guppies. PLoS One 5:e13285
Roni P, Bennett T, Morley S, Pess GR, Hanson K, van Slyke D, Olmstead P (2006) Rehabilitation of bedrock stream channels: the effects of boulder weir placement on aquatic habitat and biota. River Res Appl 22:967–980
Rusello PJ, Lohrmann A, Siegel E, Maddux T (2006) Improvements in acoustic Doppler velocimetery. 7th Int Conf Hydrosci Eng (ICHE 2006), Sept 10-13
Silva AT, Santos JM, Ferreira MT, Pinheiro AN, Katopodis C (2011) Effects of water velocity and turbulence on the behaviour of Iberian barbel (*Luciobarbus bocagei*, Steindachner 1864) in an experimental pool-type fishway. River Res Appl 27:360–373
Smith DL, Brannon EL, Odeh M (2005) Response of juvenile rainbow trout to turbulence produced by prismatoidal shapes. Trans Am Fish Soc 134:741–753
Sousa WP, Grosholz ED (1991) The influence of habitat structure on the transmission of parasites. In: Bell SS, McCoy ED, Mushinsky HR (eds) Habitat Structure. Population and Community Biology Series, Springer Netherlands, 8: 300-324
Upatham ES (1974) Dispersion of St. Lucian *Schistosoma mansoni* cercariae in natural standing and running waters determined by cercaria counts and mouse exposure. Ann Trop Med Parasitol 68:343–352
van Oosterhout C, Mohammed RS, Hansen H, Archard GA, McMullan M, Weese DJ, Cable J (2007) Selection by parasites in spate conditions in wild Trinidadian guppies (*Poecilia reticulata*). Int J Parasitol 37:805–812
Voigt R, Carton AG, Montgomery JC (2000) Responses of anterior lateral line afferent neurones to water flow. J Exp Biol 203:2495–2502
Wahl TL (2011) Win ADV. A Windows-based viewing and post-processing utility for ADV files
Webb PW (1998) Entrainment by river chub *Nocomis micropogon* and smallmouth bass *Micropterus dolomieu* on cylinders. J Exp Biol 201:2403–12

Chapter 7. Downstream migratory behaviour of European eels *Anguilla anguilla* in an open channel flume

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A version of this chapter has been submitted to the Journal of Fish Biology with LN as lead author. The experiment was designed by LN and FH. Behavioural trials were completed by LN and post mortem examination by FH, CW and AR. Supervision and comments on the manuscript were provided by JC and PK.

Abstract

With the European eel (*Anguilla anguilla*) stocks in dramatic decline, river managers are increasingly under pressure from the EU to improve river structure to aid migration. Although upstream passage for juvenile eels have generally been a success through placement of eel ladders around obstructions such as dams, weirs and shipping locks, downstream passage success of adult eels has seen considerable variation. Many individuals have been recorded to display recurrent behaviour on approach to river obstructions whereas others pass directly through uninhibited. This study aimed to explain the variation in swimming behaviour in a population of *A. anguilla* from the River Avon, Hampshire, UK in terms of individual morphology and parasite infection. By using a laboratory open channel flume with a narrowed constriction to generate water velocities similar to a downstream fish bypass entrance, it was found that the orientation at which the fish entered the constriction was the most important predictor for downstream success. Older eels and those with a higher silvering index were more likely to move downstream faster and had a reduced tendency to demonstrate recurrent behaviour. Eels with higher total parasite burdens showed a stronger downstream migratory tendency, which may be a result of increased exposure to parasites due to increased activity, an indirect effect of age and maturity, or an energetic drain caused by the parasite resulting in passive drift. In contrast, eels infected with the invasive swim bladder nematode *Anguillicoloides crassus* were more likely to exhibit a reaction to the accelerating velocity. This did not necessarily result in the infected eels returning to the upstream section, suggesting that this parasite may affect burst swimming ability. It is important that the design of downstream fish passage facilities takes into consideration the approach areas to ensure the eels do not display recurrent behaviour and orientate themselves upstream to prevent recurrence upon entering the passage.

Introduction

The European eel (*Anguilla anguilla*) comprises a single panmictic stock which has been in gradual decline throughout the last half century, with a sharp fall since the early 1980s. In the last two years, glass eel recruitment has seen a slight improvement, returning to the levels observed in 2005-2006 (ICES 2013). For example, in the UK glass eel landings increased from 3.8 tonnes in 2012 to 8.6 tonnes in 2013 with similar catch effort (ICES 2013). However, stock levels continue to be far from healthy, remaining at 1.5% of the 1960-1979 reference levels for the North Sea areas and 10% of the reference levels for the rest of Europe (ICES 2013). There is no quantitative evidence which supports one single cause of the decline (Kettle et al. 2011) and so it is likely that several factors have contributed to the mass loss (Feunteun 2002). These factors include; river manipulations preventing passage for migration (Knights and White 1998), bioaccumulation of chemical contaminants (Geeraerts and Belpaire 2010), introduction of parasites (Kennedy 2007), overfishing (Feunteun 2002) and climate induced change to oceanic currents (Feunteun 2002; Bonhommeau et al. 2008). Legislation implemented by the European Union requires all member states to draw up Eel Management Plans (EMPs) with proposals for long-term management of eels throughout Europe (EC No 1100/2007) and is further supported by the EU Water Framework Directive. EMPs may also contain measures to improve river habitats, making barriers passable and may involve temporary switching-off hydro-electric power turbines to decrease adult eel mortality.

European eels have a long and complicated life cycle and are found in fresh, brackish and coastal waters throughout Europe and the Mediterranean. The longest stage of the lifecycle, about 20 years or more, is the yellow eel stage, where the eels occupy freshwater or inshore marine or estuarine waters (ICES 2013). At the end of the yellow stage, eels enter the silvering processes where the fish undergo a series of internal and external changes such as an increase in eye diameter (Pankhurst 1982) and increase in pectoral fin size (Durif et al. 2005) to prepare them for the long migration to spawning grounds (Durif et al. 2009). A critical stage of European eel lifecycle is the migration downstream through rivers from fresh to coastal waters. Their passage may be hindered by barriers such as dams, weirs and hydroelectric power plants. Rivers used for the generation of hydropower have been associated with high eel mortalities due to increase in pressure, sudden change in flow velocity, cavitation and damage from turbine rotor blades (Russon

et al. 2010). In addition, a dam can delay downstream migration by causing an accumulation of fish in the impoundment on the approach. This would lead to reduced water quality, increased disease transfer and enhanced predation risk from piscivorous birds (Larinier and Travade 2002). The upstream migration of fish has been aided by the construction of fishways and eel ladders to bypass obstacles such as dams, sluices and locks, however downstream passage facilities are much less advanced (Larinier and Travade 2002). Furthermore, bypasses designed for salmonids may be less effective for eels (Gosset et al. 2005) due to differences in swimming depth and poorer burst swimming performance (Tesch 2003; Russon et al. 2010). Screens (e.g. trash racks) across the opening of turbines are used to prevent entry of fish, however, if poorly designed, eels may become impinged on the grill and mortalities can still be high (Russon et al. 2010; Calles et al. 2010).

Downstream passage of European eels generally takes place during autumn and is thought to depend on environmental triggers such as flow discharge (Behrmann-Godel and Eckmann 2003; Jansen et al. 2007). Different individuals may chose different migration routes towards the sea, especially in modified waterways with canals, locks, dams, sluices, weirs and culverts. For example, at hydropower stations, eels have four possible routes; the fishway, overspill over the dam, directly through turbines (Jansen et al. 2007) or can they migrate for brief periods over land (Ellerby et al. 2001). Eels have been known to exhibit circulatory behaviour upstream of obstructions for several days before continuing their downstream migration (Behrmann-Godel and Eckmann 2003). The differences in route selection and recurrent behaviour can partly be explained by the river discharge (Jansen et al. 2007). However, it is likely that individual variation in morphology (such as size and silvering) and health (including parasite infection) could also have a contributing effect on behaviour.

Laboratory experiments using open channel flumes have been used to compliment telemetry field studies to monitor eel swimming behaviour on approach to obstructions (Russon and Kemp 2011). Flume based studies have the benefit of being able to control for environmental variation and allow the fish to volitionally explore the channel whilst being able to observe fine-scale behaviours (Russon and Kemp 2011). Laboratory generated information on eel behaviour can then support the design of fish passage to allow the fish to successfully negotiate obstructions to river flow during their downstream migration. For example, knowledge about the orientation in which fish enter hydropower

turbines could be used for computational fluid dynamics (CFD) modelling of fish trajectories through turbine systems (Coutant and Whitney 2000).

Eel swimming ability is thought to be impaired as a result of pathological damage and physiological stress caused by the introduced swim bladder nematode *Anguillicoloides crassus* (see Sprengel and Luchtenberg 1991; Palstra et al. 2007b). Although the spread of *A. crassus* across Europe in the late 1980s does not agree well with the initial rapid decline of eels in the 1970s, the parasite is thought to have exacerbated the decline. European eels have a higher susceptibility and have higher intensities of infection with *A. crassus* than the parasites natural hosts Japanese eels (*Anguilla japonica*) which leads to more severe pathological damage (Knopf 2006). The infective larval stages of the parasite (L3) enters a new eel host through ingestion of infected intermediate hosts such as copepods and ostracods and penetrates through the alimentary tract to reside in the swimbladder wall (Norton et al. 2005). This penetration through the swimbladder wall initiates an inflammatory response causing the wall to become thickened and fibrotic (Kirk 2003). Sanguivorous feeding by the adult nematode reduces the oxygen carrying capacity of the host's blood resulting in additional damage to the swimbladder (Palstra et al. 2007b). Pathological damage caused by the parasite is also exacerbated by environmental stressors such as an increase in hypoxia (Gollock et al. 2005a), raised temperature (Gollock et al. 2005b) and netting (Gollock et al. 2004). *A. crassus* is largely a freshwater species, but has been documented to survive and reproduce in eels in sea water for up to 6 months. In seawater, intensity of infection is lower and L4 adult stages can become slow moving and damaged after 12 weeks (Kirk et al. 2000; Norton et al. 2005). The impact of *A. crassus* on the long duration swimming ability of eels is fairly well documented. For example, eels with damaged swimbladders had lower cruising speeds, higher cost of transport and reduced maximum swimming speeds than uninfected hosts (Sprengel and Luchtenberg 1991; Palstra et al. 2007b). During the oceanic stage, silver eels are known to swim to depths as great as >800 m and return to the surface at night. The deleterious effect of the parasite on the functioning of the swimbladder may also impair this vertical migration (Kirk et al. 2000; Wahlberg et al. 2014). However, the short-term behavioural cost of infection is not clear.

This study assessed the behaviour of European eels when faced with a rapid increase in flow velocity similar to that of eel passage facilities. Using an open channel flume to control hydraulic conditions whilst taking into account morphological characters and

parasite infection, the aim was to better understand why downstream success differs between individuals and improve future eel passage design.

