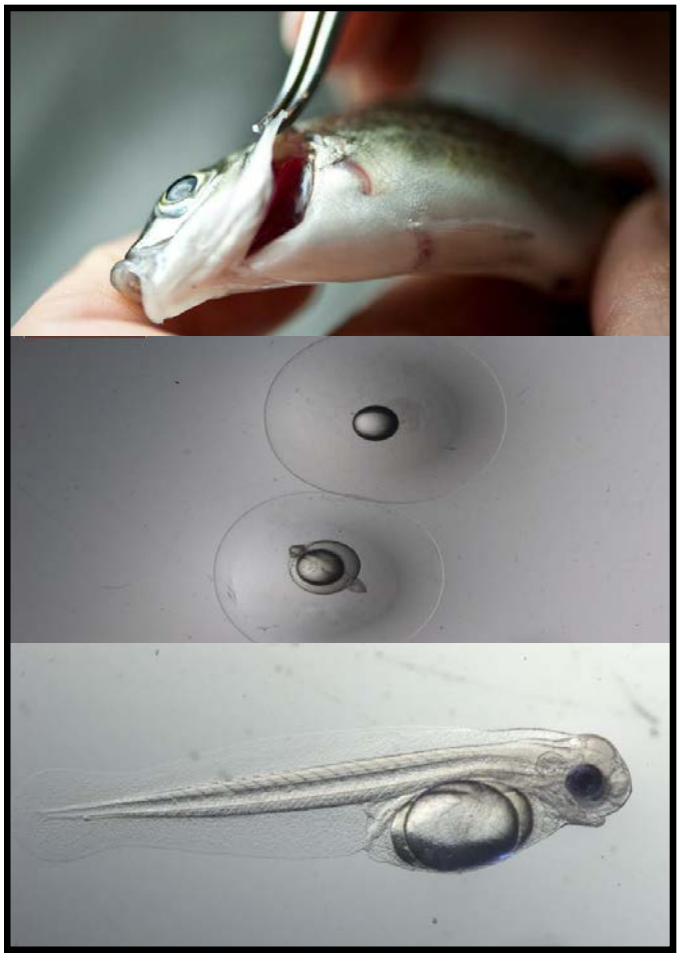
Downstream fish passage criteria for hydropower and irrigation infrastructure in the Murray–Darling Basin

Craig Boys, Anna Navarro, Wayne Robinson, Anthony Fowler, Stephen Chilcott, Brett Miller, Brett Pflugrath, Lee Baumgartner, Jarrod McPherson, Richard Brown and Zhiqun Deng



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ABBREVIATIONS

DPH days post hatch

GUI graphical user interface

LS_{10} lethal shear that would kill 10% more fish than the handling control (strain rate of zero) within 24 hours of exposure

LS_{75} lethal shear that would kill 75% more fish than the handling control (strain rate of zero) within 24 hours of exposure

MDB Murray–Darling Basin

MW megawatts

NFC Narrandera Fisheries Centre

NSW New South Wales

PC pharyngo-clitheral

PSFI Port Stephens Fisheries Institute

RPC ratio of pressure change

SE standard error

GLOSSARY OF TERMS AS DEFINED IN THIS REPORT

Acclimation pressure/depth	The pressure/depth within the water column at which the density of a fish is equal to the density of the surrounding water. At greater depths/pressure, a fish must secrete more gas into its swim bladder to maintain its density.
Barotrauma	Injury caused by rapid or extreme changes in pressure, typically a reduction or decompression.
Downstream fish passage	The movement of fish in the direction of water flow, which may include movements down a river channel, but also may involve moving laterally into and out of floodplains.
Emphysema	A condition in which air is abnormally present within the body tissues.
Exophthalmia	Abnormal protrusion of the eyeball or eyeballs.
Hydropower	The generation of electricity from the kinetic power of moving water. The kinetic energy of water is typically generated by having two water bodies at different heights (termed head), usually at a reservoir dam or weir. In a typical installation, water flows over a turbine and generates pressure, which causes the shaft to rotate. The rotating shaft is connected to an electrical generator, which converts the motion of the shaft into electricity.
Mini hydropower	The definition of a mini hydro project varies, but a generating capacity of up to 10 megawatts (MW) is generally accepted as the upper limit. This makes the technology suitable for low-head applications.
Nadir pressure	The lowest point of pressure measured.
Ratio of pressure change (RPC)	The change in pressure that a fish experiences between the pressure it is acclimated at (neutrally buoyant) before passage, and the lowest pressure (nadir) it is exposed to during infrastructure passage. RPC can be expressed in one of two ways; when comparing RPC between scientific studies, it is important to know which calculation of RPC has been used. When calculated as ***exposure ÷ acclimation pressure (E/A),*** RPC relates to the proportion that the exposure pressure is of the acclimation pressure. E.g. a RPC of 0.3 means that the fish was exposed to 30% of the pressure at which it was acclimated. In contrast, when calculated as ***acclimation ÷ exposure (A/E),*** RPC relates directly to the degree of expansion of gas governed by Boyle's law. E.g. when A/E = 3, the swim bladder would expand three times in volume. ***Unless otherwise stated, E/A is commonly referred to as RPC in this report,*** and is the predictor variable against which all injury and mortality models have been generated.
Pharyngo-clitheral membrane	Tissue which lines the gill cavity to form a semi-transparent membrane which separates the body cavity from the gills.
Physoclistous	Lacking a direct connection between the swim bladder and oesophagus, so that pressure within the swim bladder must be adjusted at different depths by gas diffusion into or out of the blood via a vascular system.
Physostomous	Having a duct connecting the swim bladder to the oesophagus, enabling gas to be quickly taken into or vented from the swim bladder through the mouth.

River infrastructure/s	Refers to any artificial structure placed within a natural or artificial waterbody for the purposes of intercepting, regulating or diverting river flow (e.g. dams, weirs, regulators, hydropower facilities).
Sensor Fish	An autonomous device containing gyrometers, accelerometers and pressure and temperature sensors that is released through river infrastructure to study the hydraulic conditions experienced by fish during passage.
(Fluid) shear (stress)	The force exerted when two masses of water with different velocities and/or direction intersect, causing friction at the interface.
Strain rate	One measure of shear stress, expressed as change of velocity over distance.
Sub-atmospheric or negative pressure	A pressure below that of the surrounding atmospheric pressure (<~101 kPa) at a specific point; a partial vacuum.
Surface or atmospheric pressure	The pressure exerted by the weight of the atmosphere, which at sea level has a mean value of ~101 kPa.
Swim bladder	A gas-filled sac present in the body of many bony fishes, used to maintain and control buoyancy.
Weir – Overshot	A weir in which water flows over the top of a crest, which may be fixed or adjustable.
Weir – Undershot	A weir in which water flows under a gate that is typically adjustable.

NON-TECHNICAL SUMMARY

Downstream fish passage criteria for hydropower and irrigation infrastructure in the Murray–Darling Basin.

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NON TECHNICAL SUMMARY:

For many fish species, downstream migration is required to satisfy important life history requirements, such as feeding and breeding. However, river infrastructure (e.g. dams, weirs, hydropower turbines) can block these migrations. The provision of safe downstream passage of fish at these structures is therefore a significant challenge worldwide. Fish are exposed to a range of stresses when they pass river infrastructure that are not encountered in natural flowing, unregulated rivers. Two stresses that can combine to cause significant injury and mortality to fish are decompression (rapid, extreme drops in water pressure) and fluid shear stress (when water of differing velocities and direction intersects, causing distortion of fish).

Within the Murray–Darling Basin (MDB), many fish species undertake extensive downstream migrations as eggs, larvae, juveniles or adults. Passage through river infrastructure has been shown to affect their survival, but the relative contribution of different stresses (such as rapid decompression and fluid shear) to overall injury and mortality remains poorly understood. In turn, this makes it difficult to assess the risk associated with infrastructure projects, or to develop engineering and operation guidelines to reduce the risks of downstream fish passage.

This report details laboratory experiments that determined the tolerance of various species and life stages of fish from the MDB to rapid decompression (in hypo/hyperbaric chambers) and elevated fluid shear (in a shear flume). Fish were exposed to a wide range of conditions to model the probability of injury and mortality. Our ultimate goal was to determine critical thresholds for injury and mortality, and develop criteria to protect downstream migrating fish at river infrastructure. We hope these criteria can better inform policy relating to the development and management of mini-hydropower and irrigation infrastructure to protect downstream migrating fish.

Rapid decompression experiments

Barotrauma is injury sustained following a rapid decrease in water pressure. It sometimes occurs when fish are brought rapidly from depth to the surface by anglers, but can also be encountered when fish experience sudden momentary drops in water pressure as they pass dams, weirs and hydropower turbines. Barotrauma can result from the overexpansion of gas-filled organs, such as the swim bladder (Boyle's law: if the pressure of a gas is decreased, its volume increases). It can also occur if gas comes out of blood and body fluids, causing bubbles (known as emphysema) in vasculature and organs (Henry's law: fluids can hold less gas in solution at lower pressures). Research has shown that many of these injuries can lead to the eventual death of fish.

The rapid decompression experiments used chambers that could generate rapid drops in water pressure to determine the degree of decompression that eggs, larvae and juvenile fish could sustain before suffering injury or mortality. Murray cod, golden perch and silver perch were exposed to rapid decompression at the egg (golden perch and silver perch), larval (Murray cod, golden perch and silver perch) and juvenile (Murray cod and silver perch) stage. These correspond to the life stages at which the fish are likely to pass through river infrastructure during downstream migration.

The pressure scenarios we used reflected the broad range of the ratio of pressure changes (RPCs) that fish may be exposed to at infrastructure in the MDB. At the lower end of the spectrum, this included what may be expected as fish momentarily encounter slightly sub-atmospheric (negative) pressure while they pass undershot weirs. The more extreme ranges were more reflective of the sudden, momentary and very low pressures that could be expected as fish pass the blades of a hydropower turbine. For eggs and larvae, the most extreme RPC tested was ~0.1 (i.e. fish were exposed to pressures as low as ~10% of the pressure at which they were acclimated. Juvenile fish were tested over a slightly larger range of RPCs, up to ~0.05 (i.e. exposure pressures falling to ~5% of acclimation pressure).

For eggs and larvae, there was little evidence that simulated infrastructure passage led to barotrauma that resulted in immediate mortality (within 24 hours). There was, however, evidence of non-lethal injuries or pressure effects in larvae at the 24-hour mark. More fish were affected as the RPC fell, with injury typically occurring once exposure pressures fell below 40% the acclimation pressure. Much of this injury involved the deflation of swim bladder, with internal haemorrhaging observed in one species and age class. The deflation of the swim bladder was undoubtedly a result of rapid decompression, which resulted in a corresponding increase in the volume of gas in the swim bladder. However, it was unclear whether the deflation was caused by over-inflation and subsequent rupturing of the swim bladder. We present evidence of an alternative explanation, where deflation of the swim bladder may have been a result of venting or 'burping' of gas through the gut and mouth. Regardless of the explanation, the longer-term implications (beyond 24 hours) of this forced deflation of the swim bladder, as well as haemorrhaging, remains unknown and warrants further investigation.

Immediate mortality (within 5 minutes) was not observed in many Murray cod or silver perch juveniles following simulated infrastructure passage. However, both species experienced a variety of injuries resulting from rapid decompression. These included swim bladder rupture, eyes protruding from the sockets (exophthalmia), and haemorrhaging of (or emphysema in) internal and external organs, such as the heart, liver, kidney, mouth, eye and fins. The percentage of fish injured increased as the RPC fell, and threshold responses were typical, where the probability of injury increased substantially once the RPC exceeded a certain level. Threshold levels varied substantially between species and injury types, ranging from more modest levels of decompression (where exposure pressures fell below 70% of acclimation pressure) to more severe scenarios (where pressure fell below 10% of acclimation pressure).

Fluid shear experiments

The effect of exposure to shear stress was tested on eggs (silver perch and golden perch), larvae (Murray cod, silver perch and golden perch) and juveniles (Murray cod, silver perch and golden perch) in a shear flume. In this flume, fish were exposed to shear of a predefined strain rate created by a submerged jet.

Eggs were extremely susceptible to damage and mortality when exposed to a shear stress; physical damage to the cell membrane or cellular contents prevented normal hatching. Once strain rate exceeded ~150 cm s^{-1} cm^{-1}, 100% mortality of golden perch and >40% mortality of silver perch eggs occurred. Larval fish were also susceptible to injury and mortality following shear exposure. However, lower values of shear were more tolerable than higher levels, and susceptibility tended to reduce as larvae aged and approached juvenile metamorphosis. Injuries were observed in more than one-third of larvae studied, and predominantly involved fin damage. It was not possible to determine which injuries were associated with eventual death in most cases. But, it was clear that Murray cod between the ages of 9 and 13 days post hatch were vulnerable to injury of the yolk sac, which would lead to eventual death. Once the three species had reached the juvenile stage, they had become quite resistant to shear stress. Although fin damage was observed in more than one-third of juveniles studied, there was little evidence that shear resulted in mortality up to 24 hours post-exposure.

Implications for fisheries managers and infrastructure engineers

This study provides downstream fish passage criteria based on the best available data from fish passage survival studies for MDB species. Identifying the mechanisms responsible for fish passage risks will help fisheries managers and infrastructure engineers determine the appropriateness of infrastructure works, and structural and operational conditions, to reduce these risks.

The following downstream fish passage criteria are based upon thresholds of decompression and fluid shear. Exceeding these thresholds will lead to a substantial increase in injury or mortality. The criteria are general in nature, and attempt to synthesise results that often varied across multiple species and injury types. The criteria should therefore only be applied after careful consideration of the results and discussion contained within individual chapters of this report.

Note that these criteria provide guidance only. Specific decisions regarding acceptable levels of mortality, and which species and age classes are of most importance, may differ between projects and should be considered by fisheries managers and engineers on a case-by-case basis. Nevertheless, the criteria provide a good indication to where certain risks lie, and suggestions have been provided as to how to reduce these risks.

Generalised downstream fish passage criteria for Murray–Darling Basin species relating to levels of decompression and fluid shear required to minimise injury and mortality of fish

Life stage	Decompression	Fluid shear
Egg	No threshold recommended	Should not exceed 150 cm s^{-1} cm^{-1}
Larvae	No threshold recommended, but caution should be exercised once exposure pressures fall below 40%[a]	Should not exceed ~10 cm $s^{-1}$$cm^{-1}$ in areas and times of suspected larval Murray cod larval drift. Otherwise should not exceed ~620 cm s^{-1} cm^{-1}
Juvenile	Exposure pressures should not fall below 60% of the acclimation pressure	No threshold recommended

[a] Based on evidence of some pressure effects where long-term impacts on survival are uncertain

1. GENERAL INTRODUCTION

1.1 Downstream fish passage at river infrastructure

River infrastructure, such as dams, weirs, regulators and hydropower facilities, play an important role in regulating variable and often unpredictable river flows and generating electricity, thus improving water, food and energy security. However, instream infrastructure has become so pervasive in the world's river systems that it has affected freshwater ecosystems and led to significant declines in the value of fisheries (Gehrke *et al.* 1995, Dudgeon *et al.* 2006, Venter *et al.* 2006, Barlow *et al.* 2008). Much of the impact has come though a change in natural flow regimes, degradation of habitat and water quality, and an interruption of important upstream and downstream fish migrations (Kingsford 2000, Agostinho *et al.* 2008).

Significant investment has been made over the last century into the restoration of upstream passage for juvenile and adult fish. This has focused on the research and development of upstream fishways at dams and weirs (Gough *et al.* 2012). But, for many species, safe downstream passage can be just as critical at various stages of their life (Lucas and Baras 2001). Some species migrate downstream as larvae or juveniles from freshwater spawning grounds to the sea (anadromy, e.g. salmonids), while others undertake these seaward breeding migrations as adults (catadromy, e.g. eels and bass) (McDowall 1988). No less important are the downstream migrations undertaken by larval, juvenile and adult fish residing entirely within freshwater (potamodromy), with seasonal downstream movements being linked to either breeding, feeding or dispersal (Lucas and Baras 2001).

A downstream mode of migration exposes many fish species of different life stages to injury and mortality when they encounter river infrastructure (Larinier and Travade 2002). Field and laboratory studies suggest that the primary causes of fish injury during downstream passage through instream structures include elevated fluid shear and turbulence, rapid and excessive pressure reduction (decompression), and collision with fixed or moving objects (e.g. turbine blades, gates or piers) (Cada 1990, Neitzel *et al.* 2000, Neitzel *et al.* 2004, Deng *et al.* 2006, Deng *et al.* 2010, Brown *et al.* 2012a).

Fish injuries often result from barotrauma and fluid shear. Barotrauma (rapid decompression) injuries typically include swim bladder rupture or emphysema (formation of bubbles) and haemorrhaging (bleeding) in the fins, musculature and organs (Brown *et al.* 2012a). Fluid shear occurs when two water masses of different velocities and direction interact (Cada *et al.* 1999). When a fish is caught between two interacting water masses and the combined force exceeds the critical threshold of fluid shear that the fish can withstand, then it is likely to be injured. Fluid shear can result in loss of scales, haemorrhaging, and eye, skin and skeletal damage (Neitzel *et al.* 2004).

In some instances, the data gathered relating to these mechanisms of injury have been used to refine the design and operation of hydropower facilities to reduce damage to fish (Cada 2001). But, much of the research has been undertaken in North America and Europe, and has typically focused on high-dam hydropower turbines, and diadromous species such as salmonids and eels (e.g. Cada 1990, Larinier 2001, Stephenson *et al.* 2010, Brown *et al.* in press). There has been comparatively far less consideration of other migratory fish species, or of river infrastructure other than large hydropower dams (Gough *et al.* 2012, Brown *et al.* in press), including smaller weirs and mini-hydropower plants.

1.2 Concerns for fish welfare at river infrastructure in the Murray–Darling Basin

The Murray–Darling Basin (MDB) has seen significant investment in restoring upstream fish passage (Mallen-Cooper and Brand 2007, Koehn and Lintermans 2012). Despite increasing recognition of the importance of downstream migration to MDB species, there has been little consideration given to how best to design and manage instream weirs and water diversions to afford greater protection to downstream migrants (Lintermans and Phillips 2004).

Several potamodromous fish species inhabiting the MDB exhibit extensive downstream migrations. Golden perch (*Macquaria ambigua*) and silver perch (*Bidyanus bidyanus*) have buoyant eggs, which disperse large distances downstream (Reynolds 1983). These species, along with others such as Murray cod (*Maccullochella peelii*), use downstream drift to disperse over large distances during the larval stage (Humphries *et al.* 2002, Humphries and King 2004, Koehn and Harrington 2005). In addition to eggs and larvae, a significant number of small and large-bodied adult fish species also undertake large-scale downstream movements, frequently encountering weirs and other infrastructure (O'Connor *et al.* 2003, Lintermans and Phillips 2004, O'Connor *et al.* 2005, O'Connor *et al.* 2006).

Recognition of the importance of downstream migrations to many MDB fish species has raised concern that the prevalence of river infrastructure may be contributing to population declines. Australia ranks in the top 15 countries in the world in terms of its number of large dams (n=517, ICOLD World Register of Dams data, cited in Gough *et al.* 2012). When smaller regulatory structures, such as weirs, are also considered, the number of barriers to migration number grows significantly. In the MDB alone, an estimated 10,000 dams and weirs regulate flows (Baumgartner 2005).

1.2.1. Irrigation structures

More than 80% of main channel weirs in the Murrumbidgee, Macquarie, Namoi and Gwydir rivers employ an 'undershot' design. In these weirs, water is discharged underneath a gate, as opposed to over a gate (NSW Department of Primary Industries 2006). Recent research has associated this design with the injury and mortality of fish (Baumgartner *et al.* 2006, Baumgartner *et al.* 2013). At an experimental undershot weir, large proportions of golden perch (> 90%), silver perch (> 90%) and Murray cod (> 50%) larvae died when passed downstream (Baumgartner *et al.* 2013). A large proportion of small-bodied native fish, such as Australian smelt (*Retropinna semoni*) and unspecked hardyhead (*Craterocephalus stercusmuscarum fulvus*), also died (>90%). Adult life stages of large-bodied species were also affected, but to a much lesser degree; adult golden perch (82%), silver perch (70%) and Murray cod (32%) suffered only minor injuries (Baumgartner *et al.* 2013). Baumgartner *et al.* (2013) used computational fluid dynamics modelling to determined that undershot weirs were characterised by higher values of shear, turbulence and rapid pressure changes. Follow-up research employing autonomous hydraulic sensors (Sensor Fish: Deng *et al.* 2007) at undershot weirs in the field supported these results (Boys *et al.* 2013). But despite this research, it remains unclear which, if any, of these conditions contribute to injury and mortality, in what combination and to what extent.

A significant finding of Baumgartner *et al.* (2013) was that fish mortality and injury rates could be altered at undershot weirs by changing hydraulics through simple design modifications. In that study, the addition of an upstream flow deflector and downstream hydraulic jump actually increased mortality, rather than decreasing it. Far from being a discouraging finding, this demonstrated that the design and hydraulic performance of river infrastructure could be modified to influence their impact on fish welfare. It also highlights that 'fish-friendly' design options can only be developed once we understand which hydraulic parameters should be altered, and to what degree.

1.2.2. Hydropower facilities

Hydropower facilities have created significant fish welfare issues throughout the world (Turnpenny *et al.* 2000, Dugan *et al.* 2010). Hydropower is currently the largest source of renewable energy globally, contributing nearly 16% of the world's total energy production in more than 160 countries. As of 2011, hydropower contributed 63% of the renewable energy mix for New South Wales (NSW) (NSW Government 2012). Further development of hydropower is likely to be driven through the implementation of global climate change policies (Paish 2002, Geoscience Australia and ABARE 2010). Regions such as China, North America, OECD Europe, South America and Africa are expected to continue large-scale hydroelectricity generation (Geoscience Australia and ABARE 2010).

Low topography and variable rainfall will limit further development of large-scale hydropower in south-eastern Australia (Geoscience Australia and ABARE 2010). Instead, much of the potential for hydropower expansion lies in existing weirs and irrigation supply networks for low-head (<6 m) mini-hydropower installations (typically less than 10 MW). The feasibility of such projects is being explored in the MDB and coastal catchments (Baumgartner *et al.* 2012).

Mini-hydropower is the most frequent type of hydropower within Australia, accounting for 54% of all projects in 2009 (Geoscience Australia and ABARE 2010). More than 1,000 MW in potential further generation may be possible on several dozen sites throughout NSW (NSW Government 2012). As an example, a new 3.7-MW hydropower plant was completed at Prospect Reservoir in Western Sydney in late 2012. But, the growth of mini-hydropower is being seen beyond NSW, and worldwide growth is predicted in regional and developing countries (Paish 2002).

It is often suggested that mini-hydropower may provide safer downstream fish passage than traditional high-head dam facilities, based on a lower operating head. But, there is little evidence to support this general assertion, and there is still concern over the suitability of mini-hydropower in natural river systems containing threatened populations of migratory fish (Larinier 2008). Research into the hydraulic tolerances of migrating fish species and the associated performance of different hydropower technologies is required. Such research will guide policy for the development and management of mini-hydropower projects that are sympathetic towards threatened aquatic communities in NSW (Baumgartner *et al.* 2012, Boys *et al.* 2013).

1.3 Scope and research objectives

Laboratory experiments have determined the tolerance of various species and life stages of fish from the MDB to rapid decompression and elevated fluid shear. The species under investigation were Murray cod, golden perch and silver perch, and the life stages were eggs, larvae and juveniles (Table 1). The combination of species and life stages were chosen because they exhibit downstream migrations that could expose them to river infrastructure. As explained in Section 1.2, golden perch and silver perch have buoyant eggs, which disperse large distances downstream (Reynolds 1983). These species, along with Murray cod, use downstream drift to disperse over large distances during the larval stage (Humphries *et al.* 2002, Humphries and King 2004, Koehn and Harrington 2005). In addition to eggs and larvae, these species also undertake large-scale downstream movements as juvenile and adults, exposing them to passage through weirs and other infrastructure (O'Connor *et al.* 2003, Lintermans and Phillips 2004, O'Connor *et al.* 2005, O'Connor *et al.* 2006).

In the first group of experiments (**Chapter 2**), hypo/hyperbaric chambers were used to simulate rapid decompression, which occurs during passage through irrigation and hydropower infrastructure. Egg, larval and juvenile fish were exposed to a wide range of severity of decompression to model injury and/or mortality rates and determine any critical thresholds for injury. **Chapter 3** describes experiments where eggs, larvae and juveniles were exposed to various degrees of fluid shear in a flume. As with the decompression experiments, injury and/or mortality models were created across a range of shear values to determine any critical thresholds. In **Chapter 4**, the key findings of both studies were synthesised and recommendations made to inform policies relating to the development

and management of mini-hydropower and irrigation infrastructure to protect migrating fish in the MDB.

Table 1. Murray–Darling Basin fish species and life stages examined during the rapid decompression and shear experiments.

Life history stage	Barotrauma (Chapter 2)	Fluid shear (Chapter 3)
Egg	Golden perch (*Macquaria ambigua*)	Golden perch
	Silver perch (*Bidyanus bidyanus*)	Silver perch
Larvae	Murray cod (*Maccullochella peelii*)	Murray cod
	Golden perch	Golden perch
	Silver perch	Silver perch
Juvenile	Murray cod	Murray cod
	Silver perch	Golden perch
		Silver perch

2. RAPID DECOMPRESSION

2.1 Introduction

Barotrauma is injury sustained following a rapid decrease in hydraulic pressure (Brown *et al.* 2014). Decompression can cause barotrauma in fish in two ways (Brown *et al.* 2012b). First, a reduction in pressure causes a reciprocal increase in gas volume (i.e. for every halving of pressure, gas volume doubles: Boyle's law). Injury can thus result from the overexpansion of gas-filled organs, such as the swim bladder. Second, fluids (including blood) can hold greater amounts of gas in solution when under pressure (Henry's law). Therefore, when pressure is reduced, gas may be forced out of blood and body fluids, causing bubbles (referred to as emphysema) to form in vasculature and organs.

Injuries that typically result from these phenomena can include rupture of the swim bladder, emphysema of vasculature and organs, dislocation of the eyes (exophthalmia), and haemorrhage (bleeding) associated with ruptured vasculature and damage to internal organs, such as the brain, gills and heart (Cramer and Oligher 1964, Beyer *et al.* 1976, Brown *et al.* 2012b, Pflugrath *et al.* 2012). Many of these injuries have been associated with eventual death of fish (McKinstry *et al.* 2007).

Barotrauma is often associated with marine and freshwater angling, where fish are brought to the surface from depth (e.g. Gravel and Cooke 2008, Schreer *et al.* 2009, Dowling *et al.* 2010, Hall *et al.* 2013) (Figure 1a). But, there is far greater potential for barotrauma when fish migrate downstream through river infrastructure (Figure 1b). This is because while an angled fish will never be exposed to pressures below atmospheric, sub-atmospheric pressures are not uncommon through hydropower turbines (Deng *et al.* 2010) and have also been reported at irrigation weirs (Boys *et al.* 2013). Furthermore, while angled fish are only susceptible once they are large enough to be targeted by anglers, migrating fish may be exposed to barotrauma at infrastructure as eggs, larvae, juveniles and adults.

Barotrauma injuries tend to be more frequent and severe the lower the exposure pressure is as a percentage of the pressure to which a fish is acclimated before exposure. In this report, this is referred to as the ratio of pressure change (RPC). This can be illustrated by comparing an angled fish and one migrating through a hydropower turbine. A fish angled from a depth of 100 m (~1109 kPa) and brought to the surface (atmospheric or ~100 kPa) encounters a minimum exposure pressure of 9% its acclimation pressure (100÷1109). However, a fish acclimated in a dam reservoir at 20 m depth (~300 kPa) that then passes a hydropower dam may be exposed to minimum pressures as low as 7 kPa at the turbine (Deng *et al.* 2010), or 2.3% of its acclimation pressure. According to Boyle's law governing gas volume at different pressures, the swim bladder of the angled fish would have expanded to 11 times the initial volume, whereas the swim bladder of the turbine-passed fish would have expanded to 43 times its initial volume. The turbine-passed fish will also experience this decompression in less than a quarter of a second (Deng *et al.* 2010), whereas the angled fish experiences it at a much slower rate (more than ~1.6 minutes, assuming a typical retrieval rate of 1 m/s: Hughes and Stewart 2013).

When trying to make general predictions and comparisons of the risks to fish posed by the broad range of current and future infrastructure developments, it is prudent to compare barotrauma rates over a large range of RPCs. As illustrated by the previous example, the severity of exposure to barotrauma will depend both on the depth at which a fish is migrating (acclimation pressure) and the lowest (nadir) exposure pressure during infrastructure passage. Both of these pressures can vary substantially. The nadir pressure that fish are exposed to can vary significantly between different migration paths (e.g. a spillway, versus an undershot weir, versus a turbine). For most species, the depth of migration (and therefore acclimation pressure) is poorly understood (Pflugrath *et al.* 2012, Brown *et al.* 2014).

Figure 1. Barotrauma injury in adult fish and can occur following offshore angling or after infrastructure passage
a) Angled snapper (*Pagrus auratus*) with prolapsed swim bladder (Photo: Julian Hughes, NSW DPI).

b) Catfish (*Pimelodus maculatus*) with a prolapsed stomach caught in the tailrace of a hydropower dam in Brazil (Photo: Carlos Bernardo M. Alves, Bio-Ambiental Consultancy).

In this chapter, we outline experiments conducted on eggs, larvae and juveniles of three potamodromous fish species native to the Murray–Darling Basin (MDB) of south-eastern Australia: Murray cod, golden perch and silver perch. Hypo/hyperbaric chambers were used to simulate rapid decompression as experienced by fish during downstream passage through river infrastructure, ranging from below what may be expected at low-head weirs to what could be experienced at large-head hydropower turbines. The relationships between the RPC and the probability of different barotrauma injuries were quantified. The data was used (where possible) to predict key thresholds for decompression, which once exceeded, resulted in significant increases in the types and probability of injury.

2.2 Methods

2.2.1. Fish production and handling

Egg and larval experiments at the Narrandera Fisheries Centre

Adult golden and silver perch were induced to spawn at the Narrandera Fisheries Centre (NFC) during their natural reproductive season (January to February) using Ovaprim®, an analogue of salmon gonadotropin-releasing hormone. Two days after induction, the adults spawned overnight and within six hours the eggs were collected and placed in an aerated 15-L bucket until they were either used within 24 hours for the egg experiments, or subsequently hatched for the larval experiments.

Once hatched, silver perch larvae were kept prior to experimentation in aerated trays (50 cm long, 50 cm wide and 15 cm deep). Experiments were undertaken on silver perch larvae at 10 days post hatch (DPH) and 22 DPH. From 3 DPH, larvae were sustained using a daily feed of newly hatched *Artemia nauplii* and a pulverised commercial pellet. Unlike silver perch larvae, golden perch are more difficult to maintain in hatchery conditions. Therefore, at 3 DPH, golden perch were stocked into a large pond (3,600 m^2 and ~ 3 ML) at the NFC and allowed to feed naturally on plankton. At 10 and 18 DPH, larvae were collected from the pond using a hand net and kept in aerated trays (50 cm long, 50 cm wide and 15 cm deep) for 24 hours prior to experimentation. No supplementary feeding occurred while in these trays.

Murray cod eggs were harvested from specially designed spawning boxes in earthen ponds at the NFC in November 2012. The eggs were kept for 7–10 days in incubating tanks and once hatched, larvae were kept prior to experimentation in aerated trays (50 cm long, 50 cm wide and 15 cm deep). Experiments on Murray cod larvae began 3 DPH and concluded at 25 DPH prior to the completion of juvenile metamorphosis. Because Murray cod undergo direct development (*sensu* Balon 1984), they have no true larval interval, and free embryos begin to feed while still retaining stores of yolk (King 2002). From 17 DPH, larvae were sustained using a daily feed of newly hatched *A. nauplii*.

All tanks or trays holding parental fish, eggs and larvae at the NFC were supplied with flow-through, bore-drawn water (~10 L min^{-1}). Daily water quality measurements were taken from the larvae holding trays, supply tank and barotrauma chambers. Throughout the study, the mean (± SE) pH was 8.08 ± 0.02 (range of 7.95–8.24); conductivity was 14.6 ± 1.38 ms cm^{-1} (9.40–20.10); dissolved oxygen was 7.15 ± 0.28 mg L^{-1} (5.07–7.67); total dissolved gas saturation was 101.41 ± 0.34% (99.72–101.86); and temperature was 18.46 ± 0.23 ^{o}C (17.0–19.10). There were no observations of disease and the fish appeared healthy throughout the entire study.

Juvenile experiments at the Port Stephens Fisheries Institute

Juvenile Murray cod were obtained from Uarah Fisheries, Grong Grong, New South Wales (NSW), and juvenile silver perch from Silverwater Native Fish, Grong Grong, NSW. Fingerlings were transported to the Port Stephens Fisheries Institute (PSFI) in plastic bags (approximately 200 fish per bag) filled with 20 L dam water and sealed with a pure oxygen atmosphere. To minimise the risk of stress-related disease following transport, the fish were given a prophylactic salt treatment. This

involved raising the salinity of the system to 5 ppt for three weeks, after which it was maintained at approximately 4 ppt throughout the study.

While at PSFI, the fish were held in 2,000-L circular polyethylene holding tanks (Figure 2). Bore-drawn water was used to fill the tanks and the total volume of the system (9,000 L) was constantly exchanged between all tanks via a heat exchanger and biological filter. A 5% water change was carried out every two weeks, which was adequate to maintain water quality, given the low stocking density (which did not exceed ~0.9 g L^{-1}). Water quality (temperature, pH, dissolved oxygen, total dissolve gas saturation, salinity, conductivity, ammonia and nitrite) were measured daily. Throughout the study, the mean (± SE) pH was 8.11 ± 0.04 (range 7.94–8.21); conductivity was 8.59 ± 0.23 ms cm^{-1} (7.47–9.20); dissolved oxygen was 7.11 ± 0.07 mg L^{-1} (6.92–7.46); total dissolved gas saturation was 101.8 ± 0.50% (100.39–104.39); and temperature was 26.84 ± 0.29 ^{o}C (25.90–27.90).

Fish were fed twice daily on a mix of frozen blood worms and commercial pellet (Ridley Aqua-Feed Native Fish Start, 1–3 mm). Murray cod were housed at PSFI for approximately two months prior to experimentation in March 2013. At the time of experimentation, their average length was 66.1 mm total length (± 0.3 SE and ranging from 80.0–54.0 mm) and average weight was 3.2 g (± 0.1 SE and ranging from 5.8–1.7 g). Silver perch were housed at PSFI for approximately seven months prior to experimentation in April 2013. At the time of experimentation, their average length was 80.0 mm fork length (± 0.7 SE and ranging from 112.0–45.0 mm) and average weight was 7.6 g (± 0.2 SE and ranging from 19.6–0.9 g). Once the barotrauma experiments had begun for a given species, they were completed within 10 days.

Figure 2. Experimental facilities at the Port Stephens Fisheries Institute showing 2000-L tanks in which juvenile fish were housed, adjacent to the mobile laboratory containing the hypo/hyperbaric chambers

2.2.2. Hypo/hyperbaric chambers

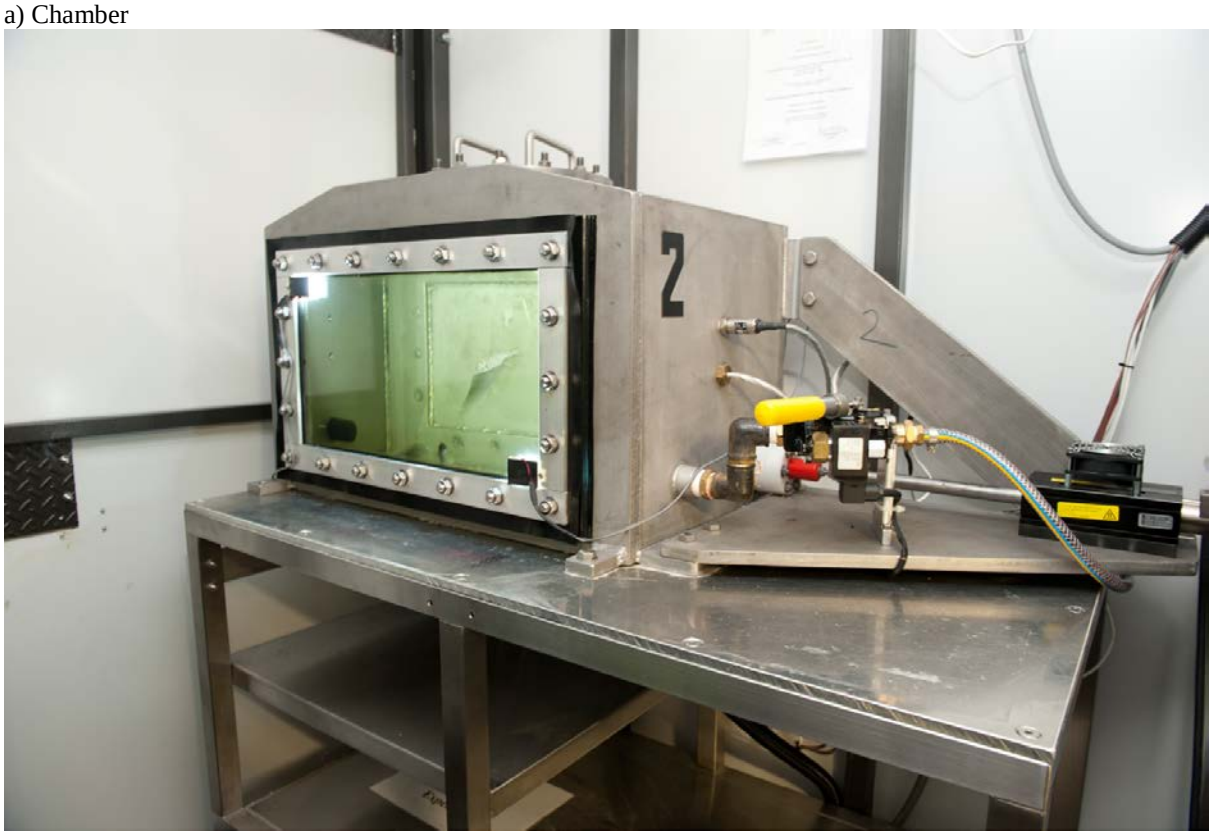
Two chambers capable of generating rapid and sustained changes in water pressure were used to simulate decompression during infrastructure passage (Figure 3). Each chamber was constructed from 16-mm-thick stainless steel plate with a 16-mm-thick laminate glass viewing window and a sealable hatch through which fish could be loaded (Figure 4). Flow-through water was delivered to each chamber (averaging ~50 L min^{-1}) using a pump (Model I91340615; Grunfos Ptd Ltd, Adelaide, Australia), being drawn and recirculated through the same tanks in which the fish were being held prior to experimentation.

A computer program with a graphical user interface (GUI; LabView, National Instruments Corporation, Austin, Texas, United States) controlled an electric actuator valve (Model OM-1; AVFI, Bundoora, Victoria, Australia) on the outlet of each chamber, to generate pre-programmed acclimation pressures within the chambers and to simulate a particular depth of migration prior to simulated passage. The desired pressure was maintained by the GUI using an active feedback loop with two pressure sensors sampling chamber pressure at 2000 Hz. The readings of the pressure sensors were validated by running various decompression scenarios with Sensor Fish (Deng *et al.* 2007) in the chambers, and comparing the pressure data collected by the Sensor Fish with that collected by the chamber sensors.

After fish were placed in a chamber, the chamber was sealed and all air removed using a bleed valve on the lid. A pre-programmed acclimation pressure was maintained by the actuator valve under control of the GUI. After the desired acclimation time had been reached (applicable to the juvenile experiments, see sections 2.2.5 for further details), ball valves on the chamber inlet and outlet were simultaneously closed and the pump switched off, effectively isolating the chamber at the set acclimation pressure prior to decompression. Decompression was achieved by the GUI using a pre-loaded pressure profile (.csv file) to activate a Linmot® linear motor (Model HS01-37x166; NTI AG Linmot, Sprietenbach, Switzerland) that withdrew and/or inserted a piston from the chamber to create the desired pressure profile. Immediately following decompression, the GUI displayed the actual pressure measurements sampled at 2000 Hz and saved this data to an exportable file. This exportable file was used to obtain both the acclimation and the nadir exposure pressure to calculate the RPC.

Figure 3. Hypo/hyperbaric chamber used to simulate rapid decompression during river infrastructure passage

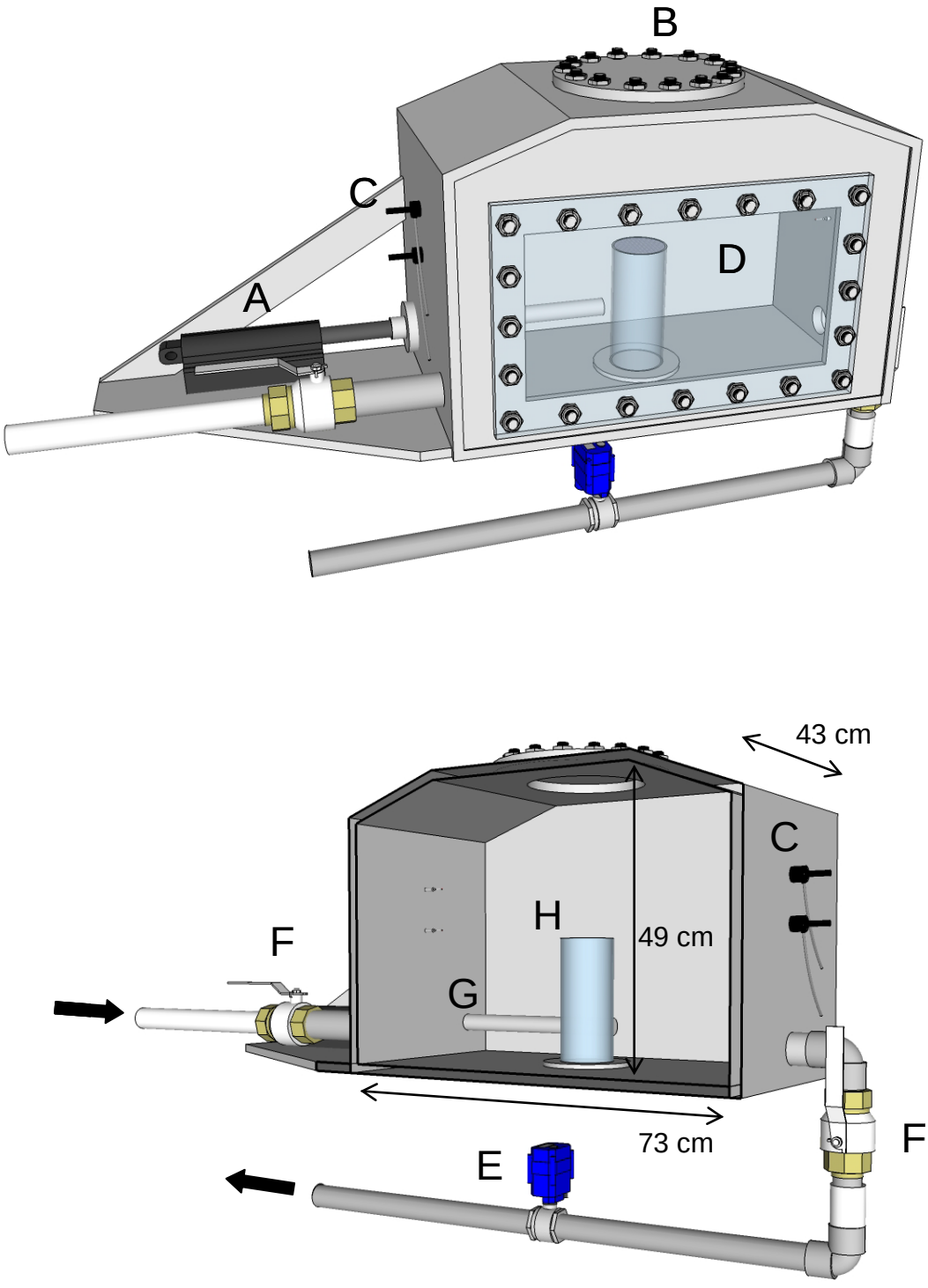
a) Chamber



b) Graphical user interface

Figure 4. Hypo/hyperbaric chamber showing the main components

A) actuator rod, B) removable hatch, C) temperature and pressure sensors, D) laminated glass viewing window, E) actuator valve, F) ball valves, G) piston, and H) acrylic holding cylinder, which was used to hold larval fish, but not eggs or juveniles



2.2.3. Egg experiments

Silver perch and golden perch eggs (Figure 5) were rapidly decompressed in hypo/hyperbaric chambers from atmospheric pressure (~101 kPa) to one of a range of nadir pressures to simulate infrastructure passage (Figure 6) over a range of RPCs (Table 2). Decompression occurred at speeds between 0.1 and 0.5 seconds, equating to RPCs between 63.9 and 359.1 kPa s^{-1}. Such rates and RPCs correspond to ranges often observed at river infrastructure such as undershot weirs (Boys *et al.* 2013) and hydropower facilities (Deng *et al.* 2010, Boys *et al.* 2013).

Twenty-four hours prior to experimentation, viable eggs were siphoned from their holding trays using vinyl tubing and held in aerated 700-mL plastic jars (Figure 7). Ten eggs were placed in each jar, with each jar corresponding to a different test group of eggs subjected to one of 10 RPC treatments (Table 2). Each treatment was replicated three times, making a total of 30 jars of test groups. The aerated jars were placed in trays supplied with flow-through bore-drawn water to maintain a constant water temperature (Figure 7). The jars of eggs were decompressed one at a time in the chambers. This involved replacing the plastic lid of a jar with a fine mesh cover and sealing the jar in a chamber for 5 minutes at atmospheric pressure (~101 kPa), before subsequently decompressing to one of 10 possible nadir pressures, ranging from 101 to 10 kPa. Following decompression, the jar of eggs was brought back to atmospheric pressure, removed from the chamber and placed back into the holding trays for 24–48 hours. After this time, the eggs were examined to determine how many had successfully hatched and how many had died.

Figure 5. Golden perch eggs

Figure 6. The exposure profile used to simulate rapid decompression during infrastructure passage. In this example, a rapid fall from an acclimation pressure 101 kPa to a nadir pressure of ~10 kPa is experienced in 0.3 seconds, corresponding to a ratio of pressure change RPC of ~0.1 (10÷101)

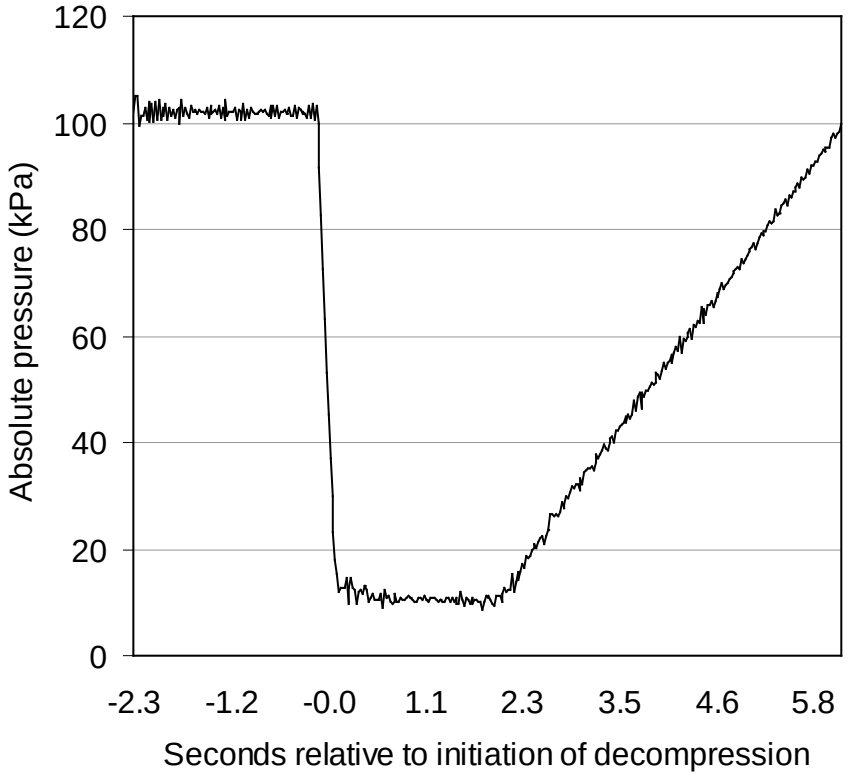


Table 2. Test groups of egg and larvae fish were subjected to one of 10 rapid decompression scenarios to simulate river infrastructure passage. Three replicate groups were tested at each ratio of pressure change to ensure a sufficient spread of data for regression and the generation of mortality and injury models

Pressure[a] (kPa)		Ratio of pressure change (RPC)[ab]	
Acclimation (A)	Exposure nadir (E)	(E/A)	(A/E)
101	101	1.00	1.00
101	79	0.78	1.28
101	61	0.60	1.65
101	48	0.48	2.12
101	37	0.37	2.72
101	29	0.29	3.49
101	23	0.23	4.48
101	18	0.18	5.75
101	14	0.14	7.39
101	11	0.11	9.49

[a] The pressures presented in this table are a guide to the approximate pressures achieved. Measurements obtained from pressure sensors in the chambers were used to determine the actual exposure acclimation and exposure nadir pressures for the calculation of RPCs for the regressions.