Methods

Study system

Downstream migrating adult female European eels *Anguilla anguilla* (n = 176, mean standard length 594 ± 6 mm, mean mass 409 ± 16 g) were caught at a permanent eel trap on 6th September 2010 from the River Avon, Hampshire, UK (NGR SZ 16103 95736) and transported to the International Centre for Ecohydraulics Research Laboratory, University of Southampton, UK.

Behavioural experiments

Behavioural trials were conducted in a re-circulatory flume (21.4 m long, 1.37 m wide, 0.6 m deep), with a maximum flow discharge of 0.47 $m^3 s^{-1}$. Flow depth was maintained at 0.24 m and temperature ranged between 11.7 – 16.5°C. A rapid increase in velocity was created by a narrow constriction 6 m long and 0.77 m wide along the central section of the flume using 0.3 m thick polystyrene foam sheeting (Figure 7.1A). Four low light cameras were mounted used to record behaviour under infrared illumination (5 W, 850 nm) and a blackout screen was used to prevent disturbance from movement or light. Longitudinal water velocity was characterised using a Nortek Vectrino II downwards-looking acoustic Doppler velocimeter (ADV) profiler at 60% depth with a sample rate of 50 Hz for 60 sec with a sampling volume of 3.1 mm height. Spurious data was filtered using a maximum/minimum threshold filter (see Cea et al. 2007). Time averaged velocity for each reading plotted in ArcGIS (ESRI, Redlands, USA) and interpolated using spline methods (Figure 7.1B). The time averaged velocity increased from 0.46 m s^{-1} to 1.06 m s^{-1} over the 1 m entry into the constriction. Downstream of the constriction was a recirculation region adjacent to the flume wall where negative velocities were generated (Figure 7.1B).

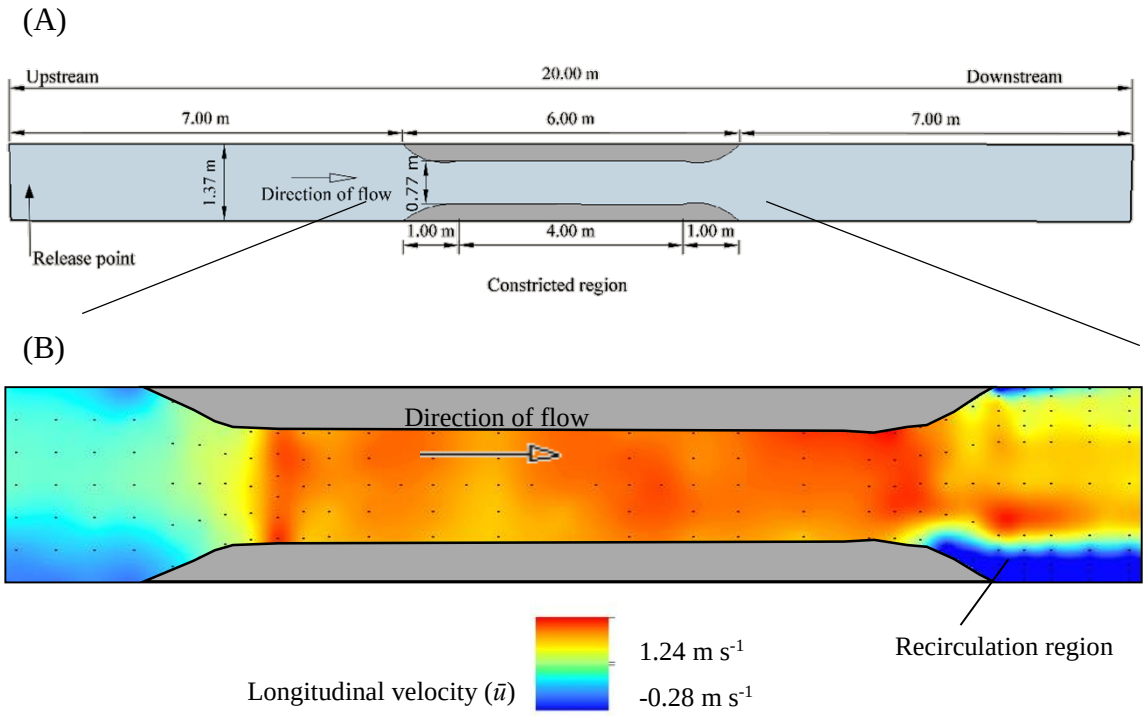

Figure 7.1. Plan view schematic of the recirculating open channel flume in the International Centre for Ecohydraulics Research Laboratory, University of Southampton, UK. (A) Scaled diagram of flume with constricted region mid-way down the channel. (B) Enlarged diagram of constricted region showing time-averaged longitudinal velocity ($\bar{u}$) profile at 60% depth.

Trials took place during the time of highest activity after sunset (19:00 – 03:00 GMT) between 12th October and 3rd November 2010. Following one hour acclimatisation at the upstream end of the flume, the eels were released at the release point (indicated in Figure 7.1A) and allowed 30 min to volitionally move downstream. The seven individuals which did not swim away from the release point and did not enter the cameras field of view were excluded from the final dataset.

Behavioural rheotaxis upon entering the constricted region of the flume was recorded as positive (entering tail first with head facing oncoming current) or negative (head downstream) determined from the most downstream part of the body on entry into the constriction. For those eels which entered the constriction, the behavioural reaction to the accelerating velocity was recorded. A reaction was defined as either a sudden increase in tail beat frequency and burst of upstream movement or a change in orientation from negative to positive rheotaxis. Of the eels which demonstrated a behavioural reaction, it was recorded whether they returned to the upstream section or continued downstream.

Exploratory behaviour was further investigated once the eels reached the downstream section. Eels were observed for a further 30 min to record attempts to return back upstream. An upstream attempt was recorded if the eel re-entered the constricted region and this attempt was recorded as successful if they exited the constriction at the upstream section.

Following swimming trials, eels were removed from the flume and anaesthetised in 1% 2-phenoxyethanol to insert a passive inductive transponder (PIT) tag for identification purposes.

Post-mortem examination

The eels were transported live to the School of Biosciences, Cardiff University, UK or the Environment Agency, Brampton, UK and dispatched by submersion with an overdose of Benzocaine. Detailed parasitological screening of internal and external organs was conducted using low and high-powered microscopy. In all eels (n=176) the heart, gills, swim bladder, spleen, gall bladder, musculature and intestinal tract were examined. A sub-sample of eels (n=93) were examined more thoroughly with additional examination of the skin, buccal cavity, nasal cavity, eyes, liver and kidney. Where appropriate, parasites were fixed, cleared or stained to confirm identification using keys (Brown et al. 1986; Chubb et al. 1987; Moravec 1994; Gibson et al. 2002; Jones et al. 2005). Tissue samples of the gills, brain, kidney and spleen were stored at -80^{o}C and tested for *Herpesvirus anguillae* (HVA) using the methods described in Armitage et al. (2014).

Infection parameters followed standard parasite definitions (Bush et al. 1997). *Anguillocoloides crassus* intensity was calculated for both adult and larval stages. The gill monogeneans were identified as *Pseudodactylogyrus bini* and *P. anguillae* however for the purpose of data analysis were recorded collectively as *Pseudodactylogyrus* spp. Intensity of *Pseudodactylogyrus* spp. was determined by doubling the count of worms from the right gill arches assuming equal distribution across right and left sides (Hockley et al. 2011). To examine parasite community effects on host behaviour, the Simpson's Diversity Index (1-*D*) was calculated:

$$D = \frac{\sum n\,(n-1)}{N(N-1)}$$

Where *n* = total number of organisms of a species and *N* = total number of organisms of all species. Species richness (number of parasite species) and total intensity (*N*) were also calculated for each eel.

Morphology of the eels was also recorded by measuring total length (*L*), weight (*W*), horizontal eye diameter (*Eh*), vertical eye diameter (*Ev*) and pectoral fin length (*PL*). These parameters were used to calculate three morphometric indexes, the Fulton's condition index (*K*) (Fulton 1904) and two indexes of silvering the eye (I_E) and fin index (I_F) (Durif et al. 2005):

$$K=100(W/L^3)$$

$$I_E = 100*[((Eh + Ev)/4)^2\pi/L]$$

$$I_F = 100*(PL*L)^{-1}$$

To determine the approximate age of the eels, otoliths were removed and aged using the 'burn and crack' technique (Christensen 1964).

Data analysis

All analyses were conducted using R v2.1.0 statistical software (R Core Team 2013).

Only eels for which behaviour data could be matched with parasite data (i.e. able to match PIT tag codes) were included in the behavioural analysis. Individuals that made contact with the walls of the constriction were also excluded from the behavioural analysis (n=22) as it was unclear whether the behavioural response was a result of hydraulic conditions or physical contact. This resulted in a total of 143 eels included in the behavioural analysis.

Factors influencing the swimming behaviour of European eels were assessed using general linear models or binomial generalised linear models (GLMs). For each behavioural response (orientation upon entering constriction, reaction to accelerating velocity, return upstream, time to reach downstream section, attempt to return upstream and success of upstream attempt) three sub-models were run. The fixed terms in each model included morphological parameters (*K*, I_E, I_F and age) and parasite infection. The three sub-models included either parasite intensity, prevalence or parasite community (species richness, Simpsons Diversity Index and total intensity). Only parasite taxa with

prevalence >10% recorded from the full post mortem examination were included in the intensity and prevalence sub-models (therefore excluding protozoa), see Table 7.1.

The sub-models were standardised using the arm library (Gelman and Su 2013) and the models with the most important explanatory variables determined using model averaging based on the top models with corrected Akaike Information Criterion (AICc) <2.5 using the MuMIn library (Barton 2013) as described by Grueber et al. (2011).

Results

Eel morphology and parasite infection

European eels (n=176) sampled from the River Avon, Hampshire, UK were infected with 21 parasite taxa of which 12 were identified to species level (Table 7.1). The most prevalent taxa were the gill monogenean *Pseudodactylogyrus* spp. infecting 97.4%, the skin protozoa *Ichthyophthirius* spp. infecting 87.6% and the swim bladder nematode *Anguillicoloides crassus* infecting 81.2% of the eels.

Table 7.1. Prevalence and mean intensity of parasites on wild-caught European Eels *Anguilla anguilla* (n=176), NA=not applicable (counts not possible). Greyed rows are taxa with prevalence >10% recorded from the full examination and included in the behavioural data analysis.

		Prevalence	**Mean intensity**	**Range**
Virus	*Herpesvirus anguillae* (HVA)	2.84%	NA	NA
Protozoa	*Ichthyophthirius* spp.	87.64	NA	NA
	Myxidium spp.	53.26%	NA	NA
	Trypanosoma spp.	23.91%	NA	NA
	Myxobolus spp.	10.75%	NA	NA
	Trichodina spp.	9.52%	NA	NA
Monogenea	*Pseudodactylogyrus* spp.	97.39%	153.13	2-836
Trematoda	*Dipostomum* sp.	11.96%	NA	NA
	Nicolla gallica	8.70%	2.00	1-3
	Rhipidocotyle campanula	0.57%	1.00	1
Cestoda	*Bothriocephalus claviceps*	7.97%	1.55	1-3
Nematoda	*Anguillicoloides crassus*	81.21%	9.36	1-58
	(Adult)	49.70%	7.85	1-47
	(Larvae)	75.85%	3.33	1-34
	Daniconema anguillae	1.45%	11.00	2-20
	Spinitectus inermis	13.77%	8.00	1-30
	Raphidascaris acus	7.97%	1.36	1-2
	Pseudocapillaris sp.	1.45%	2.00	2
	Camallanus lacustris	0.72%	1	1
	Eustrongylides sp.	0.71%	1	1
Acanthocephala	*Pomphorynchus laevis*	26.09%	4.14	1-41
	Acanthocephalus lucii	8.70%	1.67	1-3
	Acanthocephalus anguillae	1.45%	1.00	1

There was a significant positive relationship between eel age and silvering index I_F, with older eels having a high silvering index (Figure 7.2). There was a negative relationship between eel age and parasite species richness, with older eels having fewer parasites. There was no relationship between eel condition factor or silvering index and parasitism.