[b] The RPC (E/A) was used in the regression modelling and relates to the proportion that the lowest exposure pressure was of the acclimation pressure. E.g. a RPC of 0.3 means that the nadir exposure pressure was 30% of the acclimation pressure. The ratio of pressure change is also shown as A/E, as this number relates directly to the degree of expansion of gas governed by Boyle's law. E.g. when A/E= 3, the swim bladder would expand three times in volume.

2.2.4. Larval experiments

Silver perch, golden perch and Murray cod larvae were rapidly decompressed in hypo/hyperbaric chambers to simulate river infrastructure passage as per the experimental design outlined for the egg experiments (Table 2). Approximately 12–24 hours before experimentation, larvae were individually siphoned using vinyl tubing from their holding trays into aerated 700-mL plastic jars (Figure 7). Ten larvae were placed in each jar, with each jar corresponding to a different test group of fish. For experimentation, a test group of fish was poured from its holding jar into a clear acrylic cylinder (11 cm Ø and 20 cm high) with a mesh screen lid. The cylinder was then placed in the chamber (Figure 4). Following decompression, larvae were returned to individual aerated jars. Immediately following decompression and then again at ~24 hours post experimentation, the number of dead larvae in each test group were counted. At the 24-hour point, all remaining larvae were euthanased using a solution of 100 mg L^{-1} ethyl-p-amino benzoate (benzocaine), transferred to a Petri dish, and examined under a dissecting microscope fitted with a digital camera to determine the presence of external and internal injuries. The injuries looked for included exophthalmia; decapitation; internal haemorrhaging; herneation of the gut, or expulsion of digestive contents or bile; emphysema of the body cavity, eyes or fins; and evidence of swim bladder rupture. Because the larvae were too small to identify rupture points in the swim bladder, a visible swim bladder was considered to be intact, whereas the absence of a visible swim bladder was treated as being deflated, and thereby an indication of swim bladder rupture.

Figure 7. Aerated jars used to hold test groups of eggs and larvae before and after decompression in the chambers

2.2.5. Juvenile experiments

Silver perch and Murray cod juveniles were rapidly decompressed (Figure 6) in hypo/hyperbaric chambers and subjected to one of a combination of various acclimation and nadir pressures to simulate infrastructure passage over a range of RPCs (Table 3). Ten fish were sealed in a chamber at a time, corresponding to a single test group. Since juvenile silver perch and Murray cod are physoclistous, and can regulate their swim bladder volume through a vasculature rete without the need to physically gulp air. They are likely to have the capacity to migrate at greater depths than larvae (who have not fully developed an active rete) or eggs. Therefore, juveniles were tested over a larger range of RPCs than the earlier life stages (Table 3). To achieve the three larger RPCs, it was necessary to acclimate fish at pressures above atmospheric (ranging from ~122–200 kPa or ~2–10 m depth) for approximately 24 hours. After this time, all fish were judged to have acclimated fully, because they had changed from being negatively buoyant (swimming head up), to neutrally buoyant (able to maintain their vertical position in the water column without actively swimming and tail-beating). The speed of pressure change achieved was as reported for the egg experiments (Section 2.2.3).

Table 3. Test groups of juvenile fish were subjected to one of 13 rapid decompression scenarios to simulate river infrastructure passage. Three replicate groups were tested at each ratio of pressure change to ensure a sufficient spread of data for regression and the generation of mortality and injury models

Pressure[a] (kPa)		Ratio of pressure change (RPC)[ab]	
Acclimation (A)	Exposure nadir (E)	(E/A)	(A/E)
101	101	1.00	1.00
101	79	0.78	1.28
101	61	0.60	1.65
101	48	0.48	2.12
101	37	0.37	2.72
101	29	0.29	3.49
101	23	0.23	4.48
101	18	0.18	5.75
101	14	0.14	7.39
101	11	0.11	9.49
122	10	0.08	12.20
156	10	0.06	15.60
200	10	0.05	20.00

[a] The pressures presented in this table are a guide to the approximate pressures achieved. Measurements obtained from pressure sensors in the chambers were used to determine the actual exposure acclimation and exposure nadir pressures for the calculation of RPCs for the regressions.

[b] The RPC (E/A) was used in the regression modelling and relates to the proportion that the lowest exposure pressure was of the acclimation pressure. E.g. a RPC of 0.3 mean that the nadir exposure pressure was 30% of the acclimation pressure. The ratio of pressure change is also shown as A/E, as this number relates directly to the degree of expansion of gas governed by Boyle's law. E.g. when A/E= 3, the swim bladder would expand three times in volume.

After decompression, all fish were observed in the chambers for five minutes. After this time, the number of dead or disorientated fish was recorded. All fish were then removed from the chamber and euthanased in a solution of 100 mg L^{-1} ethyl-p-amino benzoate (benzocaine) and immediately taken for autopsy (Figure 8). The length and weight of fish were measured and they were inspected for various external and internal injuries typical of barotrauma (Table 4).

Figure 8. At autopsy, fish were examined for signs of barotrauma

a) External examination

b) Internal examination

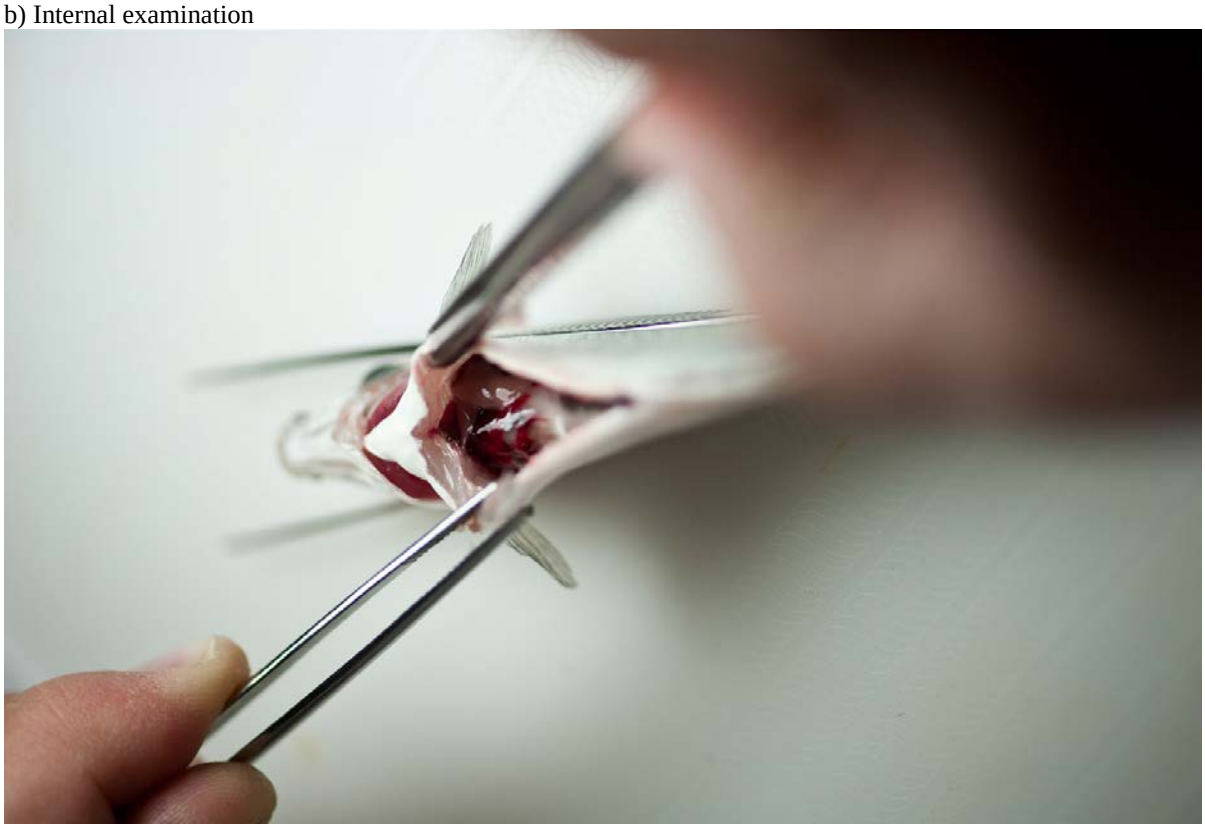


Table 4. External and internal barotrauma injuries quantified in juvenile fish

Organ – Condition	Reference photograph[a]
Skin – Haemorrhage	Figure 30
Skin – Emphysema	Figure 31
Cloaca – Bloodshot	Figure 32
Prolapsed gut	Figure 33
Abdomen – Distended	No photo
Fins – Haemorrhage	Figure 34
Fins – Emphysema	Figure 35
Pharyngo-clitheral membrane – Emphysema	Figure 36
Eyes – Exophthalmia	Figure 38
Eyes – Haemorrhage	Figure 39
Eyes – Emphysema	Figure 40
Operculum – Haemorrhage	No photo
Operculum – Emphysema	Figure 37
Gills – Haemorrhage	Figure 41
Gills – Emphysema	Figure 42
Mouth – Haemorrhage	Figure 43
Mouth – Emphysema	Figure 44
Mouth – Prolapsed gut	No photo
Viscera – Haemorrhage	Figure 45
Viscera – Mesentery emphysema	Figure 46
Stomach – Haemorrhage	No photo
Heart – Haemorrhage	Figure 48
Heart – Emphysema	Figure 47
Liver – Haemorrhage	Figure 49
Liver – Emphysema	Figure 50
Spleen – Haemorrhage	No photo
Swim bladder – Ruptured	Figure 53
Kidney – Haemorrhage	Figure 51
Kidney – Emphysema	Figure 51 & Figure 52

[a]See Appendix 1 for photographs. All reference photographs are taken from fish subjected to rapid decompression as per the methods outlined in this report, although they do include some species not covered in this report.

2.2.6. Statistical methods and modelling

For the egg experiments, logistic regression was used to determine whether the total mortality rate was influenced by RPC (E/A). For the larval experiments, logistic regression models were first fitted including DPH as a fixed factor. Both larval and juvenile injury data were also analysed using linear piecewise regression (Toms and Lesperance 2003). This approach generated 'broken-stick' models, where two lines of different slope join at a 'breakpoint'. The breakpoint was used as an objective means of estimating thresholds in RPC (x), where there was a substantial change in the probability of injury (y). Such an approach has been used previously to determine ecological and geomorphological thresholds (Ryan *et al.* 2002, Toms and Lesperance 2003). An abrupt breakpoint approach was used and confidence intervals calculated of all threshold estimates, as per the methods of Ryan *et al.* (2002). Two simple linear regression equations were fit to the data, one above and one below an estimated initial breakpoint (c). The parameters from these two initial regression equations and the estimated breakpoint were then used as the starting parameters in a non-linear model. These starting

estimates do not influence the final parameters in the broken-stick models, but were used to optimise the likelihood of the models converging, because poor starting parameters can result in models not converging (Ryan and Porth 2007). The NLIN procedure in SAS (Inc. 2011) and the Marquardt iterative method were used to determine parameter estimates for the intercept (a_1), slope of the relationship below the cut-point (b_1), slope of the relationship above the cut-point (b_2), and the cut-point (c) for each injury response variable for each species:

$y = a_1 + b_1x$ for $x \leq c$ *Eq.1*

$y = \{a_1 + c(b_1 - b_2)\} + b_2x$ *for* $x > c$ *Eq.2*

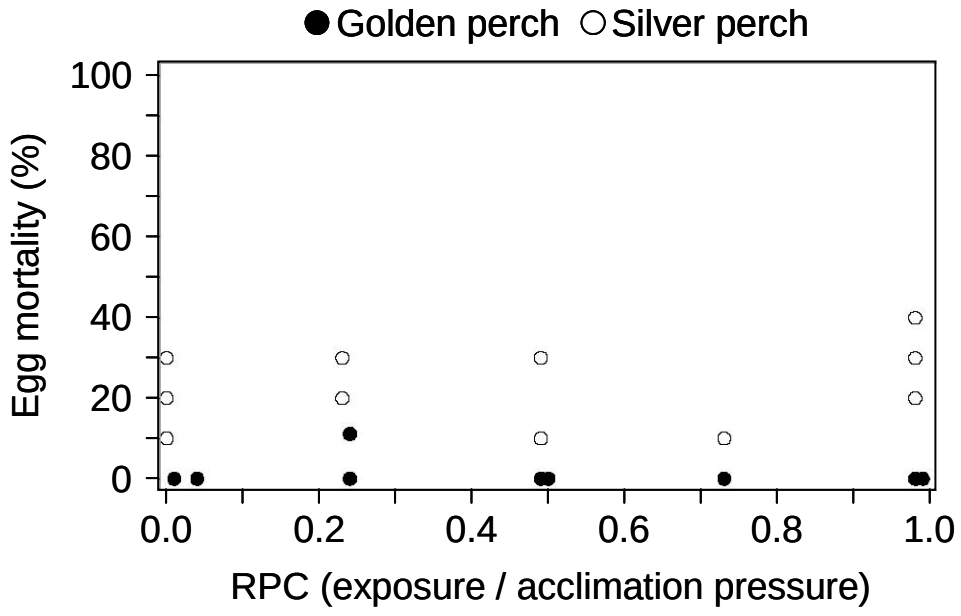
Models that did not converge were disregarded. For those that converged, equations 1 and 2 were used to generate the predicted regression lines, which were plotted with the raw data. Wald confidence intervals for the threshold cut-point (*c*) were also plotted to display the range of RPCs possibly containing the injury threshold.

2.3 Results

2.3.1. Eggs

Mortality was generally higher for silver perch eggs than for golden perch eggs (Figure 9). However, this species difference was unlikely to be related to barotrauma, because egg mortality was not significantly related to RPC for either species (golden perch $\chi^2 = 0.001$, df = 1, p = 0.993; silver perch $\chi^2 = 0.677$, df = 1, p = 0.41).

Figure 9. The probability that silver perch and golden perch **eggs** will die before hatching, after being exposed to simulated infrastructure passage across a range of ratio of pressure changes (RPC). Each point represents the percentage of that test group (10 eggs) affected



2.3.2. Larvae

For all three species, DPH (but not RPC) was significantly associated with larval mortality up to 24 hours post experimentation (Table 5). That is, larval mortality was more dependent on the age of larvae used in the experiments, rather than any effect arising from exposure to varying levels of decompression. The highest mortality was found in golden perch and silver perch at 12 and 10 DPH, respectively (Figure 10). For 5, 12 and 18 DPH golden perch larvae, the average rates of mortality were 7.7, 16.3 and 0%, respectively. Murray cod mortality averaged 5% (22 DPH) and 1.7% for (25 DPH), while silver perch had average mortalities of 7 50 and 0% for 4, 10 and 22 DPH, respectively.

Figure 10. The percentage of **larval** Murray cod, silver perch and golden perch dead within 24 hours of simulated infrastructure passage at different ages (days post hatch, DPH) and across a range of ratio of pressure changes (RPC). Each point represents the percentage of that test group (10 larvae) affected

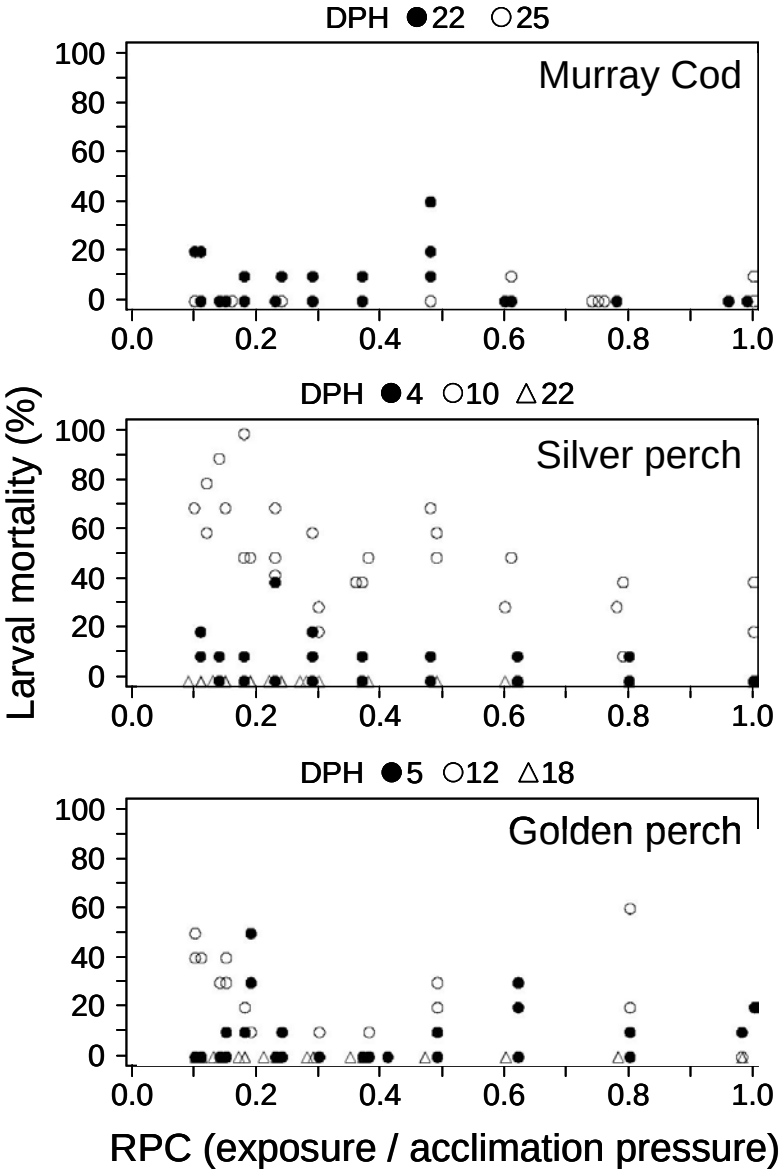


Table 5. Results from logistic regression modelling of the relationship between **larval** mortality (%) and ratio of pressure change (RPC, exposure/acclimation) and larval age in days post hatch (DPH) for golden perch, Murray cod and silver perch.

		Golden perch			Murray cod			Silver perch		
Model	Effect	χ^2	DF [a]	P value	χ^2	DF	P value	χ^2	DF	P value
RPC + DPH + (DPH × RPC)	Main	47.2	5	<.0001	8.4	3	0.0385	394.1	5	<.0001
	DPH	11.5	2	0.0032	6.2	1	0.0128	55.1	2	<.0001
	RPC	0.1	1	0.7395	0.2	1	0.6809	0.7	1	0.4001
	DPH × RPC	3.4	2	0.1785	3.7	1	0.056	1.7	2	0.432

[a] DF = degrees of freedom

Unlike mortality, the likelihood that larvae sustained an injury (but were not dead 24 hours post exposure) was significantly related to RPC in golden perch and silver perch (but not Murray cod). In these cases, the likelihood of injury increased as RPC (E/A) fell (Figure 11), but the relationship was not linear and a threshold response was observed. For silver perch, thresholds of 3.7 and 3.8 RPC were estimated for 10 and 22 DPH larvae, respectively (Table 6). For golden perch, thresholds of 0.47 and 0.56 RPC were estimated for 12 and 18 DPH larvae, respectively (Table 6). Many of these threshold estimates were associated with large 95% confidence intervals, and for some models (e.g. 10 DPH silver perch), variability in the likelihood of injury at RPCs below the estimated threshold led to a poor fit of the piecewise model (indicated by $R^2<0.5$ in Table 6).

Having a deflated swim bladder explained a large amount of the injury responses in larvae (Figure 12). For 10 DPH silver perch, 12 DPH golden perch and 25 DPH Murray cod, swim bladder deflation began below a RPC threshold of ~0.4. Deflation may occur at slightly higher RPC (~0.6) in 22 DPH Murray cod, but large confidence intervals indicate that there is a large amount of uncertainty regarding this estimate (Figure 12 and Table 6). The presence of internal emphysema (Figure 14) at lower RPCs also contributed to an increase in the likelihood of injury for all species. However, no significant threshold could be identified for this response (Figure 13 and Table 6). In 18 DPH golden perch, internal haemorrhaging was observed, with blood pooling in the cavity posterior to the swim bladder (Figure 15). Haemorrhaging increased significantly as RPC fell below an estimated threshold of 0.39 (piecewise regression, F=16.5, df=3,26, p<0.0001; R^2= 0.66) (Figure 16).

Figure 11. The percentage of **larval** Murray cod (top), silver perch (middle) and golden perch (bottom) injured at two different ages (days post hatch, DPH) following simulated infrastructure passage over a range of ratio of pressure changes (RPC). Piecewise regression lines are shown if there was convergence in the piecewise linear regression model and the relationship were statistically significant. The grey line shows the band between the 95% confidence intervals of the breakpoint outlined in Table 6.

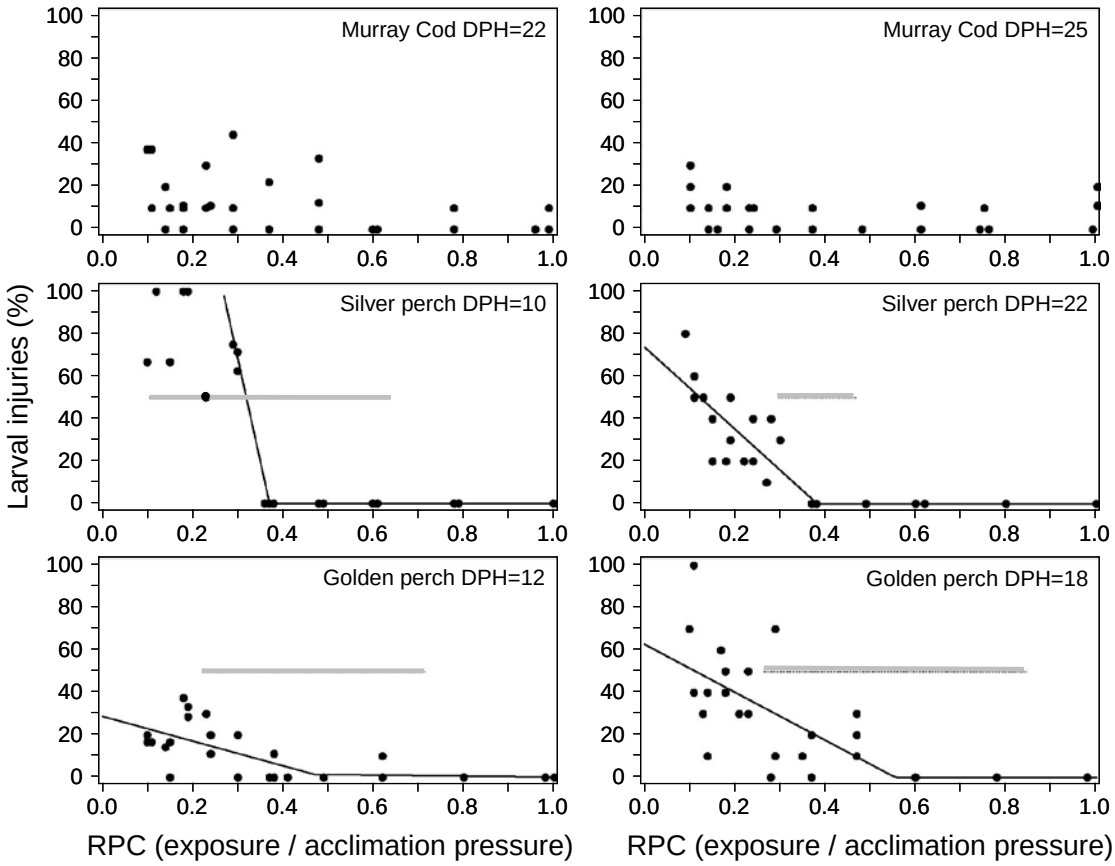


Table 6. Estimates of ratio of pressure change (RPC) threshold for barotrauma injuries in **larval** Murray cod, silver perch and golden perch following simulated infrastructure passage. F-values and probability are for piecewise regression model fit, and R^2 is proportion of variation in response explained by the piecewise model. Results are not shown where the model was not able to be fitted or was not significant for that response

Species	Response variable	Days post hatch	F-Value df = 3,26	Prob > F	R^2	RPC Threshold
Murray cod	Total injured (%)	22	–	–	–	–
		25	–	–	–	–
	Deflated swim bladder (%)	22	3.66	0.0252	0.29701	0.63
		25	3.82	0.0216	0.30494	0.42
	Internal emphysema (%)	22	–	–	–	–
		25	–	–	–	–
Golden perch	Total injured (%)	12	9.51	0.0002	0.523088	0.47
		18	10.44	0.0001	0.546429	0.56
	Deflated swim bladder (%)	12	5.62	0.0042	0.393265	0.39
		18	–	–	–	–
	Internal emphysema (%)	12	–	–	–	–
		18	–	–	–	–
Silver perch	Total injured (%)	10	3.05	0.0472	0.267773	0.37
		22	36.61	<.0001	0.808562	0.38
	Deflated swim bladder (%)	10	3.09	0.0451	0.270771	0.37
		22	1.64	0.2038	0.159259	0.33
	Internal emphysema (%)	10	–	–	–	–
		22	–	–	–	–

Note:

Only injury responses where the model was able to converge for at least one of the species are shown.

Dashes indicate where the model did not converge. i.e. the relationship did not have a breakpoint.

R^2 values indicate how well the piecewise model fit the data; those with $R^2 < 0.5$ typically display a poor fit of the breakpoint (reflected on the injury plots as a wide 95% confidence band).

Figure 12. The percentage of **larval** Murray cod (top), silver perch (middle) and golden perch (bottom) with a deflated swim bladder at two different ages (days post hatch, DPH) following simulated infrastructure passage over a range of ratio of pressure changes (RPC). Piecewise regression lines are shown if there was convergence in the piecewise linear regression model and the relationship were statistically significant. The grey line shows the band between the 95% confidence intervals of the breakpoint outlined in Table 6

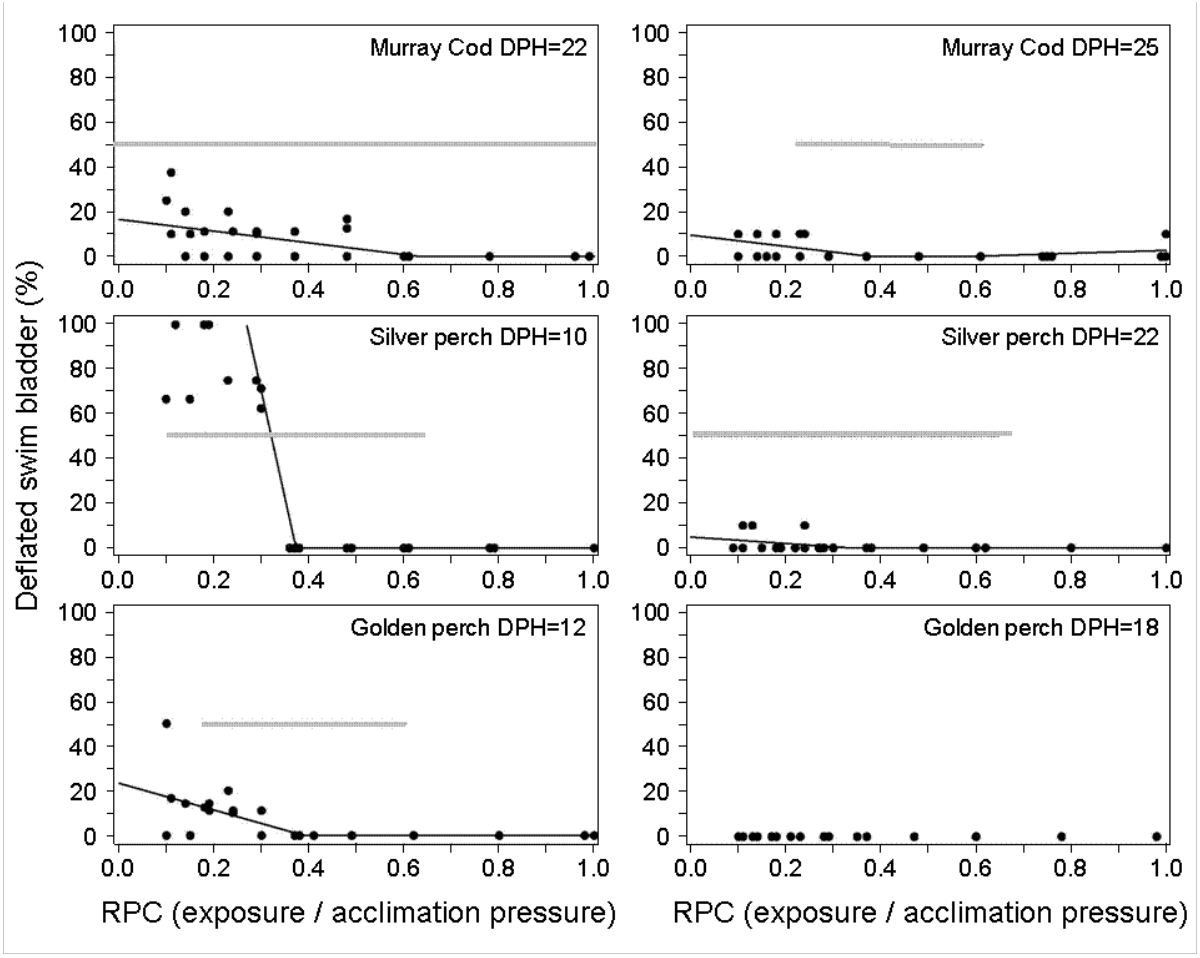


Figure 13. The percentage of **larval** Murray cod (top), silver perch (middle) and golden perch (bottom) with internal emphysema at two different ages (days post hatch, DPH) following simulated infrastructure passage over a range of ratio of pressure changes (RPC). Piecewise regression lines are not shown, because there was no convergence of models.

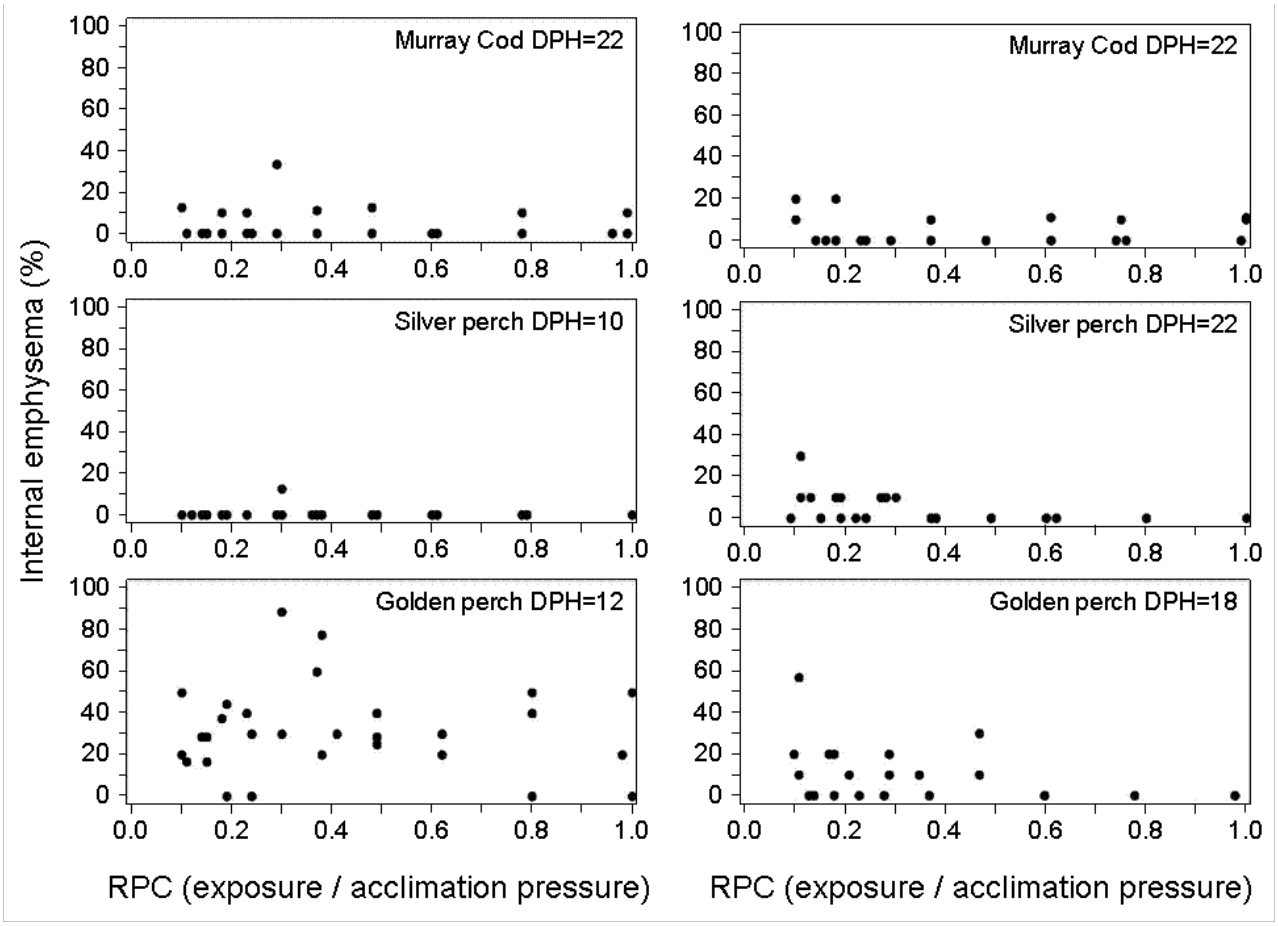
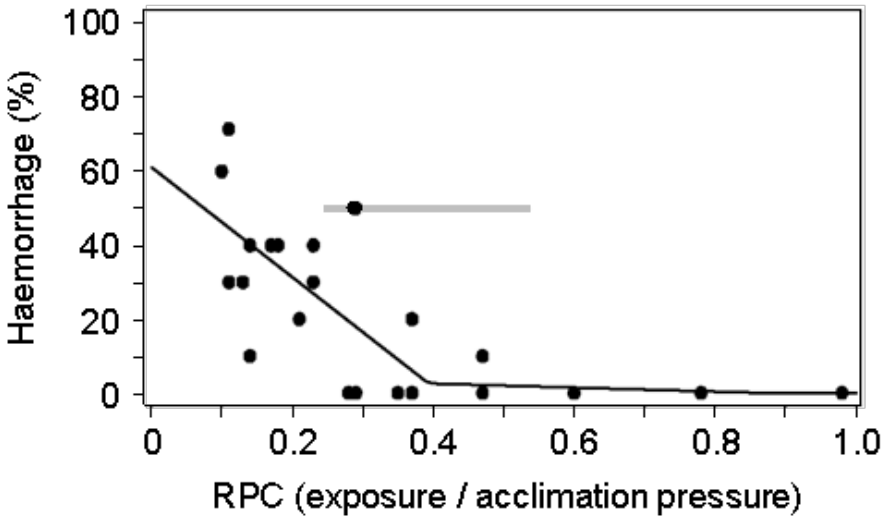


Figure 14. Internal emphysema observed in a 22-days post hatch Murray cod larvae. In this instance (as with most) emphysema was observed posterior to the intact swim bladder

Figure 15. Pooling of blood on the posterior end of the swim bladder was observed in 18- days post hatch golden perch larvae when exposed at lower ratios of pressure changes

Figure 16. The percentage of 18-days post hatch **larval** golden perch with internal haemorrhaging following simulated infrastructure passage over a range of ratio of pressure changes (RPC). A piecewise regression line is given, with the grey horizontal line showing the band between the 95% confidence intervals of the estimated breakpoint



2.3.3. Juveniles

The percentage of juvenile silver perch and Murray cod that were dead or disorientated within five minutes of simulated infrastructure passage never exceeded 30% (Figure 11). For silver perch, this probability increased as the exposure pressure fell below 42% of the acclimation pressure (RPC breakpoint 0.42: Table 7), but the confidence band of the breakpoint was wide (Figure 11), a consequence of the poor fit of the model ($R^2 = 0.21$: Table 7). Although there was no significant fit of the piecewise model to the Murray cod data ($p > 0.05$), there was a small increase in the incidence of death or disorientation at RPC < 0.35 (Figure 11).

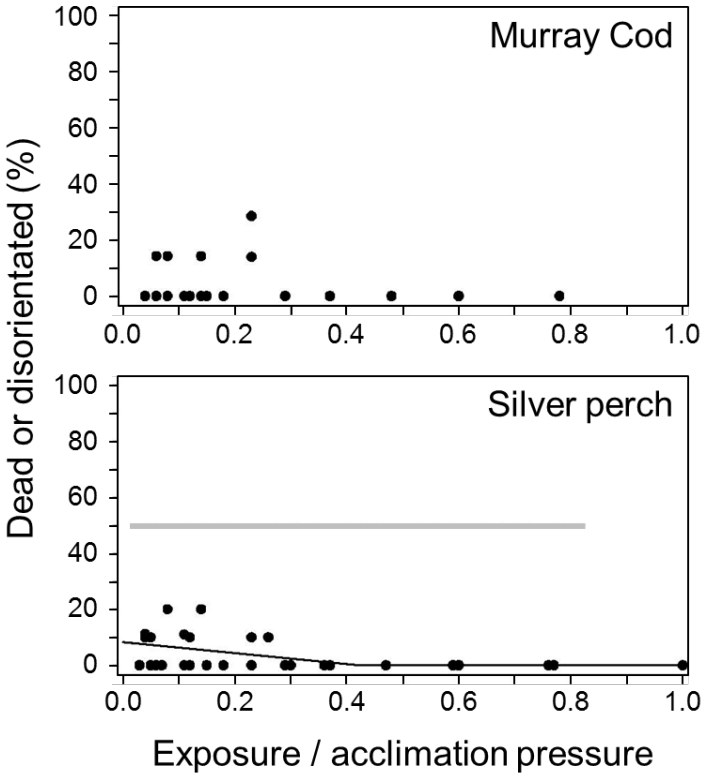
Murray cod and silver perch were not injured when held in the chambers but not decompressed (RPC=1). For most of the injury types, the incidence increased as RPC (E/A) was reduced (Appendix 2: Figure 59). For some injury types, the modelled maximum probability of injury was 80–100% at the lowest RPCs (Figure 17). For Murray cod, these injuries included swim bladder rupture, exophthalmia, haemorrhage of the viscera and mouth, and emphysema of the eye and fins. In this species, other injuries occurred to a maximum modelled probability of ~40% at the lowest RPCs, including haemorrhage of the kidney and heart, and emphysema of the viscera, heart, operculum and pharyngo-clitheral (PC) membrane. The modelled maximum probability of liver haemorrhage was ~60%.

Silver perch typically had lower maximum modelled probabilities of injury than Murray cod. Viscera haemorrhage and swim bladder rupture were the only injuries that approach 80–100% mortality at the most extreme RPCs tested (Figure 17). For all other injury types, the modelled maximum injury encountered rarely exceeded 40%, and for many it rarely exceeded 20% (including exophthalmia, haemorrhage of the eye, mouth and heart, and emphysema of the eye, fin operculum and viscera).

For thirteen different injury types, a breakpoint or threshold RPC could be estimated in at least one of the species using piecewise regression (Figure 17 and Table 7). At RPCs below the threshold, there was a substantial increase in the probability of injury. The 95% confidence interval (Figure 17) and

goodness-of-fit values (Table 7) indicate that breakpoint estimates were more reliable in determining a threshold response for some injuries than for others.

Figure 17. The percentage of **juvenile** Murray cod (top) and silver perch (bottom) that were dead or disorientated within 5 minutes of simulated infrastructure passage over a range of ratio of pressure changes (exposure/acclimation pressure). Piecewise regression lines are shown only if there was convergence in the piecewise linear regression model and the relationship were statistically significant. The grey line shows the band between the 95% confidence intervals of the breakpoint outlined in Table 7



The RPC thresholds for Murray cod were generally well-defined, with narrow confidence limits (Figure 18 and Figure 19), and the piecewise linear models fit the data well for most injuries (i.e. R^2>0.5; Table 7). A threshold range occurred between RPC 0.2 and 0.4, and once RPC had fallen to 2.0, a majority of the injury thresholds had been reached, including swim bladder rupture, haemorrhage of the liver and heart, and emphysema of the viscera and PC membrane (Figure 19). The most well-defined threshold with narrow confidence intervals occurred between RPC 0.1 and 0.2. At this RPC range, there was a substantial increase in the probability of exophthalmia; haemorrhage of the eye, mouth and kidney; and emphysema of the fins, eye and operculum. Although a threshold of RPC 0.63 was predicted to occur for viscera haemorrhage (Table 7), the confidence intervals of this estimate were wide (Figure 19), suggesting it was not a reliable threshold estimate.

For silver perch, thresholds were less consistent than they were for Murray cod and the confidence limits were much wider (Figure 18 and Figure 19). Threshold estimates ranged between RPC 0.3 and 0.7, and were identified with respect to haemorrhage of the eye, viscera, liver and kidney, as well as emphysema of the PC membrane. In the case of swim bladder rupture, there was a gradual linear response over the entire range of RPC tested and a threshold could not be identified (Figure 18). As for Murray cod, although a significant piecewise model could be fitted for viscera haemorrhage, the wide confidence limits suggest that the threshold estimate for this injury was unreliable (Figure 19).

Table 7. Estimates of ratio of pressure change (RPC) threshold for barotrauma injuries in **juvenile** Murray cod and silver perch following simulated infrastructure passage. F-values and probability are for piecewise regression model fit, and R^2 is the proportion of variation in response explained by the piecewise model. Empty cells indicate that the model was not able to be fitted or was not significant for that response

	Murray cod				Silver perch			
Injury response	F-value df = 3,35	Prob > F	R^2	RPC threshold	F-value df = 3,42	Prob > F	R^2	RPC threshold
Dead or disorientated	–	–	–	–	3.8	0.0174	0.21	0.42
Fin emphysema	123.8	<.0001	0.91	0.11	2.0	ns	0.13	
Exophthalmia	25.9	<.0001	0.69	0.15	–	–	–	–
Eye haemorrhage	13.5	<.0001	0.54	0.17	5.5	0.0028	0.28	0.39
Eye emphysema	62.6	<.0001	0.84	0.13	–	–	–	–
Pharyngo-clitheral membrane emphysema	15.6	<.0001	0.57	0.33	13.9	<.0001	0.5	0.33
Operculum emphysema	6.4	0.0014	0.36	0.13	1.3	ns	0.08	
Mouth haemorrhage	26	<.0001	0.69	0.15	–	–	–	–
Viscera haemorrhage	18.5	<.0001	0.61	0.63	24.8	<.0001	0.64	0.32
Viscera emphysema	9.6	<.0001	0.45	0.39	–	–	–	–
Heart haemorrhage	12.9	<.0001	0.53	0.3	–	–	–	–
Liver haemorrhage	19.6	<.0001	0.63	0.26	7.3	0.0005	0.34	0.49
Swim bladder rupture	38.4	<.0001	0.77	0.24	–	–	–	–
Kidney haemorrhage	8.8	0.0002	0.43	0.12	9.3	<.0001	0.4	0.68

Note:

Only injury responses where the model was able to converge for at least one of the species are shown.

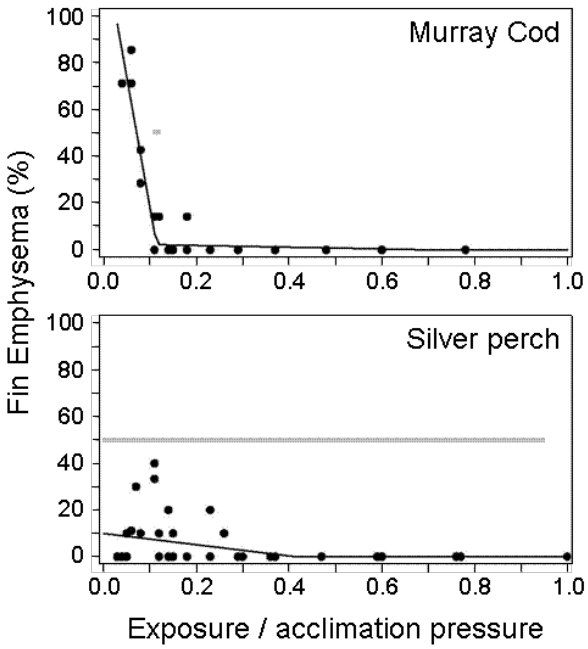
Dashes are where the model did not converge. i.e. the relationship did not have a breakpoint.

Those marked with 'ns' converged but were not statistically significant (0.05).

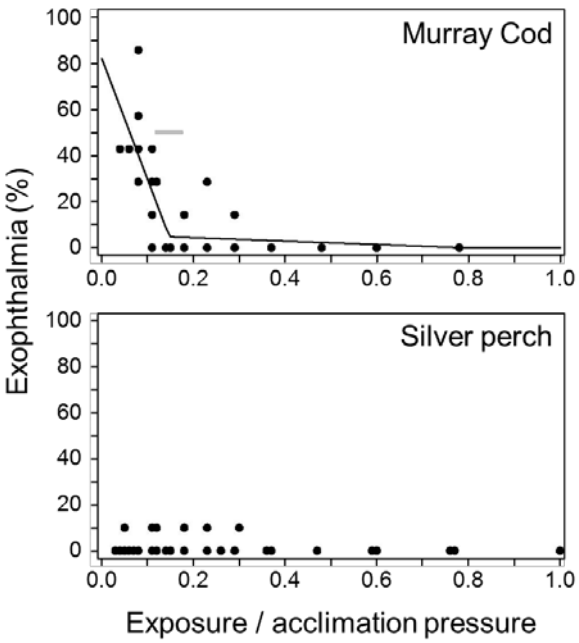
R^2 values indicate how well the piecewise model fit the data, those with $R^2 < 0.5$ typically display a poor fit of the breakpoint (reflected on the injury plots as a wide 95% confidence band).