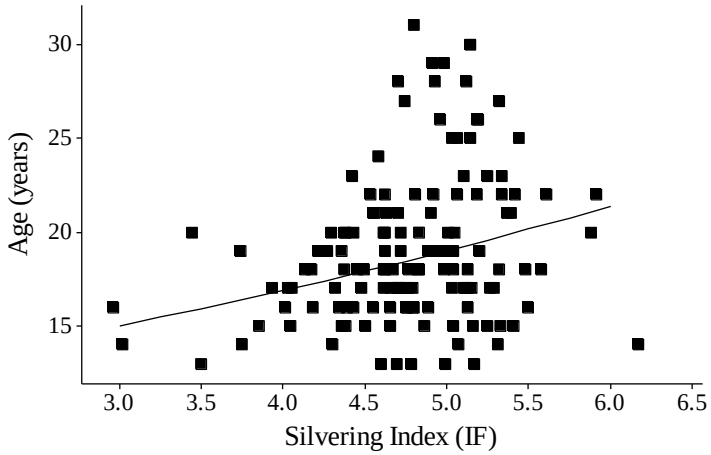

Figure 7.2. Positive relationship between eel silvering index and age. Line shows predictions from top model at mean species richness.

Orientation entering constriction

The time the eels took from the initial release to entering the constricted region ranged from 4 s to 28 min. On entry into the constriction, 104 eels entered head first (negative rheotaxis) and 39 entered tail first (positive rheotaxis). Eels that took longer to approach the constriction from release point were more likely to approach the constriction tail first. Eels entering the constriction head first were had a higher number of individual parasites (total intensity) (Figure 7.3A, Table 7.2) and were older (Figure 7.3B, Table 7.2).

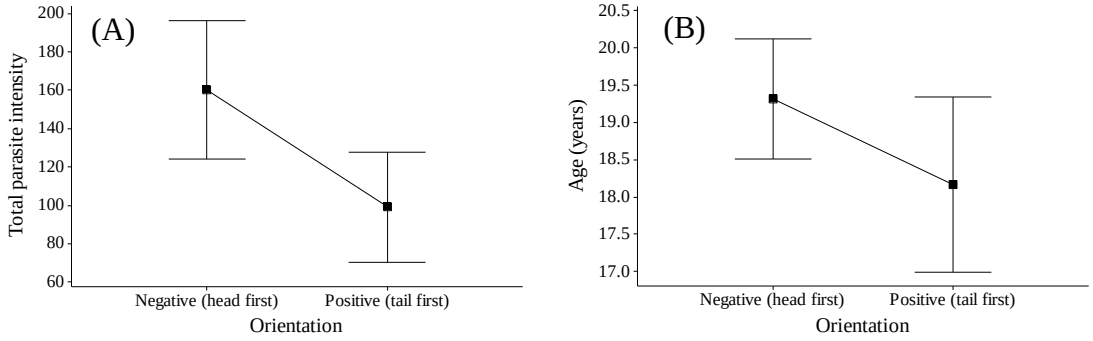

Figure 7.3. Relationship between the orientation at which European eels enter the constricted region and (A) total parasite intensity and (B) age of eels. Squares show means and error bars show 95% confidence intervals.

Time to reach downstream section

The total time the eels took to reach the downstream section from the initial release upstream ranged from 10 s to 20 min 50 s. Eels exhibiting positive rheotaxis took longer to reach the downstream section (Figure 7.4, Table 7.2) and eels with a higher eye silvering index (I_E) reached the downstream section faster (Table 7.2).

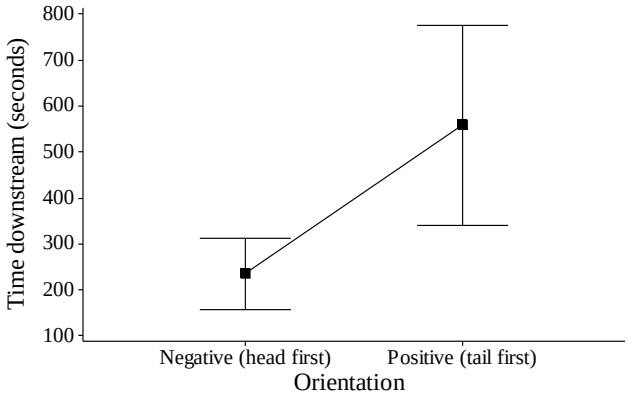

Figure 7.4. Increase in time to reach downstream section after initial release upstream in open channel flume if eels exhibit positive rheotaxis (tail first) when entering the constricted region of high velocity. Squares show mean values, error bars show 95% confidence intervals.

Reaction to accelerating velocity

On entering the higher velocity constricted section of the flume, 63 individuals exhibited at least one reaction to the change in velocity either with a burst swim or change in orientation. Of those which exhibited a reaction, 48 returned back to the upstream section. Eels which entered the constriction tail first with their head upstream (positive rheotaxis) were more likely to react (Figure 7.5A, Table 7.2) and more likely to return to the upstream section (Figure 7.5B, Table 7.2).

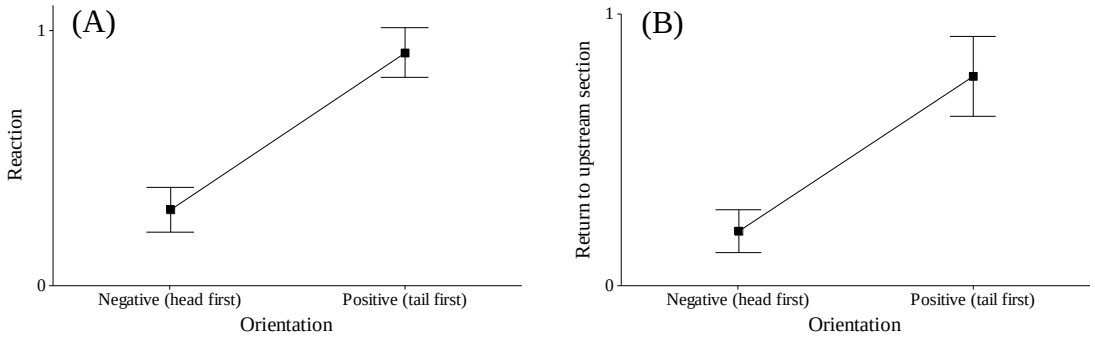

Figure 7.5. Increase in likelihood of (A) a reaction to accelerating velocity within constricted region of flume (1 = reaction, 0 = no reaction) and (B) return to upstream section (1 = return, 0 = no return) if eel enters exhibiting positive rheotaxis (tail first) compared to negative (head first). Squares show mean values and error bars show 95% confidence intervals.

Parasite effects

Parasite infection had a significant impact on eel downstream migratory behaviour. Eels with a high total parasite intensity (*N*) were more likely to enter the constricted region head first (negative rheotaxis) (Table 7.2). Eels with high parasite species richness and Simpsons Diversity Index were less likely to react to accelerating velocity and less likely to return back into the upstream section (Figure 7.6A, Table 7.2). Similarly, when infected with the nematode parasite *Spinitectus inermis* the eels were less likely to reject the accelerating velocity (Table 7.2). Infection with *Pseudodactylgyrus* spp. was associated with a shorter time to reach the downstream section (Table 7.2).

Conversely, prevalence and intensity of *A. crassus* larvae were associated with eels that were more likely to react to the accelerating velocity (Figure 7.6B, Table 7.2). However higher infection also led to a reduced time to reach the downstream section (Table 7.2), so although the eels were resisting downstream movement, they were in fact reaching the downstream section more rapidly.

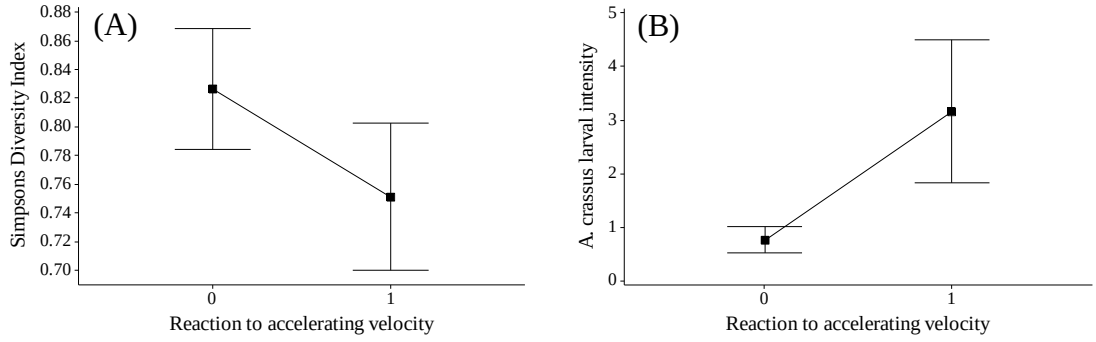

Figure 7.6. (A) The higher the parasite diversity (Simpsons Diversity Index 1-*D*) the less likely eels are to react to accelerating velocity in constricted region. However, (B) the higher *Anguillicoloides crassus* intensity the more likely the eels are to react.

Upstream movement

The majority (n=118) of eels eventually reached the downstream section and of these, 64 attempted to re-enter the constriction against the flow. Older eels were more likely to attempt to return upstream (Table 7.2), however there were no parasite or morphological factors that affected whether the eels were successful in reaching the upstream section.

Table 7.2. Summary of averaged model predictors with relative importance (RI) >0.9. SE=standard error, CI=95% confidence intervals. All=result applies to all sub-models. Greyed rows are considered significant.