Figure 18. a)–m), plots showing the percentage of juvenile Murray cod (top) and silver perch (bottom) that displayed various barotrauma injuries following simulated infrastructure passage over a range of ratio of pressure changes (Exposure/acclimation pressure). Trend lines are given if there was convergence in the piecewise linear regression model. The horizontal grey line shows the 95% confidence intervals of the breakpoint. Only injuries where the model converged for at least one of the species are shown. Estimated breakpoints and regression model tests are given in Table 7**.**

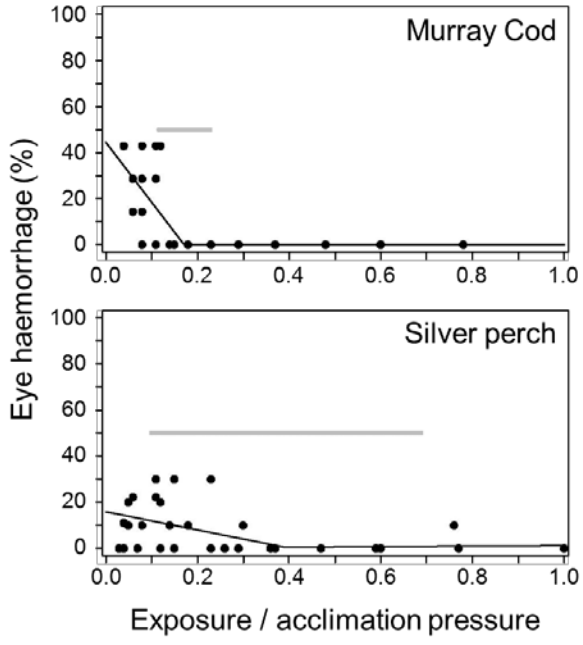
a) Fin emphysema



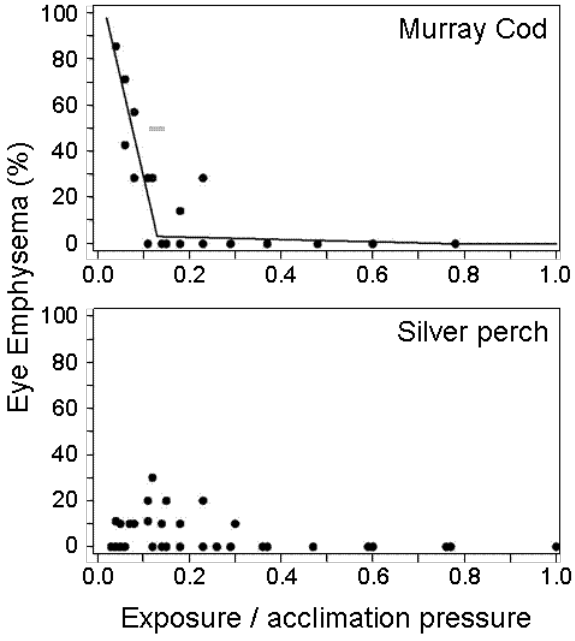
b) Exophthalmia



c) Eye haemorrhage

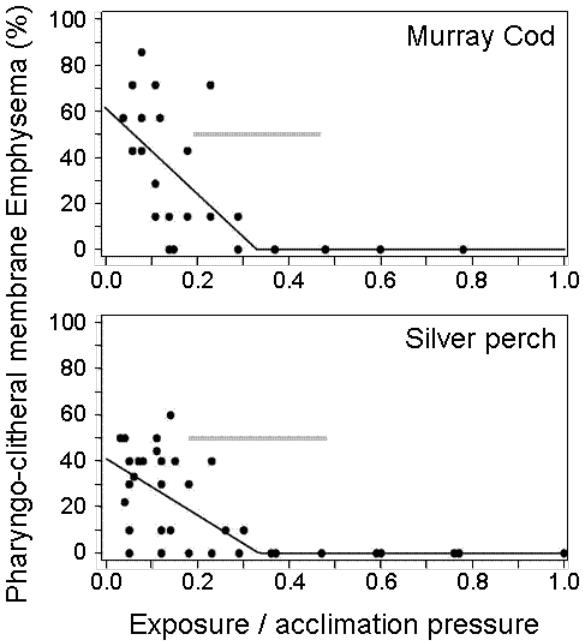


d) Eye emphysema

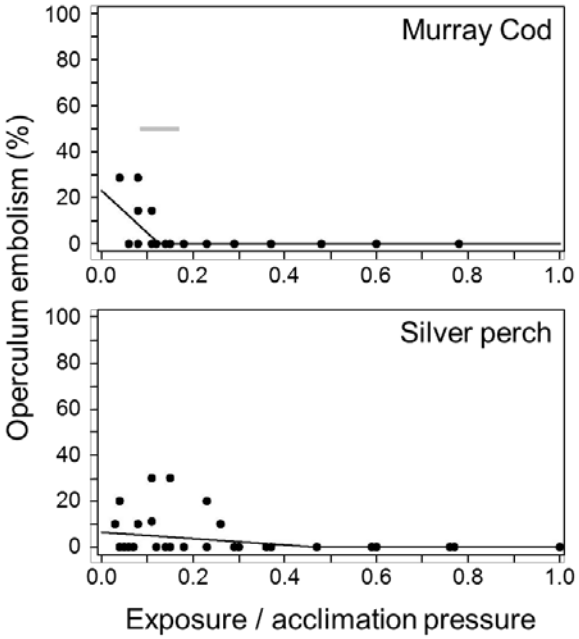


(Figure 18 continued)

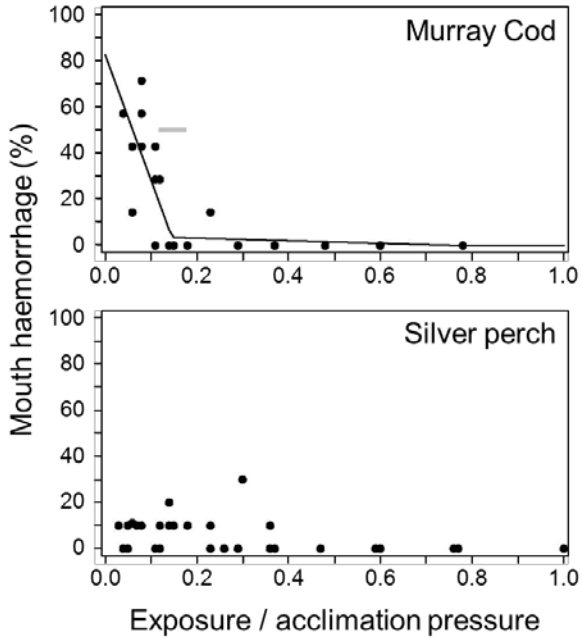
e) Pharyngo-clitheral membrane emphysema



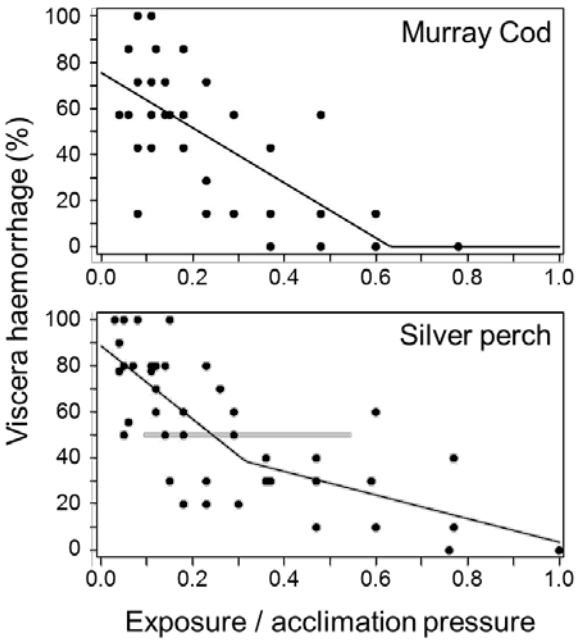
f) Operculum emphysema



g) Mouth haemorrhage

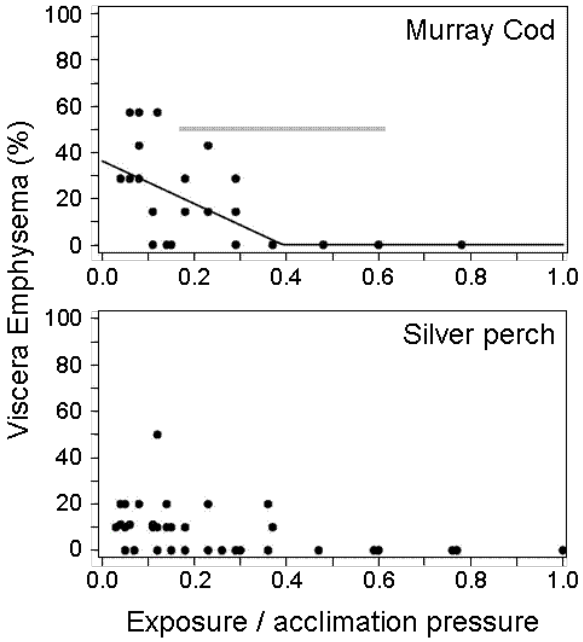


h) Viscera haemorrhage

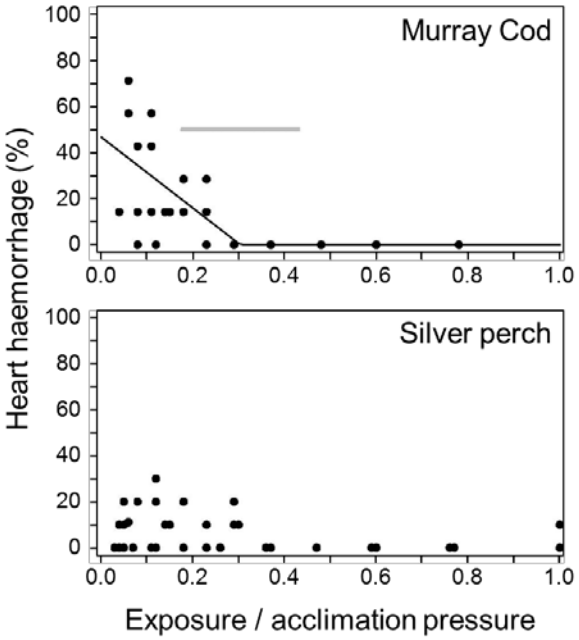


(Figure 18 continued)

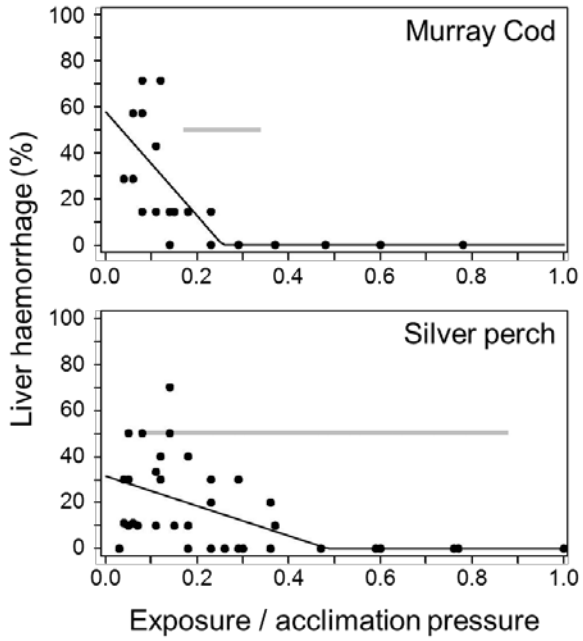
i) Viscera emphysema



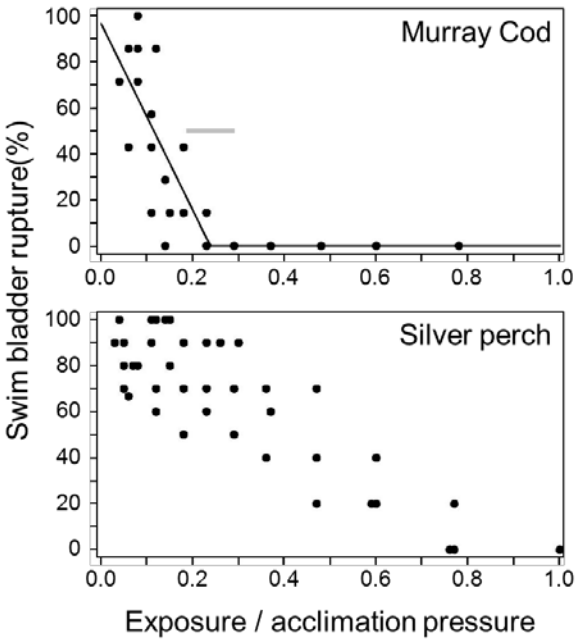
j) Heart haemorrhage



k) Liver haemorrhage



l) Swim bladder rupture



(Figure 18 continued)

m) Kidney haemorrhage

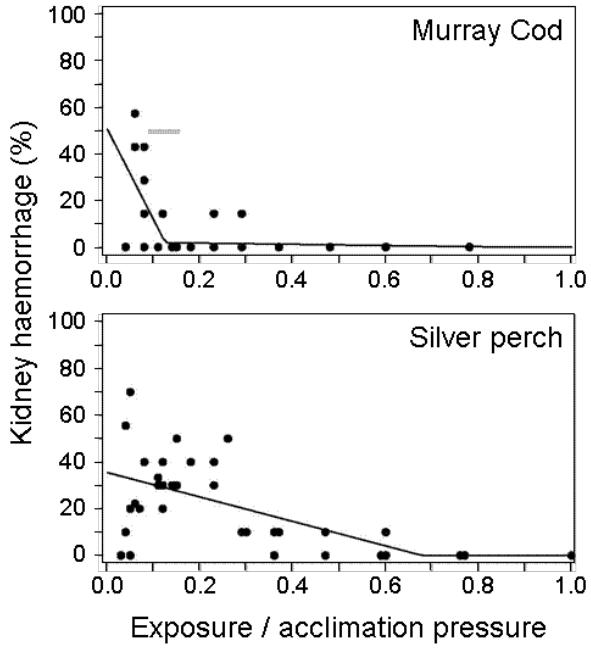
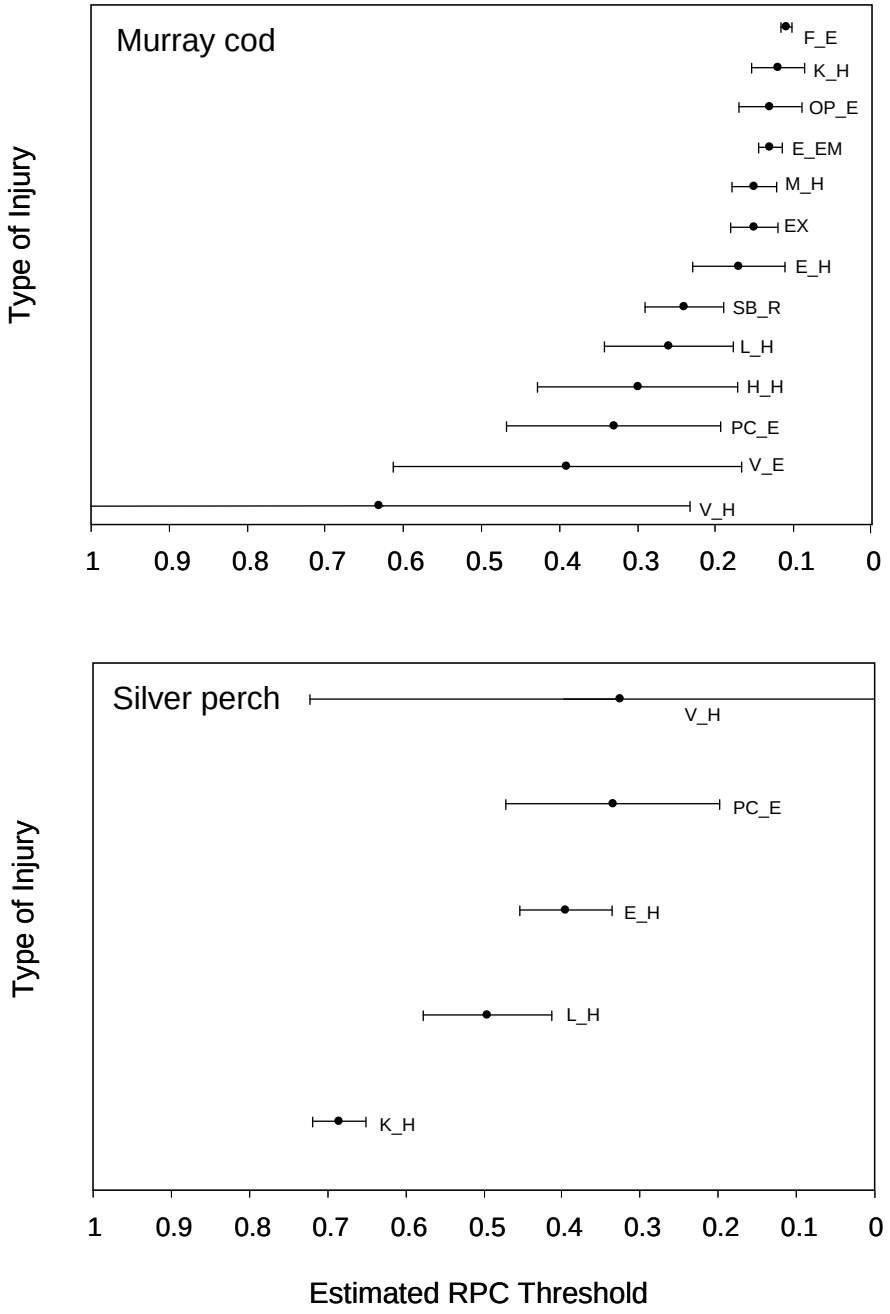


Figure 19. Summary of estimated ratio of pressure changes (RPC, E/A) thresholds **(**± 95% confidence intervals) where piecewise models could be fitted to the data. Injury types are presented bottom to top, in the order at which they were first estimated to occur (those with thresholds at less severe or lower RPCs to those at more severe RPCs)



Label codes: Kidney haemorrhage (K_H), liver haemorrhage (L_H), eye haemorrhage (E_H), pharyngo-clitheral membrane emphysema (PC_E), viscera haemorrhage (V_H), viscera emphysema (V_E), heart haemorrhage (H_H), swim bladder rupture (SB_R), exophthalmia (EX), mouth haemorrhage (M_H), eye emphysema (E_EM), operculum emphysema (OP_E) and fin emphysema (F_E).

2.4 Discussion

Murray cod, golden perch and silver perch were exposed to rapid decompression at the egg (golden perch and silver perch), larval (Murray cod, golden perch and silver perch) and juvenile (Murray cod and silver perch) stage. These correspond to the life stages at which the species are likely to be exposed to passage through river infrastructure during downstream migration. For eggs and larvae, the most extreme RPC tested was ~0.1; that is, exposure pressures that are ~10% of acclimation pressure. Juvenile fish were tested over a slightly larger range of RPCs, up to ~0.05, or exposure pressures that are ~5% of acclimation pressure.

The range of RPCs tested here reflects the vast majority of pressure scenarios that these life stages may be exposed to at river infrastructure. The release of autonomous hydraulic sensors (Sensor Fish: Deng *et al.* 2007) has revealed that fish may be exposed to slight sub-atmospheric pressures (~95 kPa) during downstream passage through 'undershot' irrigation weirs (Boys *et al.* 2013). Given this, a fish migrating at surface, 5 m or 10 m depth would be exposed to a RPC of 0.9 (95÷101 kPa), 0.6 (95÷152 kPa) or 0.5 (95÷201 kPa), respectively. Sensor Fish measurements taken at Kaplan and more advanced hydropower turbines have shown that while nadirs as low as 7 kPa could be experienced by fish, mean nadirs of ~87 kPa are more typical, with the level varying with discharge (Deng *et al.* 2010). Assuming the 87 kPa scenario, a fish migrating at surface, 5 m or 10 m depth would be exposed to RPCs of 0.9, 0.6 or 0.4, respectively. If experiencing the more extreme nadir of 7 kPa, fish migrating at surface, 5 m or 10 m depth would be exposed to RPCs of 0.07, 0.05 or 0.03, respectively.

2.4.1. Eggs and larvae

Egg and larvae seldom experienced mortality after being subjected to rapid decompression across the range of RPC tested. The vast majority of golden perch and silver perch eggs hatched successfully within 24 hours of being exposed to simulated infrastructure passage. Murray cod eggs were not tested; unlike silver perch and golden perch, Murray cod lay sticky eggs that adhere to a substrate and would not pass weirs, dams or hydropower plants like the pelagic, drifting eggs of the other two species.

While the lack of mortality may seem unexpected given the fragility of eggs and larvae, it is consistent with several laboratory studies that have exposed egg and larvae to sub-atmospheric pressures (reviewed in Cada 1990). Beck *et al.* (1975) exposed egg and larval striped bass (*Morone saxatilis*) to sub-atmospheric pressures (44 and 14 kPa) with little resultant mortality. Similarly, common carp larvae (*Cyprinus carpio*) exposed to 53 kPa suffered no mortality (Ginn *et al.* 1978). Bluegills (*Lepomis macrochirus*), largemouth bass (*Micropterus salmoides*) and channel catfish (*Ictalurus punctatus*) are other species that have displayed low or no mortality when exposed to hydraulic stresses, including decompression to pressures as low as ~50 kPa (Kedl and Coutant 1976, Cada *et al.* 1981).

Although larval mortality was rarely observed in this study, there was evidence of pressure-induced effects. Internal injury rates were typically higher as RPCs fell; that is, the rate of injury increased as the exposure pressure fell to a smaller proportion of the acclimation pressure. In many cases, this relationship was not linear and for silver and golden perch a threshold response was observed. For silver perch, the rate of injury substantially increased as the exposure pressure fell below ~40% of the acclimation pressure (RPC ~0.4). For golden perch, this threshold occurred at exposure pressures of ~50% of the acclimation pressure.

The primary observation driving the increase in injury was whether a fish had a deflated swim bladder. Jones (1951) showed that slow decompression to 40% or less of the acclimation pressure can cause swim bladder rupture in euthanased redfin perch (*Perca fluviatilis*). This has subsequently been applied as a rule of thumb by others when defining decompression thresholds for the protection of fish at hydropower turbines (e.g. Cada 1990). Brown *et al.* (2012a) exposed juvenile Chinook salmon to rapid decompression more characteristic of turbine passage, and noted that rupture occurred once

exposure pressures fell below 50% of acclimation pressures. Our observed thresholds for swim bladder deflation in silver perch, golden perch and Murray cod larvae were all around an RPC of 0.4, which seems to support the findings of these studies.

In this experiment, we considered a deflated swim bladder as a potential sign of swim bladder rupture, because the fish were too small to identify actual rupture points. The most extreme RPC tested in this study would have resulted in a larvae's swim bladder expanding to ~9.5 times its original size: a magnitude that has been shown to cause significantly high rates of rupture in other species (e.g. Chinook salmon: Brown *et al.* 2012a). However, it is unclear whether the swim bladder deflation we observed in larvae was due to rupture via an alternative mechanism, such as the venting of gas through the gut (referred to hereafter as burping). While the species examined here were physoclistous and have 'closed' swim bladders as juveniles and adults (preventing burping), it is possible that a rudimentary connection may still exist between the swim bladder and gut at the larval stage. This connection at an early life stage allows for initial swim bladder inflation by gulping air at the surface, a behaviour that has been documented in the larvae of many physoclistous species, including percichthyids (Hadley *et al.* 1987, Chapman *et al.* 1988, Battaglene and Talbot 1990, Battaglene *et al.* 1994).

Burping of gas could be beneficial for larval survival, because it may prevent swim bladder rupture and barotrauma injury. There are multiple lines of evidence for burping in the larvae we studied. First, deflated swim bladders seldom led to mortality within 24 hours of exposure, and there was little evidence from this study to suggest that these injuries will be of any consequence to the long-term survival of the fish. Second, swim bladder rupture has also been associated with the presence of internal emphysema in other studies (Brown *et al.* 2012b), because bubbles of gas released from the swim bladder become trapped in the viscera or embedded in surrounding tissue. Although we observed some cases of internal emphysema in larvae at lower RPCs, this observation was often independent of a fish having a deflated swim bladder (Figure 14). Without having any evidence of an association between a deflated swim bladder and the presence of internal emphysema, we must conclude that there is a substantial possibility that the deflation of the swim bladder resulted from burping, rather than swim bladder rupture.

The question then remains that if internal emphysema were not caused by swim bladder rupture, then what was the cause of this in some of the larvae we decompressed? Others have noted that emphysema can result either from free gas being displaced from elsewhere, or having come out of solution (Brown *et al.* 2012b). The latter is most likely in the larvae we studied, since the swim bladder was the only source of free gas in these larval fish. Although the data suggests emphysema was more likely to be observed at lower RPCs, no threshold response was evident, and emphysema was also observed in a small number of fish that had not been subjected to decompression (RPC=1). In 12 DPH golden perch, 50% of one of test group of fish not decompressed displayed signs of internal emphysema (Figure 13). This raises further doubt about whether the internal emphysema observed in this study can be considered barotrauma related.

We found some evidence that susceptibility to injury may vary between species and age classes. For example, internal haemorrhaging was only observed in 18-DPH golden perch. Larvae undergo rapid anatomical and physiological development. Therefore, vasculature and organs may be more vulnerable to barotrauma at some developmental stages than at others. In larval white sturgeon (*Acipenser transmontanus*), barotrauma susceptibility may be higher at the time of first exogenous feeding than at other times (Brown *et al.* 2013). As with swim bladder deflation, the critical threshold in larvae that were affected appears to be when exposure pressure falls below 40% of acclimation pressure.