Model	Sub model	Predictor	Estimate	SE	CI	RI
Age	(All)	Intercept	1.26	0.01	1.25 – 1.28	
		I_F	0.05	0.02	0.02 – 0.08	1.00
		Species richness	-0.04	0.02	-0.07 - -0.00	0.94
Orientation entering constriction	Intensity	Intercept	-1.58	0.42	-2.39 - -0.76	
		Age	-1.27	0.63	-2.51 - -0.04	1.00
		Time from release	1.44	0.56	0.34 – 2.54	1.00
		Pseudodactylogyrus spp	1.622	0.87	-3.32 – 0.77	0.96
	Prevalence	Intercept	-1.74	53.53	-106.66 – 103.18	
		S. inermis	-2.02	1.12	-4.21 – 10.75	1.00
		Age	-1.28	0.65	-2.56 – -0.00	1.00
		Time from release	1.63	0.67	0.31 – 2.04	1.00
	Diversity	Intercept	-1.42	0.31	-2.02 – 0.82	
		Age	-1.10	0.63	-2.33 – 0.13	1.00
		Time from release	1.58	0.58	0.45 – 2.72	1.00
		Total intensity	-1.78	0.87	-3.48 - -0.07	1.00
Reaction to accelerating velocity	Intensity	Intercept	0.11	0.17	-0.21 - -.44	
		Orientation	1.70	0.39	0.93 – 2.46	1.00
		A. crassus larvae	2.35	0.77	0.83 – 3.89	0.94
	Prevalence	Intercept	-0.06	0.14	-0.33 – 0.22	
		A. crassus larvae	0.61	0.29	0.05 – 1.17	1.00
		Orientation	1.59	0.37	0.86 – 2.31	1.00
	Diversity	Intercept	-0.04	0.14	-0.32 – 0.24	
		Orientation	1.80	0.40	1.02 – 2.58	1.00
		Age	0.01	0.30	-0.58 – 0.59	1.00
		1-*D*	-0.83	0.32	-1.46 - -0.20	1.00
		Species Richness	-0.77	0.34	-1.43 - -0.11	1.00
Return upstream following reaction	Intensity	Intercept	-0.67	0.25	-1.17 - -0.18	
		Orientation	1.28	0.32	0.65 – 1.91	1.00
		S. inermis	-1.93	1.56	-4.98 – 1.12	1.00
	Prevalence	Intercept	-0.55	0.15	-0.85 - -0.25	
		Orientation	1.16	0.33	0.52 – 1.80	1.00
		S. inermis	-1.18	0.59	-2.33 - -0.02	1.00
		Age	-0.04	0.30	-0.62 – 0.54	1.00
	Diversity	Intercept	-0.53	0.15	-0.82 - -0.24	
		Orientation	1.44	0.34	0.77 – 2.11	1.00
		Age	-0.06	0.30	-0.64 – 0.52	1.00
		Species richness	-0.88	0.35	-0.58 - -0.19	1.00
Time to reach downstream section	Intensity	Intercept	4.70	0.14	4.44 – 4.97	
		Orientation	1.30	0.33	0.65 – 1.96	1.00
		Age	0.46	0.28	-0.09 – 1.02	1.00
		I_E	-0.62	0.28	-1.16- -0.08	1.00
		A. crassus larvae	-1.17	0.49	-2.12 - -0.21	0.91
	Prevalence	Intercept	4.75	0.13	4.49 – 5.02	
		Orientation	1.20	0.33	0.56 – 1.85	1.00
		Pseudodactylogyrus spp.	-1.49	0.69	-2.84 - -0.14	1.00
		Age	0.31	0.29	-0.26 – 0.88	1.00
		I_E	-0.61	0.29	-1.17 - -0.05	0.94
	Diversity	Intercept	4.76	0.14	4.49 – 5.02	
		Orientation	1.10	0.34	0.44 – 1.76	1.00
		Age	0.44	0.29	-0.13 – 1.02	1.00
		I_E	-0.58	0.29	-1.14 - -0.02	0.90
Attempt upstream	(All)	Intercept	0.21	0.25	-0.27 – 0.70	
		Age	1.16	0.54	0.10 – 2.22	1.00
Upstream success	(All)	Intercept	0.46	0.32	-0.17 1.09	
		Age	-0.98	0.67	-2.30 – 0.34	1.00

Discussion

The downstream passage success of European eels (*Anguilla anguilla*) through European watercourses can be highly dependent on the number and structure of obstructions such as hydropower dams. Although upstream migration can be facilitated by eel ladders and fish passes, these are often less effective for downstream migrants. Flow velocity in the passes may be too low attract fish and this may result in fish passing directly through hydropower turbines (Enders et al. 2009a). A detailed understanding of behavioural, temporal and spatial patterns of movement of downstream migrating eels is critical for the design and implementation of measures to reduce passage through hydroelectric turbines and encourage through bypasses (Richkus 2001; Enders et al. 2009a). There is a paucity of knowledge about individual variation eel behaviour in response to accelerating velocity through constricted such as eel passes. This study aimed to fill some of these knowledge gaps by using an open channel flume to assess the downstream swimming behaviour of European eels. A constricted region in the central section of the flume generated an increase in flow velocity. The behaviour of the eels on approach to the constriction, taking into account morphological characteristics and parasite infection was measured over a 30 min period. Downstream passage success was highly dependent on the orientation in which the eels entered the constriction, but also was significantly related to age, silvering index and parasite infection.

Although the design of fish passes for downstream success of eels is poorly understood, Turnpenny et al. (1998) recommended intake flows which are 3-5% of the channel flow. This agrees with the 2-5% of turbine flow commonly recommended for non-anguillid fish at hydropower sites (Turnpenny and O'Keeffe 2005). For example, the hydropower station described by Pedersen et al. (2012) had a the fish ladder with flow rate of 0.15 m^3 s^{-1} and tube bypasses which had maximum discharge of 0.6 m^3 s^{-1} which is 2.9% of the mean total river discharge of 21 m^3 s^{-1}. In the current study, a similar discharge of 0.47 m^3 s^{-1} was selected. Adam et al. (1997) (translation available by Richkus 2001) determined from experimental flume studies that downstream swimming eels at velocities 0.3-0.5 m s^{-1} showed typically active movement with phases of controlled drift. At a higher velocity of 1.0 m s^{-1}, it became more difficult for the eels to swim in a controlled manner with the flow or to successfully swim against the current. In the current study,

flow velocities were similar to the range of those used by Adam et al. (1997) with an acceleration from 0.46 m s^{-1} at the upstream section to 1.06 m s^{-1} within the constriction.

European eels display negative rheotaxis when swimming in freshwater (Hain 1975), which explains why in the current study the majority of individuals entered the constricted region head first and reached the downstream section within a short period of time. Older eels were more likely to enter the constriction head first exhibiting negative rheotaxsis and eels with a higher silvering index took less time to reach the downstream region of the flume. There was a significant correlation between age and silvering index I_F, suggesting that older eels have a larger pectoral fin size relative to body length and therefore were more likely to be developed into silver eels and initiating the oceanic migratory phase (Durif et al. 2005).

The orientation in which *A. anguilla* entered the constricted region of the flume was the most important factor in determining whether the fish would continue downstream or exhibit recurrent behaviour and return to the upstream region of the channel. The eels entering head first were less likely react to the accelerating velocity by an increase in tail beat and burst swim to eventually return back upstream. If the eels initially spent more time at the upstream end of the flume before entering the constriction, they were more likely to enter the constriction tail first (positive rheotaxis) and therefore delay downstream migration through recurrent behaviour. It is therefore of key importance that fish passage design incorporates measures in which the flow of water into the passage is continuous from the main river so there are no areas in which the eels can delay entry into the passage and orientate themselves upstream.

Parasite infection had a significant effect on downstream swimming behaviour. Eels with a higher total parasite intensity were more likely to enter the constriction head first and those with higher species richness and higher Simpsons Diversity Index were less likely to react to the accelerating velocity. A higher species richness and infection with *Spinitectus inermis* resulted in the eels being less likely to return upstream. Additionally eels infected with *Pseudodactylgyrus* spp. were more likely to reach the downstream region faster than uninfected eels. It may be that the eels with higher migratory tendency have a higher exposure to infective stages of parasites due to increased movement between habitats from their home range to the location of the eel trap. However, from the

video analysis it was not possible to distinguish between passively drifting eels and those actively swimming downstream (as described in Richkus 2001). It is possible that the more heavily infected eels did not exhibit reactive behaviours to the accelerating velocity due to energetic costs imposed by the parasites and therefore passively drift downstream.

Interestingly, infection with *Anguillocloides crassus* larvae had the opposite effect to the other parasites on eel behaviour. Increased prevalence and intensity of the parasite larvae made the eels more likely to react to the high flow velocity in the constriction by a change in orientation from negative to positive rheotaxis or with a burst swim. However, there was no apparent effect of the larvae on the likelihood of returning back upstream. This indicates that although the infected eels are more likely to initiate a burst swim, they do necessarily achieve their target in returning upstream. In fact, increased infection intensity of the parasite larvae resulted in a reduced time to reach the downstream region. Although this finding is speculative at this stage, it is possible that this parasite is having a negative effect on the burst swimming ability of the host and requires further investigation. An alternative explanation is that the eels hosting the larval stages of the parasite are more likely to have recently been infected with the parasite through eating infected intermediate hosts such as copepods and ostracods. Silvering eels are known to cease feeding at the start of the migration (Kirk et al. 2000; Palstra et al. 2007a), so it is possible that the individuals infected with larval *A. crassus* are not yet fully developed into the migratory stage of development and therefore exhibit lower downstream migratory tendency. However again this theory is speculative as *A. crassus* larvae are known to remain in the swimbladder wall for extended periods before entering the lumen (Kirk et al. 2000).

To our knowledge, this study is the first to investigate the combined effects of parasitism and morphology on the short-term downstream behaviour of European eels. In addition, this study highlights the importance of orientation on the approach to a narrowed channel for downstream success. The possible effect of *A. crassus* on burst swimming and recurrent behaviour of the eels needs to be further investigated. European eels have relatively weak burst swimming capabilities compared to other migratory fish (e.g. Atlantic salmon *Salmo salar* smolts) which may inhibit their ability to avoid entering turbine intakes or impingement on trash racks (Russon et al. 2010). If burst swimming ability is further inhibited by *A. crassus* infection, this may partly explain differences in mortality through hydropower systems.