In this study, 24 hours post exposure was treated as the end point. Research into the longer-term survival of eggs and larvae following rapid decompression may be warranted. In particular, it is unclear whether injuries that appear non-lethal at the 24-hour point (e.g. haemorrhage and swim bladder deflation) have longer-term implications on survival. While burping may reduce the

susceptibility of larval fish to swim bladder rupture, larvae that fail to initially fill their swim bladder can suffer reduced growth, skeletal deformities and increased susceptibility to stress-induced mortality in aquaculture conditions (Spectorova 1976, Al-Abdul-Elah *et al.* 1983, Weppe and Bonami 1983). Early-stage larvae that are forced to burp during decompression may never regain the capacity to refill the swim bladder and develop as normal. With respect to eggs, there is evidence that prolonged increases in pressure shortly after fertilisation can lead to triploid progeny that are incapable of reproducing (Chourrout 1984, Goudie *et al.* 1995). While this is a very different profile of pressure change when compared to the decompression experienced by fish at river infrastructure, it highlights the vulnerable nature of fish to pressure effects at this early ontogenetic stage. To date, no one has attempted to keep fish hatched from eggs that have been exposed to rapid decompression for long enough to evaluate potential impacts on future reproduction.

2.4.2. Juveniles

While few juvenile Murray cod or silver perch were dead or disorientated immediately following simulated infrastructure passage, autopsies determined that there was a high frequency of barotrauma injury. This supports the findings of Brown *et al.* (2012a), who highlighted the importance of using both internal and external observations to assess the impacts of hydropower turbine passage, since fish suffering from barotrauma may not die immediately. The clinical signs of barotrauma most commonly observed were swim bladder rupture; exophthalmia; haemorrhage of the viscera, heart, liver, kidney, mouth and eye; and emphysema of the fin, eye, viscera, PC membrane and operculum. The longer-term impacts of these injuries on survival are poorly understood for these species. However, liver, kidney and viscera haemorrhage, swim bladder rupture, exophthalmia, and emphysema of the fins have been linked with mortality in juvenile Chinook salmon (McKinstry *et al.* 2007).

In most cases, there was a significant negative relationship between the percentage of fish injured and RPC (E/A). At the lowest (most extreme) RPCs tested, the percentage of fish affected ranged between 40 and 100%, depending on the type of injury. The relationship was typically non-linear, and discrete thresholds could be seen in most cases. For many of the injuries, we found that the breakpoint generated by the piecewise regression approach allowed us to objectively define a threshold in RPC that once exceeded, resulted in a significant increase in the probability of injury. There tended to be more uncertainty around the threshold estimates for silver perch, when compared to Murray cod. In silver perch, some injuries began when exposure pressure fell below 70% of the acclimation pressure, while others were not seen until exposure pressures fell below 30% of the acclimation pressure. Thresholds were more distinct in Murray cod. Generally, Murray cod required greater levels of decompression before clinical signs of barotrauma were observed. However, once thresholds were exceeded, the incidence of injury increased substantially more in Murray cod than in silver perch. For Murray cod, the vast majority of injuries were noted once exposure pressure fell below 40% of acclimation pressure, and by 20%, half of the clinical signs of barotrauma we had noted had occurred. By the time RPC had fallen below 10–20% of acclimation pressure, the remaining barotrauma injuries had begun occurring.

The difference between Murray cod and silver perch in the probability of some injury types may be explained by anatomical or physiological differences. For example, exophthalmia was regularly observed in Murray cod once exposure pressure fell below 15% of acclimation pressure, but rarely observed in silver perch. Reports of susceptibility to barotrauma-induced exophthalmia vary greatly in the literature, even within the same genus. For example, exophthalmia was frequently reported in angled golden perch (*Macquaria ambigua*) (Hall *et al.* 2013), but not in Australian bass (*M. novemaculeata*) brought to surface from similar depths (Roach *et al.* 2011). For all species predisposed to exophthalmia, the incidence always increases as RPC (E/A) decreases, whether this be angled fish surfacing rapidly from greater depths (Rummer and Bennett 2005, Hannah *et al.* 2008a), or migratory fish being exposed to sub-atmospheric pressures (Brown *et al.* 2012b).

The head shape of Murray cod is substantially different from that of silver perch; this may make the cod more predisposed to exophthalmia. The eyes of Murray cod protrude from a ventrally compressed head, whereas the eyes of silver perch protrude to a lesser extent, within a laterally compressed head (Figure 38). To our knowledge, the role of head shape and eye placement in mediating susceptibility to exophthalmia has not been investigated. However, fish with a large choroid body that secretes oxygen to the eye (similar to the rete mirabile in the swim bladder, Wittenberg and Wittenberg 1974) seem more susceptible to exophthalmia (Stephens *et al.* 2001). This has not been linked directly with decompression injuries, but rather with spontaneous exophthalmia under aquaculture conditions, which relates to the release of oxygen into the orbital cavity during stress-induced blood acidosis (Herbert *et al.* 2002, Wells and Dunphy 2009). Others have attributed exophthalmia during rapid decompression to the release of gas from the swim bladder during rupture (Brown *et al.* 2012b). The location of rupture on the swim bladder may determine the likelihood of exophthalmia. Rupture points on the anterior-dorsal region of the bladder are more often associated with gas travelling to the orbital cavity (Hannah *et al.* 2008b). In comparison, in species less susceptible to exophthalmia, air released from the swim bladder often travels caudally, and may be more associated with abdominal bloating and cloacal prolapse (Rummer and Bennett 2005, Rogers *et al.* 2008).

Rupturing of the swim bladder occurred in both Murray cod and silver perch, although its relationship with RPC differed substantially between species. In silver perch, the relationship was fairly linear. The first observations of rupture occurred once exposure pressure fell below 80% of acclimation pressure, and became progressively more frequent as RPC fell. Although no distinct threshold was determined using the piece-wise model, only two fish out of 60 displayed a ruptured swim bladder if exposure pressure was kept above 60% of acclimation pressure. Decompression beyond that level led to a steady increase in the probability of rupture, until close to 100% of fish had sustained ruptured swim bladders at exposure pressures that were 10% of the acclimation pressure. In comparison, juvenile Murray cod appear to have a much higher threshold for swim bladder rupture, which did not occur until fish were exposed to pressures lower than ~25% of acclimation pressure. Like silver perch, 100% rupture rates were evident in Murray cod once exposure pressure was close to 10% of the acclimation pressure.

The obvious cause of swim bladder rupture is the expansion of gas inside it during decompression, causing it to overinflate and burst. The degree of gas expansion for a given level of decompression can determined using Boyle's law. This law states that P1V1=P2V2 (where P1 and V1 are the initial acclimation pressure and volume and P2 and V2 are the exposure or final pressure and volume). Put simply, for every halving of pressure, gas volume will expand to twice its original volume. Therefore, while silver perch sustained ruptures from only minor pressure-induced increases in swim bladder volume (1.25 times), Murray cod appear to have more robust bladders, which can expand four times in volume before rupture occurs. Physoclistous species with closed swim bladders are often suggested to be more susceptible to rupture than physostomes, which have the ability to vent or burp gas during rapid decompression (Cada 1990, Becker *et al.* 2003, Brown *et al.* 2012a). This assertion is not supported by our result, when they are viewed within the context of other studies. For example, larger falls in pressure were required to rupture the bladders of Murray cod (a physoclist) than Chinook salmon (a physostome in which rupture occurs when pressure is halved; Stephenson *et al.* 2010). Therefore, when predicting the swim bladder's vulnerability to rupture, the morphology or strength of the swim bladder may be equally or even more important than whether the fish is physoclistous or physostomous. At the very least, it appears that generalisations about thresholds for swim bladder rupture should be treated with some degree of caution, as mentioned earlier with respect to the use of Jones' (1951) findings by Cada (1990).

Although the long-term effects of swim bladder rupture on fish survival were not investigated in this study, it has been linked to eventual mortality in some species: for example, Chinook salmon (McKinstry *et al.* 2007) and redfin perch (*Perca fluviatilis*; Harden Jones 1951). Mortality may not occur directly from the rupture itself, because swim bladder healing following rupture has been reported for several species. These include Atlantic cod (*Gadus morhua* L.; Midling *et al.* 2012), rainbow trout (*Oncorhynchus mykiss*; Bellgraph *et al.* 2008) and Pacific cod (*G. macrocephalus*

Tilesius; Nicho and Chilton 2006). Instead, displaced gas may cause associated damage of other organs, or lead to sub-lethal impacts that can subsequently cause death: e.g. loss of buoyancy regulation once gas becomes trapped in the body cavity, leading to exhaustion or predation (Brown *et al.* 2012b). The long-term effects of the injuries observed in this study on the survival of Australian species should be further investigated, along with any sub-lethal effects on vulnerability to predation, feeding, growth and reproduction.

3. FLUID SHEAR

3.1 Introduction

Water velocity plays an important role in mediating the transfer of food, nutrients and organisms through rivers, as well as creating a diversity of habitats (Gordon *et al.* 1992). Fish tend to be well-adapted to the range of water velocities (Vogel 1994), and use these during both downstream and upstream migrations (McLaughlin and Noakes 1998). However, fish are generally more tolerant of flow in a uniform direction. If the water velocity changes abruptly, shear stress and turbulence can cause injury and disorientation (Cada *et al.* 1999).

Shear stress occurs when two masses of water with different velocities and/or direction intersect. This causes friction at the interface of the two masses (Davies 1988). When a fish is exposed to this intersection at a scale comparable to its body length, it is exposed to frictional forces on its body. Fluid shear occurs naturally in river systems, particularly through riffle and rapid habitat. But, it can be substantially elevated as water passes near or through river infrastructure, such as weirs, regulators, dam spillways and hydropower facilities (Cada 2001, Baumgartner *et al.* 2006).

Once shear stress becomes elevated to a level that cannot be tolerated by fish, injury and mortality can result (Cada 2001). Studies simulating the shear environment generated in hydropower turbines have documented an increase in the mechanical injury of fish, with typical injuries including torn opercula, dislocated eyes, haemorrhaging, scale loss and behavioural stress (Neitzel *et al.* 2004, Deng *et al.* 2005). Most studies have focused on North American species and the impact on juvenile (Neitzel *et al.* 2004, Deng *et al.* 2005) or larval (Cada *et al.* 1981, Cada 1990, Killgore *et al.* 2001) fish at shear levels comparable to those produced by hydropower turbines. The data collected has been used to provide guidance on thresholds for fish injury and mortality, which can inform more 'fish-friendly' turbine design (Cada 2001).

Comparatively little is known about the role of shear as a mechanism for fish injury at infrastructure in areas outside North America, and at instream structures other than hydropower turbines. In Australia, there is concern that significant numbers of fish are being injured or killed when passing downstream through irrigation weirs (Baumgartner *et al.* 2006, Baumgartner *et al.* 2013). Computational fluid dynamics modelling suggests that elevated shear levels can occur in areas where water is discharged under a gate (i.e., an undershot weir; Baumgartner *et al.* 2013). While this may contribute to fish injury during weir passage, the theory remains to be tested. So too does the importance of shear stress as a mechanism for injury relative to other hydraulic stresses, such as barotrauma resulting from rapid decompression. It is important to determine the potential for shear-induced injury at existing instream structures, as well as to predict the potential risk of injury faced by migrating fish, as new facilities are proposed and considered for construction. Within New South Wales (NSW), this is currently the case for mini-hydropower at a number of river sites (Baumgartner *et al.* 2012).

In this chapter, we report on experiments in which various Murray–Darling Basin (MDB) fish species (Murray cod, silver perch and golden perch) and life stages (egg, larvae and juvenile) were subjected to shear stresses of varying magnitudes in a flume. Using this approach, we were able to model the relationship between increasing shear stress and injury and mortality.

3.2 Methods

3.2.1. Shear flume

A cylindrical plexiglass flume (1.95 m long and 0.44 m diameter) was connected to a submerged jet at one end, and to a fibreglass reservoir tank (2.10 m long x 2.10 m wide x 0.9 m) at the other end (Figure 20). An electric pump (Grundfos® NBG 125), capable of pumping water at 153 $m^3 h^{-1}$, circulated water through the flume via a 15-cm diameter PVC pipe. On entry to the flume, a conical plastic nozzle reduced the diameter of flow from 15 cm to 5 cm over a distance of 26 cm (Figure 21),

effectively accelerating the flow and creating a submerged jet into the flume. This approach has been used by others to reduce the non-uniformity of velocities inside the flume, and generate a quantifiable shear environment where water from the flume becomes entrained in the jet stream (Neitzel *et al.* 2000).

Maximum velocities of 18.3 m s^{-1} were possible in the centre of the nozzle. A valve was used to reduce this velocity and subsequently alter the shear environment. An inline flow meter (Wollman Silver Turbo Water Meter, ARAD Waterworks®) was used to set the jet at various flow rates, which were pre-determined as generating particular levels of shear strain at the interface of the jet and the surrounding water (Figure 21). A clear polycarbonate deployment tube fixed above the submerged jet at an angle of ~30° was used to introduce eggs, larvae and juveniles to the point in the flume where the strain rate was known. The end of the deployment tube was located 30 mm above the centreline of the jet (Figure 21).

3.2.2. Characterisation of the shear environment

The level of shear that experimental fish were exposed to was quantified using an approach similar to that adopted in previous studies (e.g. Neitzel *et al.* 2004). Using a pitot tube attached to a calibrated pressure gauge, velocity measurements were taken along a radial axis. The axis started 90 mm in front of the nozzle and radiated out from the jet's centreline at 5 cm increments, encompassing the point where fish were first exposed to the jet (Figure 21). Although fluid shear can occur downstream of this point, strain rates have been quantified at multiple points downstream (Neitzel *et al.* 2004), and these will never be as high or constant as the strain rates at the zone of flow establishment closest to the jet nozzle. It is also impossible to determine an exact and consistent path of fish downstream in the flume, and therefore impossible to quantify a standardised measure of shear for the fish's entire time in the flume. As a result, when we refer to strain rates in this study, we are referring to the maximum strain rate that a fish was exposed to within the zone of flow establishment (as per Neitzel *et al.* 2004). We are not referring to a measure of the complete, complex and stochastic shear environment that a fish was exposed to during its entire time in the flume.

Flow velocities (m s-^{1}) were calculated by converting the velocity head (m) measured with the pitot tube and using a Bernoulli's equation, where H is the total head (m), υ is the velocity (m s^{-1}) and g the gravitational constant (m^2 s^{-1}):

$$H = \frac{\upsilon^2}{2g} \qquad \textit{Eq. 4}$$

Velocity measurements at the exit of the jet were taken for a variety of flow rates generated by the flume pump (5, 10, 15, 20, 25, 30, 35 and 37 L s^{-1}) (Boys *et al.* 2013). These generated mean jet velocities (measured 90 mm in front of the nozzle) of 3.9, 7.78, 14.71, 18.97, 24.82, 28.92, 32.36, 33.81 and 34.5 m s^{-1}, respectively. Shear strain (τ) was calculated for each of these flows based on the radial velocities measured with the pitot tube. Shear strain rate (cm $s^{-1}$$cm^{-1}$) can be described as the change of water velocity ($\vec{\mu}$) produced over distance (y), as shown in Equation 5:

$$\tau = \frac{\delta\vec{\mu}}{\delta y} \qquad \textit{Eq. 5}$$

The distance across which changes of velocities are measured has an important influence on the calculation of shear strain. During our study, the distance used to calculate shear strain was 5 mm ($\Delta y = y_f - y_i$). This is of higher resolution than that used by Neitzel *et al.* (2004), who calculated strain using a Δy = 18 mm. We used a finer scale measurement in this study, because we were interested in testing smaller fish, including eggs of ~ 2mm diameter and larvae ranging from ~9–20 mm in length. The resultant strain rates that fish were tested at in this study were 18.20, 148.35, 446.22, 629.49, 880.80, 1056.90, 1204.94, 1267.02 and 1296.87 cm $s^{-1}$$cm^{-1}$ (Table 8).

Figure 20. Overview of the shear flume, showing the main components

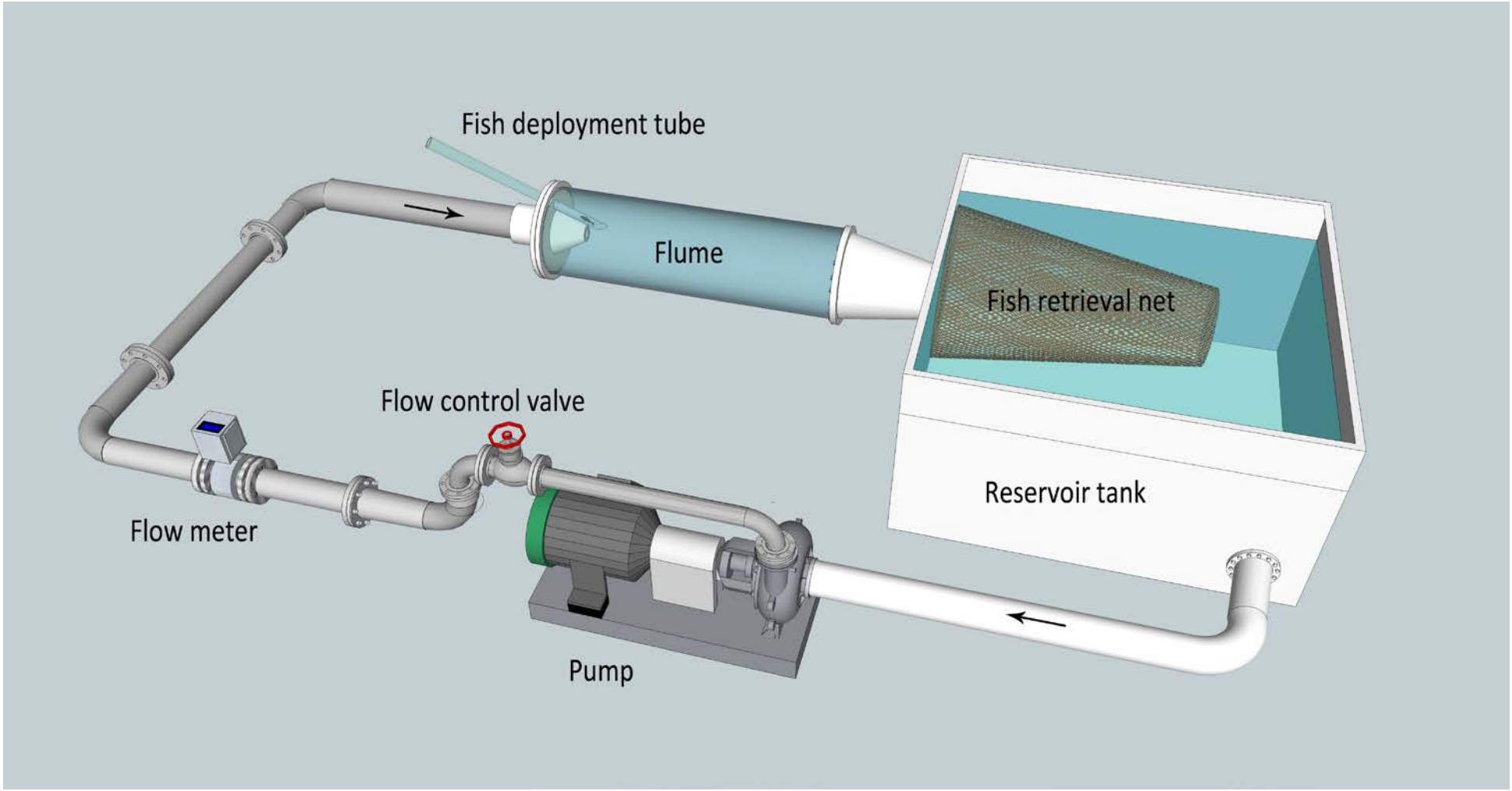


Figure 21. Schematic of the flow establishment zone of the flume, showing the nozzle (A), the deployment tube (B), the edge of the jet and fish exposure point (C) and location of the flow establishment zone (D)

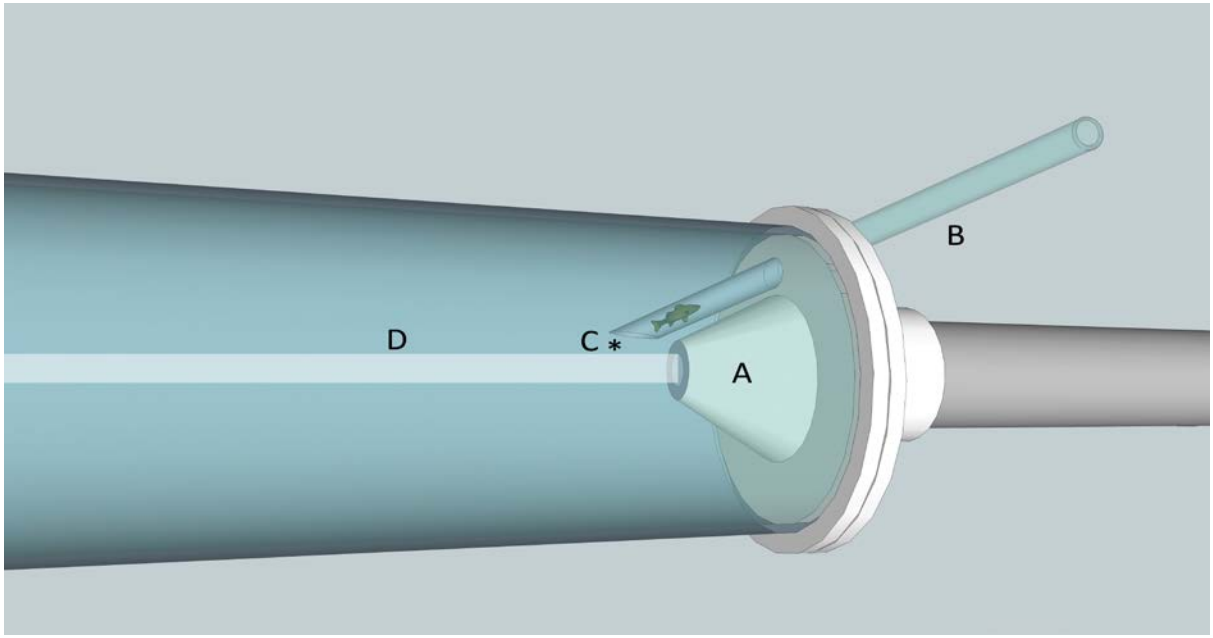


Table 8. Velocities of the jet stream in front of the nozzle and the calculated shear strain rate for a given flow generated by the pump through the flume

Flow through flume (L s^{-1})	Velocity of jet (m s^{-1})	Strain rate (cm s^{-1} cm^{-1})
3.90	3.13	18.20
7.78	4.00	148.35
14.71	5.56	446.22
18.97	6.52	629.49
24.82	7.83	880.80
28.92	8.75	1056.90
32.36	9.53	1204.94
33.81	9.85	1267.02
34.50	10.01	1296.87

3.2.3. Fish production and handling

Eggs and larvae
Larval Murray cod, golden and silver perch and eggs of golden perch and silver perch were bred, harvested and housed at the Narrandera Fisheries Centre (NFC) in accordance with the methods outlined in Section 2.2.1. Murray cod eggs were collected from earthen ponds. Once hatched, the larvae were kept in aerated trays until needed for the experiments (every second day between 9 and 29 days post hatch, DPH). Golden perch larvae of 12, 18 and 26 DPH and silver perch larvae of 13, 19 and 27 DPH were collected from rearing ponds using a dip and/or pull net. The larvae were collected 24 to 48 hours before experimentation and kept in aerated trays until required.

Juveniles
Juvenile Murray cod, silver perch and golden perch (~2 months old) from a MDB genetic strain were bred at NFC. Juveniles were collected from earthen rearing ponds within 2–3 weeks following juvenile metamorphosis using a dip net, and held in aerated holding trays supplied with flow-through, bore-drawn water (~10 L min^{-1}) for 24–48 hours prior to experimentation. At the time of experimentation, the average weight and length of Murray cod were 42 mm (± 3 SE) and 0.8 g (± 0.2); golden perch were 39±3 mm and 0.7±0.2 g; and silver perch were 29±3 mm and 0.5±0.2 g.

3.2.4. Egg experiments

Healthy golden perch and silver perch eggs were pipetted from an aerated bucket into a small plastic jar of bore-drawn water (15 eggs per test group) and transferred via the deployment tube into the shear flume. A gentle flow of water down the deployment tube ensured all eggs were flushed into the flume where they were exposed to a shear event. After exposure, the eggs were retrieved from the collection reservoir using a larval net (Figure 22). Any eggs retrieved were placed into an aerated jar and kept for 24 hours so that delayed mortality could be quantified.

Four test groups of 15 eggs each (for each species) were exposed to one of nine shear strain rates: 18.20, 148.35, 446.22, 629.49, 880.80, 1056.90, 1204.94, 1267.02 or 1296.87 cm s^{-1} cm^{-1}. Sixty additional eggs of each species not put through the flume were kept in aerated jars (20 eggs each) to be used as hatching controls. Handling effects were further quantified by introducing four test groups at a strain rate of 0 cm s^{-1} cm^{-1} into the flume via the deployment tube while the pump was not running.

Figure 22. Net used to recapture the fish and eggs after being exposed to shear strain environments

3.2.5. Larval experiments

Ten larvae were pipetted from the holding tray into a small jar of bore-drawn water and transferred via the deployment tube into the shear flume. A gentle flow of water down the deployment tube ensured all larvae were flushed into the flume, where they were exposed to a shear event. The orientation of fish entering the flume (i.e. head or tail first) was not controlled for. After exposure, larvae were collected downstream of the flume as per the methods outlined for the egg experiments. All larvae were kept for up to 24 hours in aerated jars so that delayed mortality could be quantified.

Murray cod, golden and silver perch larvae were exposed to one of nine strain rates (18.20, 148.35, 446.22, 629.49, 880.80, 1056.90, 1204.94, 1267.02 or 1296.87 cm s^{-1} cm^{-1}) at various ages (DPH: Table 9). Each strain rate treatment (along with a handling control of 0 cm s^{-1} cm^{-1}) was conducted on three replicate test groups.

Table 9. Summary of experimental design showing the age (days post hatch: DPH) of larvae tested and the mean maximum and minimum lengths of fish across the three test groups, with 10 replicates for each age group

Species	Age of larvae (DPH)	Mean length (mm)±SD	Min. length (mm)	Max. length (mm)
Murray cod	9	9.48±0.10	9.01	9.89
	11	9.62±0.08	9.22	9.97
	13	9.85±0.06	9.60	10.28
	15	9.91±0.09	9.35	10.38
	17	10.02±0.06	9.70	10.34
	19	9.86±0.07	9.33	10.24
	21	10.03±0.09	9.47	10.58
	23	9.97±0.10	9.51	10.62
	25	9.92±0.08	9.60	10.30
	27	9.95±0.08	9.48	10.34
	29	9.97±0.12	9.55	10.51
Golden perch	12	9.88±2.84	9.18	10.26
	18	11.44±0.76	9.90	13.79
	26	16.12±1.48	13.44	19.95
Silver perch	13	8.71±0.59	7.67	10.60
	19	13.74±0.6	12.45	15.20
	27	18.41±0.93	16.96	21.32

3.2.6. Juvenile experiments

Juvenile Murray cod, golden perch and silver perch were dip-netted from holding trays and transferred via the deployment tube into the shear flume. A gentle flow of water down the deployment tube ensured all fish were flushed into the flume, where they were exposed to a shear event. Although the orientation of fish entering the flume was not controlled for, fish typically orientated themselves into the flow and therefore entered the flume tail first. Each test group was exposed to one of five strain rates (18.20, 446.22, 880.80, 1204.94 or 1296.87 cm s^{-1} cm^{-1}). For each species, each strain rate treatment (along with a handling control of 0 cm s^{-1} cm^{-1}) was conducted on six replicate test groups of five fish. After exposure, all fish were collected downstream of the flume as per the methods outlined for the egg and larval experiments. All fish were kept for up to 24 hours in aerated jars to enable delayed mortality to be determined.

At the time of the experiments, the mean length for Murray cod juveniles was 42.3 mm (± 3.1 SD and ranging from 36–49 mm) and mean weight of 0.77 g (± 0.17 SD and ranging from 0.4–1.2 g). The mean length for golden perch was 39 mm (± 3.4 SD and ranging from 30–50 mm) and mean weight of 0.74 g (±0.23 SD and ranging from 0.2–1.6 g). The mean length of silver perch was 28.5 mm (± 3.2 SD and ranging from 23–38 mm) and mean weight of 0.50 g (± 0.16 SD and ranging from 0.2–1.0 g).

3.2.7. Characterisation of injuries

Eggs were determined to be mortally damaged if either torn into small pieces prior to being recovered from the net (mortality at 0 hours), or if they were recovered whole but had sustained sufficient damage to result in a failure to hatch into larvae (mortality at 24 hours). Examination of eggs under a microscope (Leica M165FC, Leica Microsystems Pty Ltd®) helped to identify mortally damaged eggs.

Numbers of dead larvae or juveniles in each test group were counted immediately following exposure and again at 24 hours, with the sum of these two counts to calculate mortality rate. During each count, any external injuries were also noted, to determine which injury types were most prevalent following exposure to fluid shear. After 24 hours, all larval and juvenile fish still alive were euthanased in a solution of 100 mg L^{-1} ethyl-p-amino benzoate (benzocaine).

3.2.8. Statistical methods and modelling

The total number of mortalities (0 + 24 hours) was expressed as a percentage and analysed using logistic regression to model the effects of shear strain rate on mortality. For the larval experiments, developmental stage (DPH) was included in the models as a covariate to test for differences in mortality rates between stages. To allow simple comparisons between developmental stages, separate models were also applied at each stage, and the actual and predicted mortalities were presented graphically.

Threshold strain rates were calculated for each larval developmental stage for each species using Equation 6:

$$(1) \quad P_i \Big/ \left(1 - P_i\right) = e^{\left(b_0 + b_1 * x\right)} \qquad \textit{Eq. 6}$$

where P_i is the threshold mortality rate, x is the strain rate, and b_0 and b_1 are coefficients in the logistic model. We calculated the lethal shear that would kill 10% and 75% more fish than the handling control (strain rate of zero) within 24 hours of exposure (referred to as LS_{10} and LS_{75}).

3.3 Results

3.3.1. Eggs

Egg mortality (0 + 24 hours) significantly increased as strain rate increased, both for silver perch (χ^2 = 333.1, df =1, p <0.0001) and golden perch (χ^2 = 123.3, df =1, p <0.005) (Table 10). Each increase in unit of strain rate (1 cm s^{-1} cm^{-1}) led to a 0.8% increase in the mortality rate of silver perch eggs, and a 7.1% increase in mortality of golden perch eggs (odds ratio: Table 10), until 100% mortality was reached. This occurred at strain rates of ≥629.49 cm s^{-1} cm^{-1} for silver perch, and ≥148.35 cm s^{-1} cm^{-1} for golden perch (Figure 23). Torn chorion and disrupted cellular protein were noted as the most probable cause of egg mortality (Figure 24).

Table 10. Logistic regression results for the prediction of mortality in golden perch and silver perch eggs exposed to increasing shear strain rates. The strain rates tested ranged from 0 to 1296.87 cm s^{-1} cm^{-1}

Species	χ^2	df	sig	Model	Values	SE	Wald	df	sig	Odds ratio
Golden perch	123.3	1	<0.001	Constant	-0.444	0.300	2.192	1	0.139	1.071
				Shear	0.068	0.022	9.314	1	0.002	
Silver perch	333.1	1	<0.001	Constant	-1.508	0.233	42.042	1	<0.001	1.008
				Shear	0.008	0.001	66.554	1	<0.001	

Figure 23. Average percentage mortality of eggs exposed to different shear strain rates for: a) golden perch and b) silver perch across different strain rates. The line defines the probability calculated by a binary logistic regression model

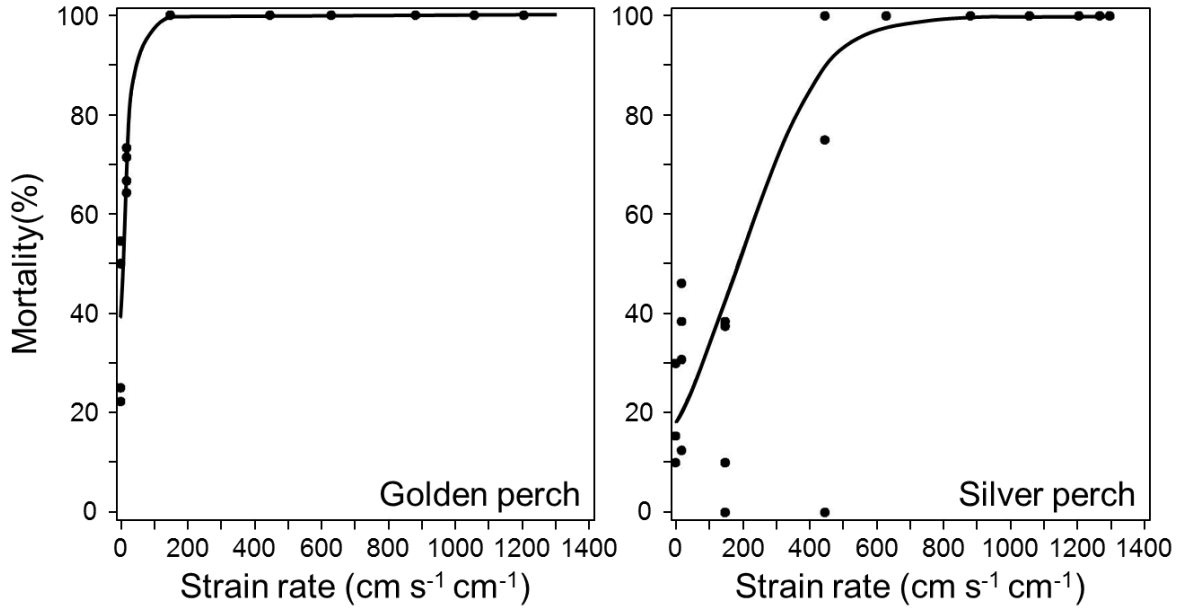
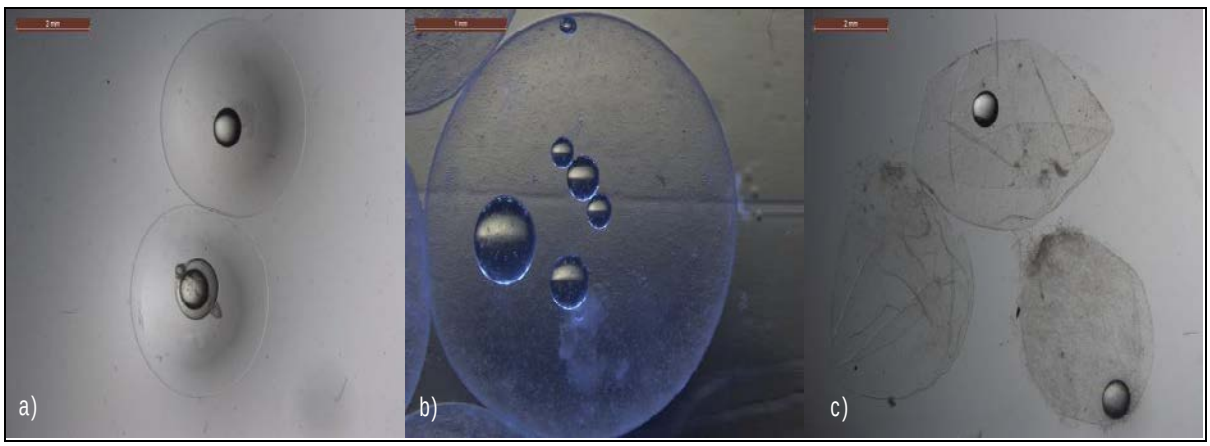


Figure 24. Images of golden perch eggs showing: a) no injury, b) disrupted cellular protein, and c) torn chorion



3.3.2. Larvae

Following exposure to shear, 92% of Murray cod, 96% of golden perch and 93% of silver perch larvae were recovered from the flume for analysis. Larval mortality for all three species significantly increased as strain rate was increased, but was not consistent across all DPH (Figure 25; Table 11). Typically, younger larvae were more susceptible to mortality when exposed to a shear environment than older larvae (Figure 25), and the degree to which increasing strain rate increased mortality changed with age (Figure 25). For example, Murray cod larvae were more likely to die across most strain rates at 9 DPH than at 19 or 29 DPH (Figure 25). Mortality increased for golden perch as strain rate increased for 12 and 18 DPH. However, once larvae were 26 DPH, exposure to a shear environment had little effect on survival across the complete range of strains tested (Figure 25) and the relationship was not significant (Table 11). Similarly, silver perch showed a strong relationship of increasing mortality with increasing strain rate at 13 DPH, but little effect was observed at 19 DPH and there was no significant relationship at 27 DPH (Table 11, Figure 25).

Of all the larvae studied, the age class of 9-DPH Murray cod were by far the most susceptible to mortality following shear exposure, having an LC_{10} of only 6 cm s^{-1} cm^{-1} and LC_{75} of 1056 cm s^{-1} cm^{-1} (Table 12). Murray cod larvae became less susceptible once they were 19 DPH, having a LC_{10} and LC_{75} of 894 and 2162 cm s^{-1} cm^{-1}, respectively, but remained more susceptible than the other two species at the oldest age class investigated (Table 12). Silver perch larvae were the next susceptible, having an LC_{10} and LC_{75} of 100 and 924 cm s^{-1} cm^{-1} at 13 DPH, and 1061 and 2159 cm s^{-1} cm^{-1} at 19 DPH, respectively (Table 12). Golden perch susceptibility decreased with age ranging from an LC_{10} and LC_{75} of 728 and 1762 cm s^{-1} cm^{-1} at 12 DPH, and 1326 and 1520 cm s^{-1} cm^{-1} at 26 DPH, respectively (Table 12).

It was not possible to determine the cause of injury for many larvae that died, because they discoloured and began to decompose relatively quickly after the time of death. When injury could be ascertained (Figure 26), fin damage was by far the most frequently observed external injury following exposure to a shear environment (Table 13). Forty-seven per cent of all Murray cod larvae had fin damage following exposure to shear, although this figure was as high as 61 and 74% for the youngest larvae tested (9 and 11 DPH, respectively). Fin damage was also noted in golden perch (31%) and silver perch (41%). The youngest age classes of Murray cod (9-13 DPH) were also susceptible to damage to the yolk sac, which was noted in more than a third of fish of this age bracket.

Figure 25. Average percentage mortality (immediate and delayed) of Murray cod[a] (top, a–c), golden perch (middle, d–f) and silver perch (bottom, g–i) larvae at three different ages (days post hatch (DPH), corresponding to columns) after exposure to various level of shear (strain rate). The line defines the probability of mortality calculated by the logistic regression model as defined in Table 12. Lines are not drawn on plots that were not statistically significant ($p>0.05$)

[a]Although Murray cod models were calculated for all ages (9 through to 29 DPH, shown in Appendix 3), for brevity only 9, 19 and 29 DPH are shown here as examples.

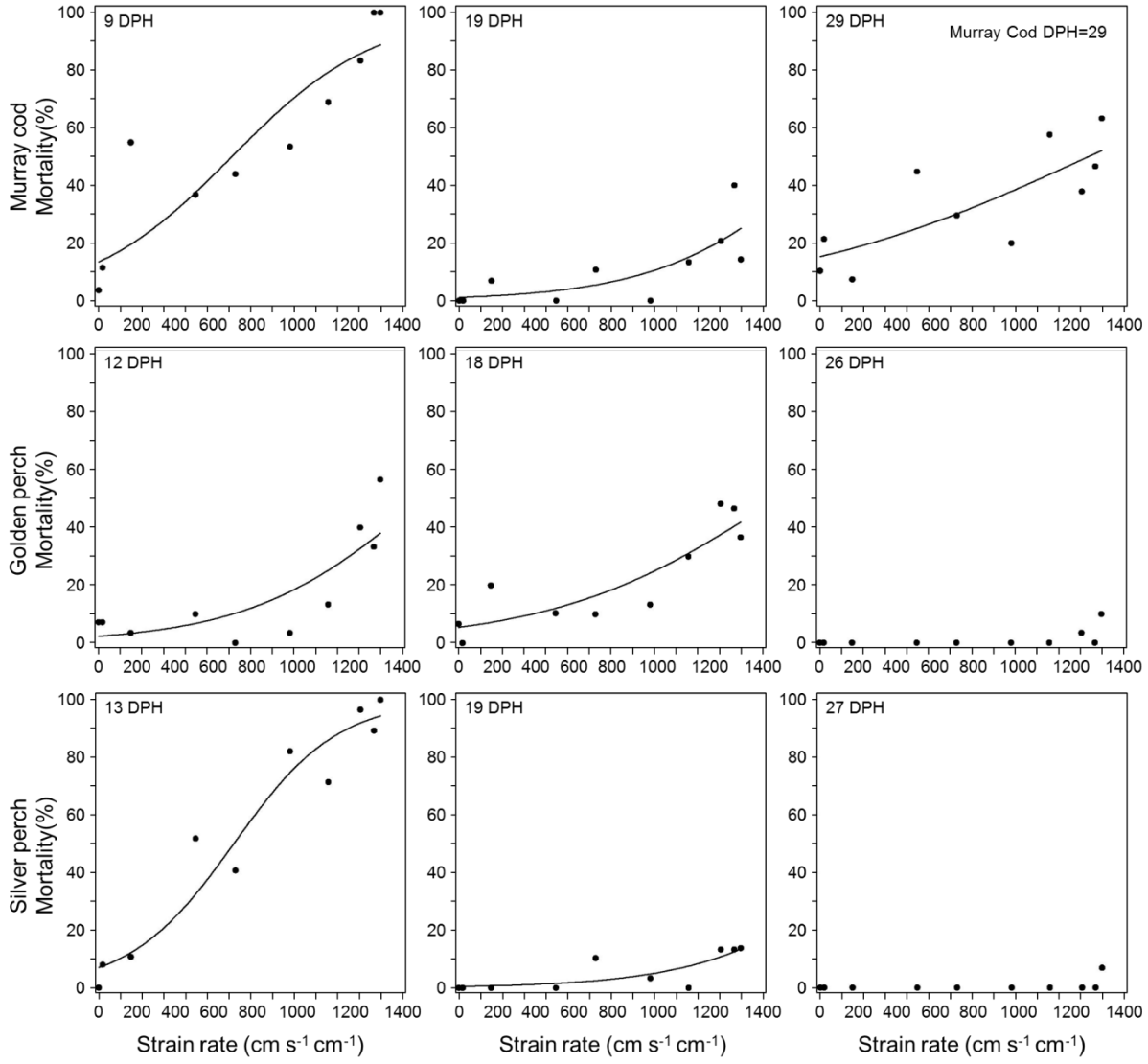


Table 11. Results of the logistic regression showing whether strain rate, days post hatch (DPH), or the interaction of both best explained variation in mortality rates of Murray cod, golden perch and silver perch larvae when exposed to a shear environment. The strain rates tested ranged from 0 to 1296.87 cm s^{-1} cm^{-1}. 'Model sig.' tests whether there is a significant relationship of mortality with strain or stage, and 'Effect sig.' tests which of strain, DPH or their interaction are significant

Species	χ^2	df	Model sig.	Effect	Co-efficients	SE	Wald	df	Effect sig.
Murray cod	447.2	3	<0.0001	Constant	–2.337	0.33			
				Strain	0.00267	0.0003	65.2	1	<0.001
				DPH	–0.00467	0.0165	0. 1	1	0.777
				Strain x DPH	–0.00004	0.00002	7.2	1	0.007
Golden perch	117.6	3	<0.0001	Constant	–2.2159	1.0353			
				Strain	0.00288	0.000976	8.7	1	0.003
				DPH	–0.0822	0.0615	1.8	1	0.181
				Strain x DPH	–0.00004	0.000058	0.6	1	0.440
Silver perch	490.3	3	<0.0001	Constant	5.0902	2.757			
				Strain	0.00477	0.00254	3.	1	0.0609
				DPH	–0.5817	0.2039	8.1	1	0.0043
				Strain x DPH	–0.00007	0.000182	0.1	1	0.707

Table 12. Results of follow-up logistic regressions for the relationship between strain rate and larval mortality rate at different developmental stages (days post hatch: DPH). The strain rates tested ranged from 0 to 1296.87 cm s^{-1} cm^{-1}. LC_{10} is the strain at which mortality was 10% more than the control (zero strain) group and LC_{75} is the strain at which mortality was 75% more than the control.

Species	DPH	Effect	Model	SE	Wald	Sig.	Odds ratio	LC_{10}	LC_{75}
Murray Cod	9	Constant	–1.8594	0.396	39.4	0.001			
		Strain	0.0030	0.0004	72.6	0.001	1.003	6	1056
	19	Constant	–4.5217	0.726	38.8	0.001			
		Strain	0.0026	0.0006	16.5	0.001	1.003	894	2162
	29	Constant	–1.7141	0.2670	41.2	0.001			
		Strain	0.00139	0.0002	23.7	0.001	1.001	251	2501
Golden perch	12	Constant	–3.759	0.522	51.8	<0.001			
		Strain	0.003	0.0005	27.4	<0.001	1.003	728	1762
	18	Constant	–2.86	0.376	57.9	<0.001			
		Strain	0.002	0.0004	28.1	<0.001	1.002	625	2177
	26	Constant	–24.741	17.463	2.0	0.157			
		Strain	0.017	0.014	1.6	0.212		1326	1520
Silver perch	13	Constant	–2.596	0.345	56.8	<0.001			
		Strain	0.004	0.0004	88.5	<0.001	1.004	100	924
	19	Constant	–5.379	1.067	25.433	<0.001			
		Strain	0.003	0.001	8.386	<0.001	1.001	1061	2159
	27	Model could not fit data							

Figure 26 Examples of injuries sustained by larvae[a] after exposure to mortal levels of shear stress: a) spinal column injuries, b) missing eye, c) yolk sac rupture and d) decapitation

[a] Shown here are 9-days post hatch Murray cod subjected to a strain rate of 1204.94 cm s^{-1} cm^{-1}

Table 13. Frequency of different types of injury (n) and the percentage of fish showing that type of injury for a given age class (days post hatch: DPH) for Murray cod, golden perch and silver perch larvae after exposure to the complete range of shear treatments

Species	DPH	Number of larvae recovered	Yolk sac		Fin damage		Eye damage		Spinal damage		Decapitation		Haemorrhage	
			n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Murray cod	9	258	39	15	158	61	1	0	3	1	1	0	8	3
	11	270	54	20	200	74	1	0	1	0	2	1	3	1
	13	266	37	14	148	56	0	0	4	2	0	0	5	2
	15	267	15	6	122	46	2	1	1	0	0	0	1	0
	17	289	6	2	112	39	0	0	2	1	0	0	1	0
	19	285	0	0	91	32	0	0	2	1	0	0	3	1
	21	281	0	0	124	44	0	0	0	0	0	0	1	0
	23	274	0	0	56	20	1	0	0	0	0	0	0	0
	25	288	0	0	147	51	0	0	0	0	0	0	1	0
	27	285	2	1	142	50	0	0	0	0	0	0	2	1
	29	284	0	0	126	44	0	0	1	0	0	0	0	0
	Total	**3047**	**153**	**5**	**1426**	**47**	**5**	**0**	**14**	**0**	**3**	**0**	**25**	**1**
Golden perch	12	291	0	0	49	17	0	0	1	0	0	0	0	0
	18	298	0	0	149	50	0	0	6	2	0	0	2	1
	26	280	0	0	70	25	1	0	0	0	0	0	11	4
	Total	**869**	**0**	**0**	**268**	**31**	**1**	**0**	**7**	**1**	**0**	**0**	**13**	**1**
Silver perch	13	271	0	0	94	35	1	0	1	0	0	0	0	0
	19	288	0	0	109	38	0	0	0	0	0	0	9	3
	27	279	0	0	142	51	0	0	0	0	0	0	7	3
	Total	**838**	**0**	**0**	**345**	**41**	**1**	**0**	**1**	**0**	**0**	**0**	**16**	**2**

N.B. Data pooled for all strain rates.