References

Adam B, Schwevers U, Dumont U (1997) Beiträge zum Schutz abwandernder Fische - Verhaltensbeobachtungen in einem Modellgerinne. Bibliothek Natur & Wissenschaft Band 16. Solingen: Verlag Natur & Wissenschaft

Armitage J, Hewlett NR, Twigg M, Lewin NC, Reading AJ, Williams CF, Aprahamian M, Way K, Feist SW, Peeler EJ (2014) Detection of *Herpesvirus anguillae* during two mortality investigations of wild European eel in England: implications for fishery management. Fish Manag Ecol 21:1-12

Barton K (2013) MuMIn: Multi-model inference. R package version 1.9.13. http://CRAN.R-project.org/package=MuMIn

Behrmann-Godel J, Eckmann R (2003) A preliminary telemetry study of the migration of silver European eel (*Anguilla anguilla* L.) in the River Mosel, Germany. Ecol Freshw Fish 12:196–202

Bonhommeau S, Chassot E, Planque B, Rivot E, Knap AH, Le Pape O (2008) Impact of climate on eel populations of the Northern Hemisphere. Mar Ecol Prog Ser 373:71–80

Brown AF, Chubb JC, Veltkamp CJ (1986) A key to the species of Acanthocephala parasitic in British freshwater fishes. J Fish Biol 28:327–334

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al revisited. J Parasitol 83:575–583

Calles O, Olsson IC, Comoglio C, Kemp PS, Blunden L, Schmitz M, Greenberg A (2010) Size-dependent mortality of migratory silver eels at a hydropower plant, and implications for escapement to the sea. Freshw Biol 55:2167–2180

Cea L, Puertas J, Pena L (2007) Velocity measurements on highly turbulent free surface flow using ADV. Exp Fluids 42:333–348

Christensen JM (1964) Burning of otoliths, a technique for age determination of soles and other fish. ICES J Mar Sci 29:73–81

Chubb JC, Pool DW, Veltkamp CJ (1987) A key to the species of cestodes (tapeworms) parasitic in British and Irish freshwater fishes. J Fish Biol 31:517–543

Coutant CC, Whitney RR (2000) Fish behavior in relation to passage through hydropower turbines: A Review. Trans Am Fish Soc 129:351–380

Durif C, Dufour S, Elie P (2005) The silvering process of *Anguilla anguilla* : a new classification from the yellow resident to the silver migrating stage. J Fish Biol 66:1025–1043

Durif C, Guibert A, Pierre E (2009) Morphological discrimination of the silvering stages of the European eel. In: Casselman JM, Cairns DK (eds) Eels at the Edge. Science, Status, and Conservation Concerns. American Fisheries Society Symposium Bethesda, Maryland, 58:103–111

Ellerby DJ, Spierts ILY, Altringham JD (2001) Fast muscle function in the European eel (*Anguilla anguilla*) during aquatic and terrestrial locomotion 204: 2231-2238

Enders EC, Gessel MH, Williams JG (2009) Development of successful fish passage structures for downstream migrants requires knowledge of their behavioural response to accelerating flow. Can J Fish Aquat Sci 66:2109–2117

Feunteun E (2002) Management and restoration of European eel population (*Anguilla anguilla*): An impossible bargain. Ecol Eng 18:575–591

Fulton TW (1904) The Growth Rate of Fishes: Twenty-second Annual Report, Part III. 141–241

Geeraerts C, Belpaire C (2010) The effects of contaminants in European eel: a review. Ecotoxicology 19:239–66

Gelman A, Su Y-S (2013) arm: Data analysis using regression and multilevel/hierarchical models. R package version 1.6-10. http://cran.r-project.org/package=arm

Gibson DI, Jones A, Bray RA (2002) Keys to the Trematoda (Volume 1). CABI Publishing, Wallingford and Natural History Museum, UK

Gollock MJ, Kennedy CR, Brown JA (2005a) European eels, *Anguilla anguilla* (L.), infected with *Anguillicola crassus* exhibit a more pronounced stress response to severe hypoxia than uninfected eels. J Fish Dis 28:429–436

Gollock MJ, Kennedy CR, Brown JA (2005b) Physiological responses to acute temperature increase in European eels *Anguilla anguilla* infected with *Anguillicola crassus*. Dis Aquat Organ 64:223–228

Gollock MJ, Kennedy CR, Quabius ES, Brown JA (2004) The effect of parasitism of European eels with the nematode, *Anguillicola crassus* on the impact of netting and aerial exposure. Aquaculture 233:45–54

Gosset C, Travade F, Durif C, Rives J, Elie P (2005) Tests of two types of bypass for downstream migration of eels at a small hydroelectric power plant. River Res Appl 21:1095–1105

Grueber CE, Nakagawa S, Laws RJ, Jamieson IG (2011) Multimodel inference in ecology and evolution: challenges and solutions. J Evol Biol 24:699–711

Hain JHW (1975) The behaviour of migratory eels, *Anguilla rostrata*, in response to current, salinitiy and lunar period. Helgoländ Wiss Meer 27:211-233

Hockley FA, Williams CF, Reading AJ, Taylor NGH, Cable J (2011) Parasite fauna of introduced pumpkinseed fish *Lepomis gibbosus*: first British record of *Onchocleidus dispar* (Monogenea). Dis Aquat Organ 97:65–73

ICES (2013) Report of the Joint EIFAAC/ICES Working Group on Eels (WGEEL). Sukarietta, Spain, 18-22 March and Copenhagen, Denmark 4-10 September, 2013, ICES CM 2013/ACOM:18. 851

Jansen HM, Winter HV, Bruijs MCM, Polman HJG (2007) Just go with the flow? Route selection and mortality during downstream migration of silver eels in relation to river discharge. ICES J Mar Sci 64:1437–1443

Jones A, Bray RA, Gibson D (2005) Keys to the Trematoda (Volume 2). CABI Publishing

Kennedy CR (2007) The pathogenic helminth parasites of eels. J Fish Dis 30:319–334

Kettle AJ, Asbjørn Vøllestad L, Wibig J (2011) Where once the eel and the elephant were together: decline of the European eel because of changing hydrology in southwest Europe and northwest Africa? Fish Fish 12:380–411

Kirk RS (2003) The impact of *Anguillicola crassus* on European eels. Fish Manag Ecol 10:385–394

Kirk RS, Lewis JW, Kennedy CR (2000) Survival and transmission of *Anguillicola crassus* Kuwahara, Niimi & Itagaki, 1974 (Nematoda) in seawater eels. Parasitol 120:289–295

Knights B, White EM (1998) Enhancing immigration and recruitment of eels: the use of passes and associated trapping systems. Fish Manag Ecol 5:459–471

Knopf K (2006) The swimbladder nematode *Anguillicola crassus* in the European eel *Anguilla anguilla* and the Japanese eel *Anguilla japonica*: differences in susceptibility and immunity between a recently colonized host and the original host. J Helminthol 80:129–136

Larinier M, Travade F (2002) Downstream migration: problems and facilities. Bull Français la Pêche la Piscic 364 sppl: 181–207

Moravec F (1994) Parasitic Nematodes of Freshwater Fishes of Europe. Kluwer Academic Publishers

Norton J, Rollinson D, Lewis JW (2005) Epidemiology of *Anguillicola crassus* in the European eel (*Anguilla anguilla*) from two rivers in southern England. Parasitology 130:679–686

Palstra A, Curiel D, Fekkes M, de Bakker M, Székely C, van Ginneken V, van den Thillart G (2007a) Swimming stimulates oocyte development in European eel. Aquaculture 270:321–332

Palstra AP, Heppener DFM, van Ginneken VJT, Székely Cm van den Thillart GEEJM (2007b) Swimming performance of silver eels is severely impaired by the swim-bladder parasite *Anguillicola crassus*. J Exp Mar Bio Ecol 352:244–256

Pankhurst NW (1982) Relation of visual changes to the onset of sexual maturation in the European eel *Anguilla anguillai* (L.). J Fish Biol 21:127–140

Pedersen MI, Jepsen N, Aarestrup K, Koed A, Pedersen S, Økland F (2012) Loss of European silver eel passing a hydropower station. J Appl Ichthyol 28:189–193

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Richkus W (2001) Review and documentation of research and technologies on passage and protection of downstream migrating catadromous eels at hydroelectric facilities. Final Report to the Electric Power Research Institute No. 1000730, November 2001, EPRI Customer Fulfillmen

Russon IJ, Kemp PS (2011) Experimental quantification of the swimming performance and behaviour of spawning run river lamprey *Lampetra fluviatilis* and European eel *Anguilla anguilla*. J Fish Biol 78:1965–1975

Russon IJ, Kemp PS, Calles O (2010) Response of downstream migrating adult European eels (*Anguilla anguilla*) to bar racks under experimental conditions. Ecol Freshw Fish 19:197–205

Sprengel G, Luchtenberg H (1991) Infection by endoparasites reduces maximum swimming speed of European smelt *Osmerus eperlanus* and European eel *Anguilla Anguilla*. Dis Aquat Organ 11:31–35

Tesch F-W (2003) The Eel, 5th Ed. Blackwell Publishing Company, Oxford

Turnpenny AWH, O'Keeffe N (2005) Screening for intake and outfalls: a best practice guide. Environment Agency Science Report SC030231

Turnpenny AWH, Struthers G, Hanson KP (1998) A UK guide to intake fish-screening regulations, policy and best practice. Contractors report to the Energy Technology Support Unit, Harwell, Project No. ETSU H/00052/00/00

Wahlberg M, Westerberg H, Aarestrup K, et al. (2014) Evidence of marine mammal predation of the European eel (*Anguilla anguilla* L.) on its marine migration. Deep Sea Res 86:32–38

Chapter 8. General Discussion

Throughout the globe, freshwater rivers and streams are increasingly under pressure from human interference through water abstraction, power generation, transport, food resource and recreation. These pressures have resulted in modifications which disrupt the natural flow regime, for example by homogenising flow (e.g. canals and culverts) or causing large fluctuations in discharge when dams are opened and closed (Murchie et al. 2008). In the UK, over 40% of river length is classified as 'severely modified' (Environment Agency 2010). With the added pressure of climate change causing more extreme rainfall patterns and variable river discharge (Ormerod 2009) which will co-vary with other environmental factors such as temperature, turbidity and overhead cover; the resultant effect on fish populations is likely to be severe (Bunn and Arthington 2002; Murchie et al. 2008). Fish disease is also an important issue, as an introduction of parasites into new areas can result in mass mortalities (e.g. *Gyrodactylus salaris* in Norway) (Bakke et al. 2007). However, it is unclear how variable flows will affect diseased fish and parasite transmission.

This thesis investigated the interaction between parasite infection and variable flows utilising two fish model systems the Trinidadian guppy (*Poecilia reticulata*), the three-spined stickleback (*Gasterosteus aculeatus*) and a species of conservation concern the European eel (*Anguilla anguilla*). Key findings were: (i) infection with directly transmitted parasites can reduce fish shoaling tendency, but the magnitude of the effect is dependent on flow condition (Chapters 2 and 3); (ii) differences in anaerobic and aerobic swimming performance may be explained by microhabitat choices, which in turn could affect exposure to infectious stages of parasites (Chapters 4 and 5); (iii) parasite infection can affect the fish host's tolerance of increased turbulence, shear stress and velocity (Chapter 6); and (iv) introduced parasites can have a detrimental effect on the host swimming performance and behaviour (Chapter 7).

In flowing water such as rivers and streams, it can be essential for resident fish to maintain position in order to stay within optimum breeding and feeding grounds, keep up with other shoal members or shelter from predators (Carlson and Lauder 2011; Bleckmann et al. 2012). Natural watercourses provide a habitat in which heterogeneous flow is generated around obstacles such as boulders and woody debris or from coarse river beds (Gerstner 1998). Turbulence and vortices generated in these habitats can be either

beneficial or detrimental to fish, depending on the size, direction and strength of the component (Hockley et al. 2014a). These regions may therefore provide areas of low flow in which fish can shelter from the higher velocities in the free stream (Przybilla et al. 2010; Carlson and Lauder 2011). This microhabitat selection is therefore vital in a fish's life history in order to conserve energy which can otherwise be allocated to feeding, courtship and avoiding predators. This was demonstrated in Chapters 3 and 4 where three-spined sticklebacks utilised the lower velocities near the bed of the flume to improve swimming performance and in Chapter 6 where guppies of different size, sex or parasite load selected different microhabitat regions in order to station hold.

The cause-or-effect relationship between parasite infection and behavioural response of the host to varying hydraulic conditions may be difficult to disentangle. On one hand, the parasite itself may be causing the host (or potential host) to behave abnormally by either selecting alternative microhabitats or responding differently to hydraulic conditions. This behaviour may be a result of direct manipulation by the parasite to increase transmission potential, indirectly through pathological damage, or as a result of social response of the host to avoid further transmission to shoal mates. The latter was demonstrated in Chapters 2 and 3 where the presence of a guppy infected with *G. turnbulli* caused reduction in shoaling behaviour, either through the infected individual preventing transmission to shoal mates, or the uninfected members avoiding becoming infected. However, this effect was only observed in the static condition, whereas in flow there was little behavioural effect of the parasite. As a result, the guppies in the flow condition reached a higher peak prevalence (Chapter 3). When infected with *G. turnbulli,* guppies were more likely to be found in the region of low turbulence intensity and moderate longitudinal velocities, suggesting that the parasite is affecting the tolerance of the fish to turbulence (Chapter 6). Individuals infected with *A. crassus* were more likely to initiate a reaction to the accelerating velocity (Chapter 7), perhaps due to the hosts having a lower tolerance to the higher flow rate and shear stress associated with the constricted region.

On the other hand, parasite infection may result from individual variation in host behaviour in response to other environmental factors. For example, guppies in Trinidad have higher infections of the directly transmitted ectoparasites *Gyrodactylus* spp. in downstream populations where predation pressure is high. The fish in these populations counteracted predation pressure by shoaling more frequently, which in turn facilitated transmission (see Stephenson et al. 2014). A similar situation where fish behaviour

indirectly affected parasite burden was demonstrated in Chapter 4. In this study, sticklebacks with a greater burst swimming ability had a higher infection level with the endoparasite *Phyllodistomum folium*. The intermediate hosts of this digenean are freshwater mussels which inhabit fast flowing water, so it is likely that the fish with enhanced swimming ability spent more time in the faster river currents and therefore had a higher exposure to the infective stages of the parasite. Similarly, in Chapter 7 the eels which demonstrated increased downstream migratory tendency had a higher parasite species richness and diversity. This may again be a result of higher exposure to infective stages of various parasite taxa from increased downstream movement through the river system.

Research into the relationship between river hydraulics and fish disease is vital in supporting decisions by river managers, fish farmers and aquarium keepers to prevent parasite introduction, spread and resultant mortality. Some of these questions can be answered using model systems (Chapters 2-6 and Appendix 1), however the drawbacks of using model species must also be considered. There may be difficulties with extrapolating results from studies with model species to other systems due to the huge global diversity of fish and parasite species and changeable environmental conditions. However when trying to answer general ecological questions, a good starting point is to study specific interactions between environmental variables and species in which their biology is well understood. This will allow scientists to have a first insight into important interactions and therefore prove a good starting point for further study.

For species of conservation concern, it is particularly important to consider the ethical sensitivity of experimental research, particularly if euthanasia is the end point of the study. Understanding eel health interactions in Chapter 7 inevitably required sacrificial sampling, so it was important to maximise data retrieval from every eel examined. This was achieved by thorough examination of all tissues and archiving unused material for future work. This study has led to the development of a standard protocol for eel health which is currently being developed further by the Environment Agency, with contributions from Cefas, Cardiff University, University of Stirling and Agi-Food and Biosciences, Belfast. The protocol will describe a standard fish health protocol to assist with collection, examination, handling and archiving of eel tissues. The aim is to maximise retrieval of data from every eel examined, to support collaborative research opportunities and to promote knowledge transfer to better integrate eel health into the

future management of eel stocks. A comprehensive understanding of eel health requires the involvement of many different disciplines. By using a consistent approach to promote good quality data collection, collaboration and tissue archive, Eel Management Plans can be better informed which will promote eel recovery into the future. This fish health protocol could then be used as a model for other endangered species.

Continuing the findings of this thesis, further studies are needed to corroborate results of open channel flow experiments with field observations. As attempted in the Lydney Weir project (Appendix 2), the ability of European eels to migrate both up and downstream through obstacles and fish passes may be achieved through tagging and recapture methods in rivers. This can be followed by further tests in laboratory flumes to verify whether swimming ability observed in the laboratory corresponds to swimming abilities observed in the wild. Regular monitoring of Trinidadian river flow and *Gyrodactylus* spp. abundance in guppies will give a clearer idea of how flow regimes may affect diseased fish with the added natural pressures such as predation, temperature fluctuation and pollution. Finally, the possible effect of the antihelminthic praziquantel on swimming ability of fish (Chapter 4 and Appendix 1) requires further experimental research. This finding may put past behavioural studies into question if the drug was used to clear parasite infection prior to experimentation.

Conclusion

This thesis has demonstrated the importance of flow heterogeneity in natural systems to promote flow refugia for fish, particularly those suffering from the adverse effects of parasitism. In addition, the work has shown that flow condition plays an important role in behavioural decisions of fish. Many past laboratory studies have taken place in static tanks where the water remains stationary except for minimal disturbance to the water by filter systems, as opposed to strong, variable and unidirectional flow action encountered in natural rivers and streams. For example, only a small difference in flow rate from 0.0 cm s^{-1} to 2.6 cm s^{-1} in Chapter 3 had a significant effect on shoaling behaviour and parasite transmission in guppies. It is therefore important that future laboratory studies investigating the effect of parasitism on host behaviour incorporates flow condition into the study. As with all laboratory studies, open channel flumes allow for a controlled approach to experimentation but also allows for manipulation of velocity and turbulence regimes more akin to natural systems experienced in the wild. For example, in this thesis,

water velocities ranged from 2.6 cm s^{-1} in Chapter 3 (akin to the lower range of flows in guppy streams in Trinidad) to 106 cm s^{-1} in Chapter 7 (akin to high flows experienced by eels in UK rivers). Use of open channel flumes therefore bridges the gap between natural systems and static aquaria.

References

Bakke T A, Cable J, Harris PD (2007) The biology of gyrodactylid monogeneans: the "Russian-doll killers". Adv Parasitol 64:161–376

Bleckmann H, Przybilla A, Klein A, Schmitz A, Kunze S, Christoph B (2012) Station holding of trout: Behaviour, physiology and hydrodynamics. In Tropea C, Bleckmann H (eds) Nature-Inspired Fluid Mechanics, Springer 161–177

Bunn SE, Arthington AH (2002) Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environ Manage 30:492–507

Carlson RL, Lauder G V (2011) Escaping the flow: boundary layer use by the darter *Etheostoma tetrazonum* (Percidae) during benthic station holding. J Exp Biol 214:1181–1193

Environment Agency (2010) River habitats in England and Wales: current state and changes since 1995-96. Environment Agency, Bristol

Gerstner CL (1998) Use of substratum ripples for flow refuging by Atlantic cod, *Gadus morhua*. Environ Biol Fishes 51:455–460

Hockley FA, Wilson CAME, Brew A, Cable J (2014) Fish responses to flow velocity and turbulence in relation to size, sex and parasite load. J R Soc Interface 11:20130814

Murchie KJ, Hair KPE, Pullen CE, Redpath TD, Stephens HR, Cooke SJ (2008) Fish response to modified flow regimes in regulated rivers: research methods, effects and opportunities. River Res Appl 24:197–217

Ormerod SJ (2009) Climate change, river conservation and the adaptation challenge. Aquat Conserv Mar Freshw Ecosyst 19:609–613

Przybilla A, Kunze S, Rudert A, Bleckmann H, Brücker C (2010) Entraining in trout: a behavioural and hydrodynamic analysis. J Exp Biol 213:2976–2986

Stephenson JF, van Oosterhout C, Mohammed RS, Cable J (2014) Parasites of Trinidadian guppies: evidence for sex- and age-specific trait-mediated indirect effects of predators. Ecology [In press]

Appendix A. Effect of Praziquantel on burst swimming response in guppies, *Poecilla reticulata*

Hockley FA, Stone C, Mitchell R, Cable J

Introduction

In a previous experiment (Chapter 4), a possible side effect of the antihelminthic praziquantel (PZQ) was observed to cause an increased burst swimming performance in the stickleback (*Gasterosteus aculeatus*). To further investigate this effect and eliminate any possible effects of ectoparasites, the burst swimming performance test was repeated on guppies (*Poecilia reticulata*).

Methods

Naïve offspring (n=11) of guppies captured from the Tacurigua River, Trinidad in 2005 with mean standard length 18.15 (SE 0.56) mm were used for the burst swimming trials. Two months prior to the experiment, fish anaesthetised with 0.02% tricaine methane sulphonate (MS222) were screened using a stereo-microscope to confirm that they were clear of ectoparasite infection. Four individuals (two male, two female) were treated with 0.04g/ml PZQ and seven individuals (three males, four females) were sham treated by adding the equivalent volume of dechlorinated water. Fish were treated individually for 24 hours in 1 L pots before the burst swimming trials were started.

Immediately following PZQ treatment or sham treatment, individual fish were placed in a 60 x 30 cm aquarium tank filled to 3 cm depth dechlorinated water and were restricted to a 12 cm x 12 cm focal arena using plastic mesh covered in thin netting. The fish was habituated in the focal arena for 15 min before a burst swim was initiated by dropping an orange ball from 25 cm height into the main section of the tank at the opposite end to the focal area. This was repeated in a series of three dropping events separated by 4 min intervals, with 15 min intervals between each series to prevent habituation to the stimulus (Garenc et al. 1999). This resulted in a total of nine burst swim initiation events per fish. Burst responses were captured using an IR LED mini CCTV camera recording at 25 frames per second. The video sequences were analysed using Ethovision XT 8.0 to record the velocity of each response and the total distance moved by the fish. The burst swim response was analysed for both the initial response (60 ms – 2-3 frames) and the full response (160 ms – 5 frames), starting from when the fish formed a C-shape. Any

responses where the fish were within 1 cm of the sides of the focal arena were disregarded. The mean velocity and distance moved of the three fastest burst swims were calculated for each fish. The total number of burst swims initiated out of the nine attempts was also recorded. Data were analysed using R statistical software (R Core Team 2013) using general linear models to analyse the effect of PZQ treatment, sex, standard length and weight on the total distance moved, velocity of response and number of responses.

Results

During the initial response (first 60 ms of the burst swim) there was a significant positive relationship between guppy standard length and the velocity of burst swim (t-value=2.702, p=0.042) (Figure A.1). However, there was no effect of fish weight, sex or praziquantel treatment during the initial response.

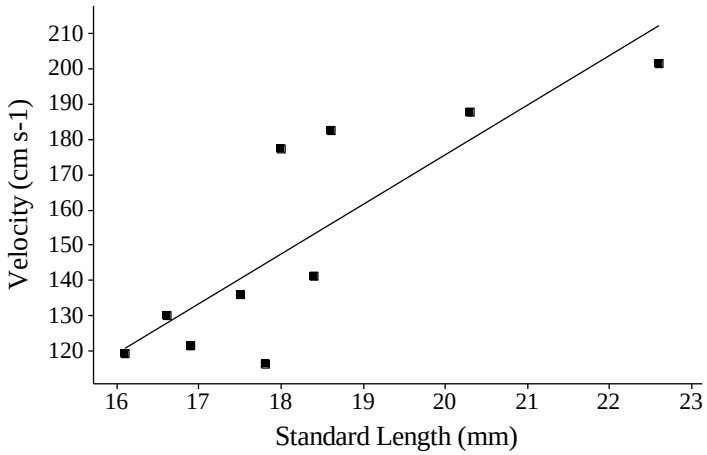

Figure A.1. Increase in burst swim velocity with increasing standard length of guppies (*Poecilia reticulata*).

For the full duration of the burst swim response (160 ms), treatment with praziquantel resulted in a significant increase in the distance moved by the guppies (t-value=-5.079, p=0.004) (Figure A.2). The velocity of the fast start response was also higher in the treated group, however this was not significant (t-value=-0.800, p=0.460). There was no significant difference in the number of burst responses by the treated and untreated groups and there was no effect of fish length, weight or sex on the full burst swimming performance.

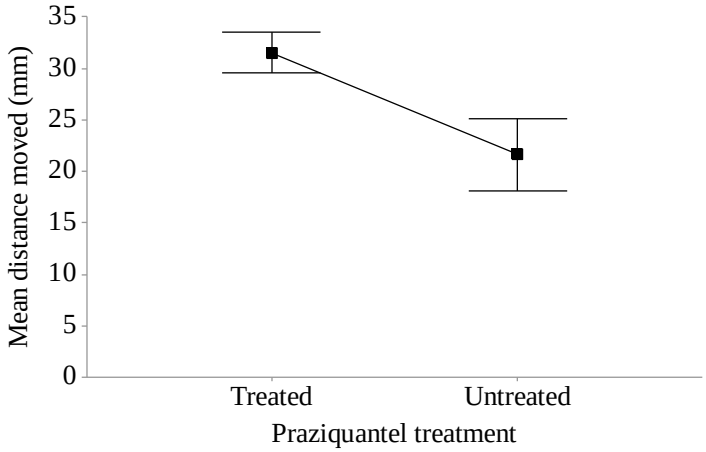

Figure A.2. Increased distance moved during burst swim response in guppies *Poecilia reticulata* when treated with praziquantel. Squares show mean and bars show 95% confidence intervals.

Discussion

Results from this trial indicate that larger guppies had a faster initial burst swimming response to the stimulus compared to smaller counterparts, however there was no size effect for the full duration of the response. This may be because the larger fish have increased muscle power to produce the initial burst swim (Wakeling et al. 1999), but increased size has a drag effect so the fish cannot reach as high velocity in the later stages of the burst swim.

The antihelminthic drug praziquantel positively affects the burst swimming performance of guppies. The fish moved significantly further when treated with the drug compared to untreated counterparts. There was also a small but insignificant increase in the velocity of the burst swim in treated fish. This result supports a previous finding whereby sticklebacks increased the velocity of burst swims when treated with PZQ (see Chapter 4). The mode of action of PZQ when used as an antihelminthic drug is thought to be through activation of voltage-gated calcium Ca^{2+} channels which results in an influx of extracellular calcium and causes the contraction of the parasite's muscles (Doenhoff et al. 2008; You et al. 2013). It could be possible that the drug is also causing a contraction of the host muscle, therefore increasing burst swimming performance. Further investigations are needed to assess how long PZQ resides in host tissue, how long swimming performance is affected post-treatment and whether the drug could also improve aerobic swimming performance in fish.

References

Doenhoff MJ, Cioli D, Utzinger J (2008) Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. Curr Opin Infect Dis 21:659–667

Garenc C, Couture P, Laflamme M-A, Guderley H (1999) Metabolic correlates of burst swimming capacity of juvenile and adult threespine stickleback (*Gasterosteus aculeatus*). J Comp Physiol B 169:113–122

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Wakeling J, Kemp K, Johnston I (1999) The biomechanics of fast-starts during ontogeny in the common carp *Cyprinus carpio*. J Exp Biol 202:3057–3067

You H, McManus DP, Hu W, Smout MJ, Brindley PJ, Gobert GN (2013) Transcriptional responses of *in vivo* praziquantel exposure in schistosomes identifies a functional role for calcium signalling pathway member CamKII. PLoS Pathog 9:e1003254

Appendix B. Lydney weir project

Introduction

European eels (*Anguilla anguilla*) have complex lifecycles which involves a migratory stage from the ocean to upstream freshwater systems. Upon reaching freshwater habitats, the young eels, known as elvers, develop into yellow eels and remain within the river system for the majority of their life before maturation and returning to oceanic spawning grounds where they die. Successful upstream passage is therefore essential for survival and development of this species. European eels have been subject to substantial declines in recent decades (ICES 2013), which have been partly attributed to riverine barriers to migration, including weirs used for flow gauging (Coe and Kibel 2014). There are an estimated 1500 gauging weirs throughout the UK (CEH 2014) which are essential for managing water flows for abstraction and forecasting flooding. Fish passage over gauging weirs can be aided by the installation of fish passes including eel ladders. However to maintain the accuracy of the gauge, the ladders must not affect the river flow by more than 1% (Coe and Kibel 2014). Vertical bristles which are commonly used as eel ladders are not suitable for flat-V weirs because the outer edges of the weir dries out at low discharge. Therefore, most flat-V gauging weirs have an ‘up and over’ eel pass which is a separate channel above the weir maintained by a submersible pump. However, the pumps require constant maintenance, repair and are relatively expensive. An alternative solution for improving eel passage over flat-V weirs is the installation of 0.5 x 0.5 m eel tiles which have been developed by the Environment Agency (Figure B.1). The tiles can be either bolted directly on to the surface of the weir or recessed into the weir surface so that the top of the protrusions are level with the existing water level.

Figure B.1. Environment Agency developed tile to aid upstream passage of eels over weirs.

The introduction of the invasive parasite *Anguillicoloides crassus* has also been attributed to the European eel decline. *A. crassus* causes severe pathological damage to the host swimbladder (Kirk 2003) and is thought to impair swimming ability during the oceanic migratory stage of the eel lifecycle. Evidence from Chapter 7 suggests that the parasite also might affect burst swimming ability in downstream migrating adult eels, however, it is unclear how the parasite might affect upstream passage in elvers.

The aim of this project was to assess the efficiency of two eel tile ladders over a flat-V weir in relation to eel size, flow rate, time of day and parasite infection. Two types of tile ladders were compared, the first was recessed into the weir surface and the second was bolted on top of the weir. A further aim was to corroborate swimming ability tests observed in laboratory flume experiments with that observed in the wild and to assess whether infection with *A. crassus* affects the ability of eels to climb the tile ladders.

Methods

Field experiments

A concrete flat-V gauging weir on the River Lyd, Lydney, Gloucesterhire (NGR SO 63161 03590) of 3.6 m width and face of 2.4 m length down the centre line was used for the field experiment. The river has a relatively high base flow due to the addition of spring water from old mines (Figure B.2). The experiment was conducted by Fishtek Consulting and Royal Haskoning DHV contracted by the Environment Agency (Coe and Kibel 2014). Two sets of eel tiles were installed either side of the gauging weir in May 2013. On one side the tiles were recessed into the surface to minimise disruption to the flow over the top of the weir (‘recessed’ ladder) (Figure B.3A) and the other side the tiles are simply bolted on top of the weir surface which allows easy installation and lower flow velocities over the tiles (‘raised’ ladder) (Figure B.3B).

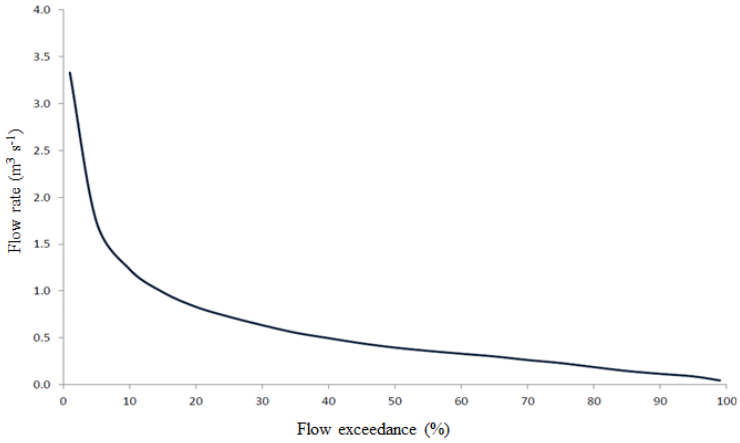

Figure B.2. Flow duration curve for the Lydney weir showing percent of time the flow equals or exceeds each discharge.

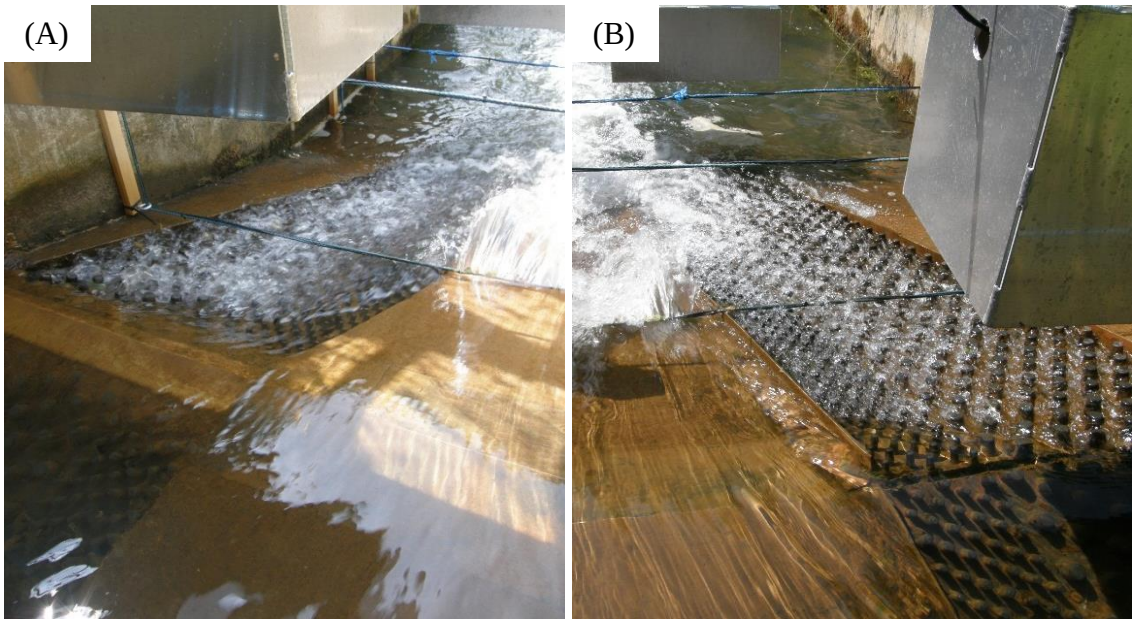

Figure B.3. Flat-V gauging weir on the River Lyd. Eel tiles have been installed either side of the weir in two configurations. (A) Tiles recessed in the weir as per hydrometric preferences and (B) raised tiles fitted on top of weir surface as per fisheries preference.

A total of five pit-tag loops (Wyre Micro Design Ltd) were installed on the weir on 11th July 2013. Two loops were positioned on the downstream face (approximately 2-2.5 m downstream from crest) on each ladder, two were positioned in the middle of each ladder (approximately 0.5 m downstream from crest) and one large loop was installed at the crest of the weir. For each set of tiles, two overhead IR cameras were placed above the weir and an underwater camera positioned at the entrance of the eel ladder (Figure B.4).

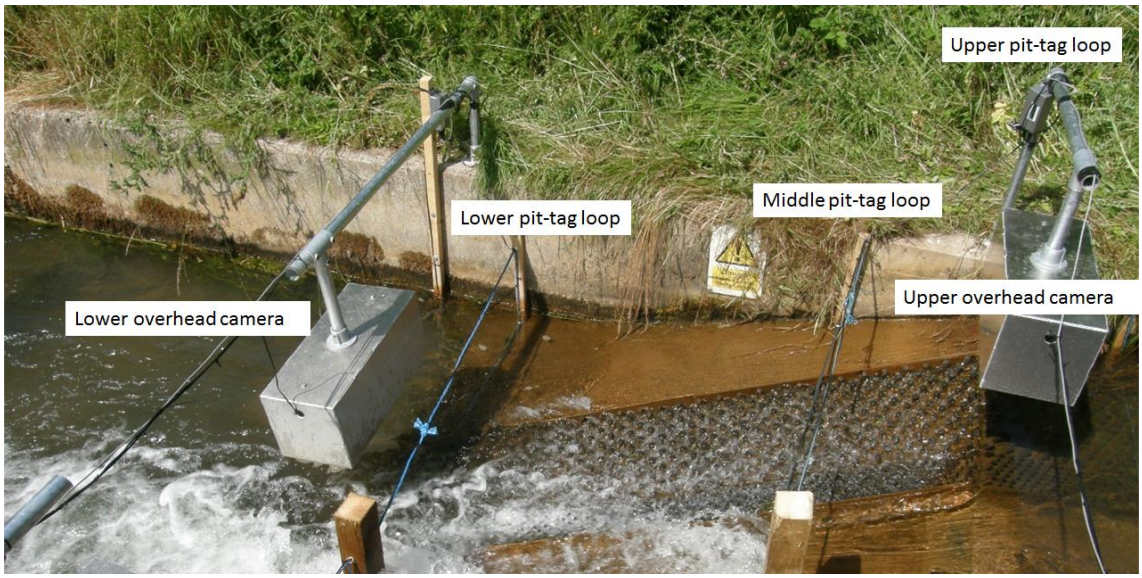

Figure B.4. Raised tile ladder showing the three PIT-tag loops and two overhead IR cameras to record upstream migration of European eel elvers.

On 10th July 2013, European eel elvers (n=72) of length 270 - 615 mm were captured by electrofishing approximately 300 m downstream of the weir between NGR SO 6363 02634 and NGR SO 6360 0321. The following day, a passive integrated transponder (PIT) tag (12 x 2 mm HDX tags) was implanted under the skin of each eel after anaesthetising with benzocaine. Each fish was weighed to the nearest 5 g and total length measured to the nearest 10 mm. The eels were allowed to recover overnight, then released in the large pool directly below the weir at 14:04 GMT on 12th July 2013.

The number of eels moving over each tile ladder was recorded from both PIT-tag recordings as tagged eels moved through the loops and from observations on the IR cameras. The time of day and flow rate was recorded for each upstream attempt and for the PIT-tagged eels, the length of the eel was also recorded.

In order to recapture the eels, a fyke net was positioned 10-15 m upstream of the weir and checked daily. Following recapture, elvers were to be transported to Cardiff School of Biosciences for further swimming tests in an open channel flume. However, no eels were captured in the net and it was subsequently removed on 19th July 2013. The PIT tag loops were removed from the site on 20th September and cameras removed on 8th October 2013.

Planned further methods

The following methods describe the planned flume trials and health screens.

Flume trials

The swimming ability of the recaptured PIT-tagged elvers would have been tested using a 10 m long, 0.29 m width glass walled recirculating flume (described in Chapters 2, 5 and 6). In the first test, the EA tiles identical to those fixed to the weir at Lydney would be placed along the length of the flume. Following acclimatisation of 1 hour at area mean velocity of 10 cm s^{-1}, the velocity would be increased to 55 cm s^{-1} and an individual eel released at the downstream end of the channel. The time taken to reach the upstream end of the flume would be recorded in addition to specific behaviours such as number and time of resting periods within the tile protrusions and number of burst swims upstream.

Following a 7 day resting period, the elvers would be subject to a second swim test. The eels would be restricted to a 1 m length section of the flume and then the critical swimming speed of each individual measured using the *Ucrit* method described in Chapter 5 without the use of a bed substrate. The vertical position of the eels would be recorded to take into account differences in flow speed close to the bed of the flume.

In order to assess the velocity and turbulent conditions experience by the elvers within the tiles, the velocity profile for all flow rates would be measured using acoustic Doppler velocimetry (ADV) with a Nortek Vectrino II as described in Chapter 5.

Health screens

Following the flume trials, the elvers would be dissected and screened for internal and external parasites as described in Chapter 7. Dissections and processing would take place at Cardiff University in line with the standard protocol of eel health developed by the Environment Agency (described in Chapter 8). The swim bladder will also be removed and fixed for histopathological processing to measure the level of damage caused to the organ by *Anguillicoloides crassus*. The eels will also be measured (length and breadth) to take into account drag effects on swimming ability.

Results and Discussion

Of the 72 tagged eels, 33 were detected by the PIT-tag loops. In total 19 eels passed over the weir crest through the top loop. There was no significant difference in upstream

passage success between the two tile ladders, with 10 eels using the recessed tiles and 9 eels using the raised tiles. Due to the turbulence generated by the raised tiles, it was not possible to detect the number of untagged eels passing the weir crest using the overhead cameras. However for the recessed tiles, 104 eels attempted to move upstream past the weir, of which 52 were successful (Figure B.5).

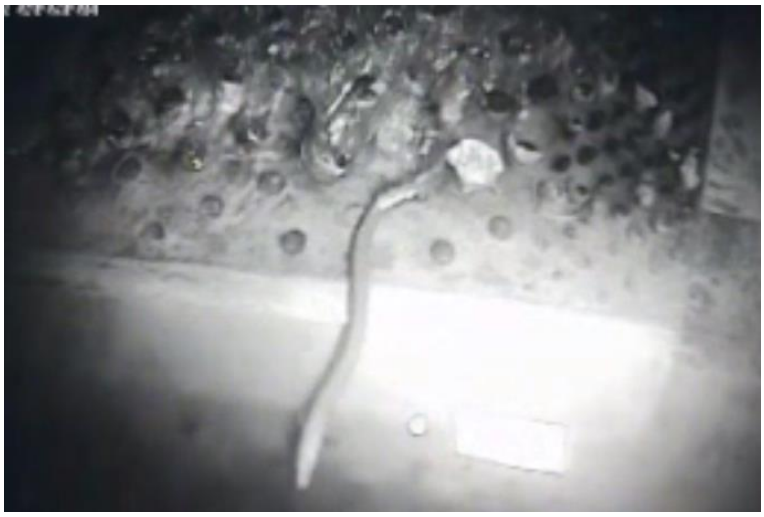

Figure B.5. Infra-red camera image of a European eel (*Anguilla anguilla*) elver moving over the crest of the flat-V gauging weir using recessed tiles.

The eels successfully attempting upstream passage over the weir were significantly larger in size than the unsuccessful eels ($U=483.5$, $p<0.01$) (Figure B.6). However, the recessed tiles appeared to be less suitable for smaller eels. Eels moving over the recessed tiles were significantly larger than those moving over the raised tiles (Mann-Whitney, $U=23.5$, $p=0.04$). Of these eels which attempted upstream movement in the recessed tiles, there were no successful attempts by eels <100 mm and only 37% of 101-150 mm eels were successful. This is in contrast to findings in Chapter 8 where there was no size effect on the downstream or upstream behaviour of adult eels in an open channel flume experiment.

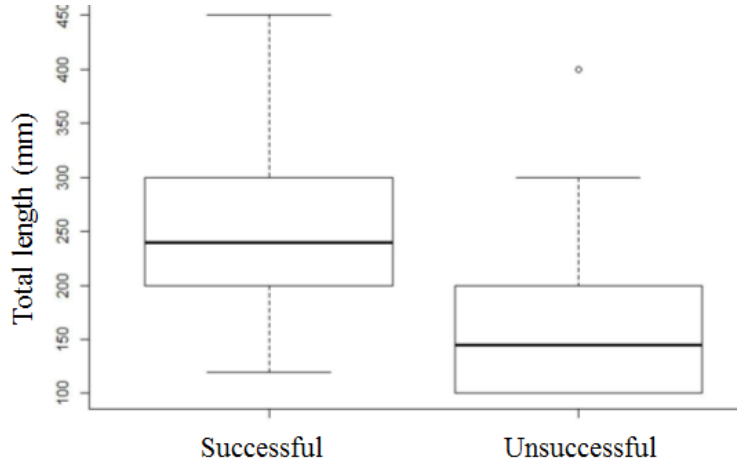

Figure B.6. Larger European eel elvers were more successful in upstream ascent over the weir. Thick lines denotes median value, boxes interquartile range and error bars show data range. Circle denotes outlier value.

Upstream passage occurred most frequently during dusk and the first few hours of darkness between 21:00-01:00. This coresponds with the increased activitiy of eels after sunset observed in previous studies (Baras et al. 1998; Riley et al. 2011). During the study period, flow rates ranged from 0.177 and 0.422 $m^3 s^{-1}$, which was equal to approximately Q80-Q45. The eels were most likely to attempt upstream passage up the weir during the most frequent flows of Q80-Q60. The eels also frequently ascended the weir when flows were low at Q99-Q80, indicating a bias towards lower flow rates for upstream migration.

References

Baras E, Jeandrain D, Serouge B, Philippart JC (1998) Seasonal variations in time and space utilization by radio-tagged yellow eels *Anguilla anguilla* (L.) in a small stream. Hydrobiologia 371/372:187–198

CEH (2014) Hydrometry in the UK. NERC - Centre for Ecology and Hydrology [online] http://www.ceh.ac.uk/data/nrfa/hydrometry/hydrometry.html

Coe T, Kibel P (2014) Assessment of eel tiles as means to improve eel passage at gauging weirs - pit-tagging study. Fishtek Consulting Ltd.

ICES (2013) Report of the Joint EIFAAC/ICES Working Group on Eels (WGEEL). Sukarietta, Spain, 18-22 March and Copenhagen, Denmark 4-10 September, 2013, ICES CM 2013/ACOM:18

Kirk RS (2003) The impact of *Anguillicola crassus* on European eels. Fish Manag Ecol 10:385–394

Riley WD, Walker AM, Bendall B, Ives MJ (2011) Movements of the European eel (*Anguilla anguilla*) in a chalk stream. Ecol Freshw Fish 20:628–635