3.3.3. Juveniles

Exposure to the complete range of shears caused no mortality to juvenile Murray cod and golden perch (χ^2 = 0.12, df = 1. P = 0.7277) (Figure 27). A small amount of mortality was observed in silver perch juveniles at strain rates in excess of ~800 cm s^{-1} cm^{-1} (χ^2 = 7.3, df = 1. P = 0.0068), but not exceeding a probability of 20% (Figure 27). The incidence of injury following exposure to shear stress was relatively consistent across the three species of juveniles (Table 14). Fin damage was the most commonly noted injury, being present in 39% of all juveniles, with scale loss seen in 14% of all juveniles.

Figure 27. Average percentage mortality of juvenile Murray cod, golden perch and silver perch exposed to different shear strain. The line defines the probability calculated by logistic regression and is only shown if significant at the p<0.05 level

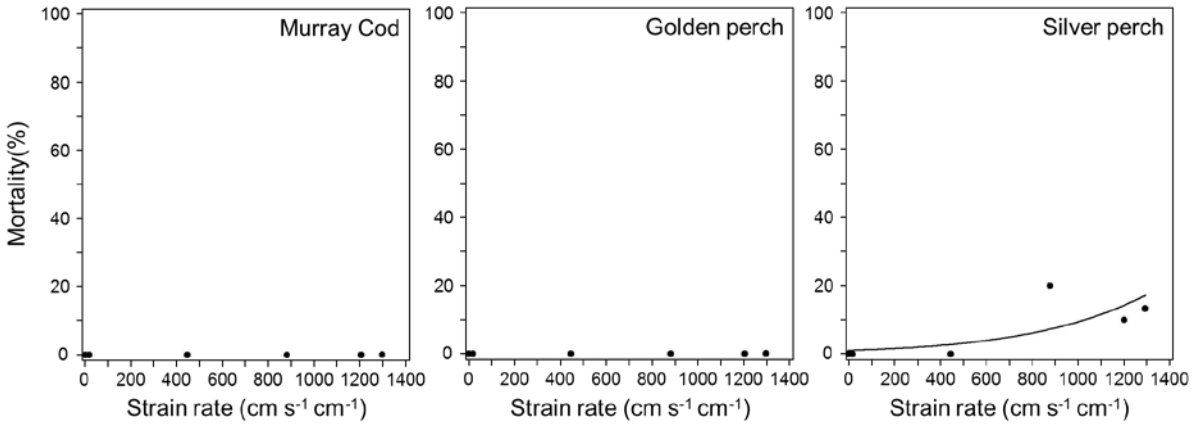


Table 14. Frequency of different types of injury (n) and the percentage of fish showing that type of injury for juvenile Murray cod, golden perch and silver perch after exposure to the complete range of shear treatments

Injury	Murray cod		Golden perch		Silver perch		Total	
	n	(%)	n	(%)	n	(%)	n	(%)
Exophthalmia	1	1	0	0	1	1	2	0.4
Fin damage	101	56	66	37	46	26	213	39
Disorientated swimming	0	0	22	12	9	5	31	6
Scale loss	16	9	26	14	34	19	76	14
Operculum damage	0	0	0	0	0	0	0	0
Spinal damage	0	0	0	0	0	0	0	0
Haemorrhage	12	7	4	2	7	4	23	4

3.4 Discussion

Eggs were found to be extremely susceptible to damage and mortality when exposed to a shear stress. Once strain rate exceeded ~150 cm s^{-1} cm^{-1}, 100% mortality of golden perch and >40% mortality of silver perch eggs occurred. Larval fish were also susceptible to injury and mortality following shear exposure. However, lower values of strain rate were more tolerable than higher levels, and susceptibility tended to reduce as larvae aged and approached juvenile metamorphosis. The only exception to this was Murray cod, which remained moderately susceptible to high levels of shear stress as late as 29 DPH, when they are on the verge of completing juvenile metamorphosis. Once the three species had reached a young juvenile stage, they had become quite resistant to shear stress. Although fin damage was observed in more than one-third of juveniles studied, there was little evidence that shear stress resulted in mortality up to 24 hours post exposure.

The mechanism responsible for mortality in eggs was related to physical damage to the chorion (or cell membrane), or when sufficient disruption occurred to cellular contents to disrupt normal hatching. Although we attempted to quantify injuries in larvae, it was not possible to determine which injuries contributed to mortality, since larvae were typically at an advanced stage of decomposition at the time of autopsy. Damage to the yolk sac was frequently observed in Murray cod larvae between the ages of 9 and 13 DPH. Murray cod undergo direct development (*sensu* Balon 1984); they have no true larval stage and free embryos leave the parental nest and begin to feed while still retaining stores of yolk (King 2002). As a result, Murray cod have been observed to be actively drifting at a stage where they have a prominent yolk sac (personal communication Zeb Tonkin Arthur Rylah Institute) (Figure 28). This will likely make them vulnerable to shear-induced injury. An interesting anomaly is that the vulnerability of Murray cod to yolk sac damage when exposed to shear stress is contrary to what has been reported for striped bass (*Morone saxatilis*) (O'Connor and Poje 1979). This may simply be due to experimental differences in jet velocity. In the striped bass study, larvae were exposed to a jet velocity of 3 m s^{-1}, which is comparable to the lowest jet velocity that larvae were exposed to in our study.

Figure 28. Nine-day post hatch Murray cod, showing the large yolk sac that was vulnerable to damage when exposed to shear stress

To determine an injury threshold for larval fish, an arbitrary level of mortality of 10% of the population (above the level noted in handling controls) was used (LS_{10}), as per previous studies (Neitzel *et al.* 2004, Deng *et al.* 2005). LS_{10} was very low (6 cm s^{-1} cm^{-1}) for 9-DPH Murray cod, likely due to the yolk sac's susceptibility to damage as previously discussed. LS_{10} was also relatively low (100 cm s^{-1} cm^{-1}) for the youngest age class of silver perch larvae and the oldest age class of Murray cod larvae studied (251 cm s^{-1} cm^{-1}). Beside these three age classes, all other thresholds for LS_{10} ranged from ~600–1000 cm s^{-1} cm^{-1}.

Other investigators have reported variable outcomes for larval fish when exposed to shear stress. High mortality has been reported in larval carp (*Cyprinus carpio*), but low mortality has been reported in species such as bluegill (*Lepomis macrochirus*), channel catfish (*Ictalurus punctatus*), large-mouth bass (*Micropterus salmoides*) and mosquitofish (*Gambusia affinis*) (Cada *et al.* 1981). In a review of laboratory studies that quantified the effects of shear stress on larval fish, Cada (1990) concluded that the weight of evidence suggests that early-life-stage fish are not susceptible to shear-induced mortality. In his paper, the author speculated that this may be due to the small size of larvae leading to smaller velocity differentials, and therefore smaller shear forces. Our findings demonstrate that such generalisations should be treated with caution. Although some larvae were not vulnerable to mortality at certain ages, at other ages they were highly susceptible. Essentially, the outcomes of any study may be driven by the age at which the larvae are tested. The findings reported in Cada (1990) are also from a very limited range of shear stress, and do not account for changes in mortality over a wide range of shear values as reported in our study.

It is difficult to directly compare the strain rate thresholds estimated during our study and values reported for other species in other studies. This is because other investigators either generate shear in a different way to our study (e.g. through pipes rather than with a submerged jet; e.g. O'Connor and Poje 1979), or express shear stress in a different way, such as flow velocity rather than strain rate (e.g. O'Connor and Poje 1979, Cada *et al.* 1981). Other studies use strain rate, but calculate it at a scale most relevant to the species under investigation (e.g. Neitzel *et al.* 2004, Deng *et al.* 2005). Where approximate comparisons can be made to studies that also used a submerged jet, the threshold values for damaging shear we estimated for most age classes of larvae (~600–1000 cm s^{-1} cm^{-1}) were close to the ranges reported by others. For example, Neitzel *et al.* (2004) reported LC_{10} values for major injury and mortality of juvenile American shad (*Alosa sapidissima*) of ~600 cm s^{-1} cm^{-1} and from ~900–1000 cm s^{-1} cm^{-1} for juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*O. mykiss*). Similarly, Deng *et al.* (2005) estimated an LC_{10} for mortality in juvenile Chinook salmon of approximately 900 cm s^{-1} cm^{-1}, and cites a comparable injury threshold determined by Turnpenny *et al.* (1992) of ~800 cm s^{-1} cm^{-1}.

Unlike these previous studies, we found juveniles to be resistant to damage when exposed to the complete range of shear stress tested. Although more than one-third of juvenile fish sustained fin damage, significant injury was rarely observed. By comparison, studies such as Neitzal *et al.* (2004) and Deng *et al.* (2005) frequently noted head and operculum damage. This is likely to be due to the differing orientation with which the fish were exposed to the shear environment. In our study, the juvenile fish were not restricted in their orientation and typically orientated themselves into the flow coming down the deployment tube, entering the jet stream tail first. If fish are forced to enter a shear environment head first, injury is greater and the force of flow coming from behind the fish can lift scales, tear open operculum, dislodge eyes and damage gills (Neitzel *et al.* 2004, Deng *et al.* 2005). The importance of orientation is illustrated nicely by Neitzel *et al.* (2004), who found that mortality of Chinook salmon was reduced from 100% to 10% if the fish was exposed tail first to strain rates of ~1000 cm s^{-1} cm^{-1}, rather than head first. The implication of this for our study is that we may not have gained a full appreciation for the impact of fluid shear on juveniles of the species studied. Future research where fish are exposed to a shear stress in a head-first manner may be warranted.

4. CONCLUSIONS AND RECOMMENDATIONS

Previous research has shown that Murray–Darling Basin (MDB) fish species may be susceptible to injury and mortality as they pass downstream through river infrastructure (Baumgartner *et al.* 2006, Baumgartner *et al.* 2013). Until now, the mechanism responsible for this was poorly understood. This has made it difficult to properly evaluate the sustainability of river infrastructure developments and reduce any impact on fish passage through sound engineering and operation (Baumgartner *et al.* 2012, Thorncraft *et al.* 2013).

This report has detailed laboratory assessments of the likelihood of varying levels of decompression and fluid shear contributing to the injury and mortality of various MDB fish species at different life history stages. Our ultimate goal was to determine critical thresholds for injury and mortality, and develop criteria to protect downstream migrating fish at river infrastructure (Table 15).

4.1 Recommended thresholds for decompression

For eggs and larvae, there was little evidence that simulated infrastructure passage led to barotrauma resulting in immediate mortality (within 24 hours). We did, however, observe evidence of non-lethal (at the 24-hour point) injuries or pressure effects in larvae. The percentage of fish affected by these increased as the ratio of pressure change (RPC) fell, but the relationship was rarely linear; typically, a threshold response was evident. Injuries usually occurred once exposure pressures fell below 40% of the acclimation pressure. Much of this injury involved the deflation of swim bladder, although in one species and age class, internal haemorrhaging was observed. While internal emphysema was observed at low-range RPCs, the source of this gas is unclear, because it does not appear related to swim bladder rupture.

It is possible that the swim bladder deflated as a result of venting or burping of gas through the gut during decompression, rather than rupture. Swim bladder deflation did not result in immediate mortality. However, it is still unclear whether burping reduces the susceptibility of larvae to barotrauma, or whether larvae lack the ability to refill the bladder at this young age, and as a result suffer longer-term effects on development, growth or survival.

Based on the results of this study, there is little evidence of susceptibility of eggs and larvae to barotrauma-induced mortality. Therefore, there appears to be little need at this stage for guidelines around the magnitude of decompression for the protection of these early life stages in the MDB. However, swim bladder deflation and haemorrhage can occur once exposure pressures fall below 40% of the acclimation pressure. Caution may need to be exercised beyond this level of decompression until the consequence of these conditions on long-term survival of larvae is established.

For juvenile fish, autopsy revealed that although many of them did not die immediately (within 5 minutes) following decompression, a large proportion displayed clinical signs of barotrauma. Symptoms included ruptured swim bladder, internal and external haemorrhaging, emphysema of internal and external organs, and exophthalmia. Other studies indicate that many of these injuries can lead to eventual death. Until the longer-term implications of these injuries on survival are known, the recommended guidelines for juveniles are based on thresholds that are likely to minimise injury.

Critical thresholds for juvenile injury varied both between injury types and between the two species studied, making generalisations difficult. Thresholds ranged from modest levels of decompression, where exposure pressures fell below 70% of acclimation pressure, to more severe scenarios, where pressure fell below 10% of acclimation pressure. When making recommendations, a precautionary approach was used. As a guide, exposure pressures above 60% of acclimation pressure should protect physoclistous fish such as the species studied here. This guideline is conservative, and is based on the desire to guard against swim bladder rupture and kidney haemorrhage in juvenile silver perch. This is

comparable to estimates and guidelines produced for other physoclistous species (e.g. 60% for bluegill, *Lepomis macrochirus*) (Becker *et al.* 2003). Further research that determines the extent that different injuries contribute to eventual mortality (see Section 4.4) may enable the 60% guideline to be changed to the less conservative recommendation of 40%.

Table 15. Generalised downstream fish passage criteria for Murray–Darling Basin species relating to levels of decompression and fluid shear required to minimise injury and mortality of fish

Life stage	Decompression	Fluid shear
Egg	No threshold recommended	Should not exceed 150 cm s^{-1} cm^{-1}
Larvae	No threshold recommended, but caution should be exercised once exposure pressures fall below 40%[a]	Should not exceed ~10 cm $s^{-1}$$cm^{-1}$ in areas and times of suspected larval Murray cod larval drift. Otherwise should not exceed ~620 cm s^{-1} cm^{-1}
Juvenile	Exposure pressures should not fall below 60% of the acclimation pressure	No threshold recommended

[a] Based on evidence of some pressure effects where long-term impacts on survival are uncertain.

4.2 Recommended thresholds for fluid shear

Our results suggest that downstream drifting eggs are highly susceptible to damage when exposed to shear stress, and would therefore be likely to suffer high levels of mortality at river infrastructure. The threshold for mortality differed between the two species studied. Silver perch eggs could survive higher levels of shear (<629.49 cm s^{-1} cm^{-1}) than golden perch (<148.35 cm s^{-1} cm^{-1}). Nevertheless, based on the protection of the more vulnerable species, we recommend that shear not exceed 150 cm s^{-1} cm^{-1}. In this study, 100% mortality of golden perch and >40% mortality of silver perch eggs occurred when this threshold was exceeded.

Strain rates that exceeded the threshold mortality of 10% of the population (above handling control levels) were used to set the recommended criteria for larval fish. The majority of age classes and species of larvae will be adequately protected if shear does not exceed ~620 cm s^{-1} cm^{-1}. The only instance where we would recommend a more conservative guideline is if the protection of 9–13-days post hatch (DPH) Murray cod is desirable. In this case, alternative measures to reduce injury should be explored if shear exceeds ~10 cm s^{-1} cm^{-1}. Since there was little evidence of significant injury or mortality of juveniles of any of the species over the range of shear tested, no thresholds are recommended for the protection of this life history stage. Further research may be warranted to establish whether we have underestimated the effect on juveniles, due to the tail-first orientation in which they were exposed to the submerged jet.

4.3 Implications for fisheries managers and infrastructure engineers

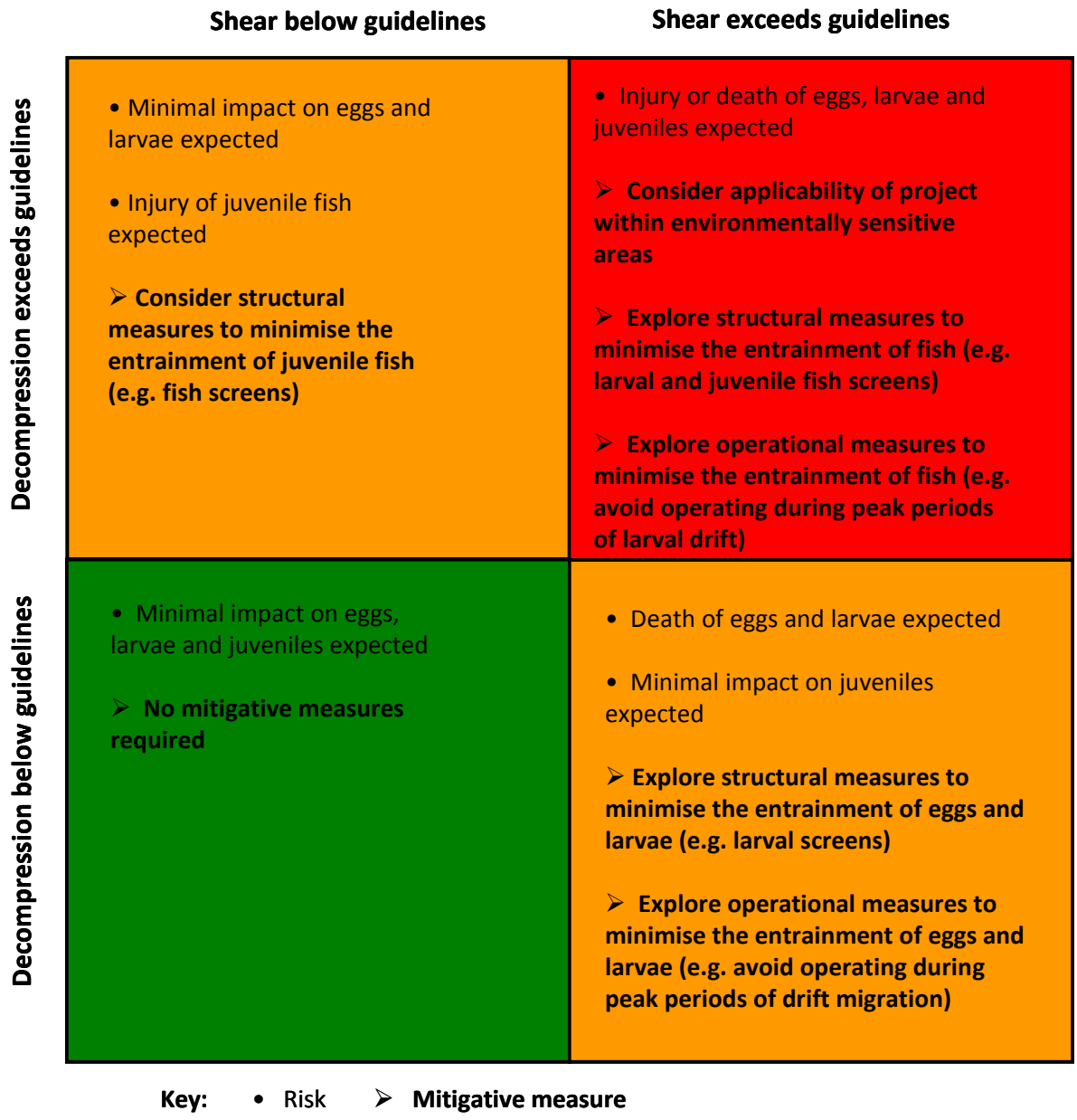
Identifying the mechanisms responsible for fish passage risks will help fisheries managers and infrastructure engineers determine the appropriateness of infrastructure works, and structural and operational conditions, to reduce these risks. The fish passage criteria presented in Table 15 and summarised in this chapter are general in nature, and are an attempt to synthesise results that often

varied across multiple species and injury types. Managers and engineers should not apply these criteria without careful consideration of the results and discussion contained within the previous chapters. In many cases, the criteria are based on a precautionary principle, where it is assumed that by protecting the species most susceptible to injury and mortality, a large range of species in an assemblage will also be protected. However, such a precautionary approach may not be desirable in all instances. At times, managers may deem it more appropriate to focus on the protection of one particular threatened species or age class.

The criteria generally relate to thresholds that once exceeded, lead to injury and mortality. In some instances, it may be ecologically reasonable to accept a higher degree of mortality and injury than is reflected by these criteria. For example, it may be demonstrated that alterations to an existing structure are likely to result in mortality that would be equal to (or even less than) that currently experienced (a 'no net loss' approach).

We acknowledge that in some cases, the design of a structure may not be able to be modified sufficiently to meet the suggested guidelines without compromising its operational efficiency. In the instance of 9-DPH Murray cod, a shear guideline of 10 cm s^{-1} cm^{-1} is extremely low, and it may not be possible to design river infrastructure to meet these levels. Initially, it may be worth considering whether a higher level of mortality of 9-DPH larval may be acceptable. For example, accepting 75% mortality would allow shear levels of up to ~1000 cm s^{-1} cm^{-1}. But, such decisions are difficult to make without understanding the critical level of mortality for larvae at different ages before recruitment to the population becomes compromised. If accepting a higher level of mortality is not desirable, managers and engineers may want to consider additional measures, such as intake screens to prevent entrainment of fish (Figure 29). An alternative approach would be to consider operational guidelines for structures within the vicinity of known areas of Murray cod spawning. Since Murray cod have a very narrow (~6 week) window each year for spawning, it may be possible to restrict the operation of hydropower facilities during these times.

Figure 29. Downstream fish passage risk assessment and decision support matrix for the assessment of river infrastructure projects



4.4 Further research needs

The downstream fish passage criteria outlined here would benefit from additional research to address knowledge gaps and clarify any unresolved uncertainty. The following research needs are recommended.

Determine mortal injury of barotrauma
Future research should aim to evaluate the effects of swim bladder deflation, haemorrhage and emphysema on longer-term development and survival of larvae. This will require holding larvae for longer than 24 hours post decompression, preferably to a point after juvenile metamorphosis. Similarly, experiments should establish the long-term survival impact of the multitude of barotrauma injuries suffered by juveniles. By establishing a more specific list of mortal injuries, it may be possible to recommend less conservative guidelines associated with the decompression of larvae and juveniles.

Investigate sub-lethal impacts of barotrauma
Experiments are needed to establish whether rapid decompression results in sub-lethal impacts that do not cause immediate death, but compromise the long-term sustainability of populations. Areas of concern may be effects on fitness and reproductive potential. It may be worth holding decompressed eggs and larvae for long enough to establish whether growth, feeding, swim bladder regulation and eventual breeding are affected. Another area of concern is whether fish that are ready to spawn lose a reproductive season if they are decompressed. The stripping of eggs from ripe females has been observed during rapid decompression in pressure chambers (Brett Pflugrath, Pacific Northwest National Laboratory, personal observation).

Include additional factors to shear testing
Further investigation regarding the tolerance of juvenile fish to head-first exposure to shear stress will determine if guidelines should be added for the protection of juveniles. Further shear testing could also incorporate acceleration as a factor for consideration (e.g. Deng *et al.* 2005), because this has been shown to be a key determinant of injury and mortality. Such testing will better enable laboratory results to be related to field measurements, using technology such as Sensor Fish.

Determine the risk of exposure in the wild to critical levels of decompression and shear
Although this study sheds some light on the tolerable limits of decompression and shear for different fish species and life history stages, the results need to be placed within the context of what may be encountered in the field. This involves gaining a better understanding of the migratory ecology of native fish, and the hydraulic conditions experienced at a greater range of infrastructure types. For instance, the true extent of egg and larval drift through weir pools, weir structures and hydropower facilities remains undocumented. In relation to determining the range of pressures experienced at infrastructure in the field, the release of autonomous hydraulic sensors (Sensor Fish: Deng *et al.* 2007) at 'undershot' irrigation weirs has revealed that fish may be exposed to slight sub-atmospheric pressures (~95 kPa) during downstream passage (Boys *et al.* 2013). Similar measurements undertaken overseas at Kaplan and more advanced hydropower turbines have shown that while nadirs as low as 7 kPa could be experienced by fish, mean nadirs of ~87 kPa are more typical, with the level varying with discharge (Deng *et al.* 2010). Many more infrastructure types and flow conditions need to be evaluated to provide a more complete understanding of the risk that fish will be exposed to injurious levels of decompression. Fluid shear is of particular interest, and has not been properly explored at NSW infrastructure in any great detail.

Extend fish passage guidelines to include coastal species
River infrastrcuture exists in coastal streams, and in some catchments infrastructure owners are exploring the use of these existing structures to generate mini-hydropower. It will be beneficial to extend research to species found in coastal systems of NSW, which are home to many species that have downstream migration from rivers to estuaries as a necessary requirement in their life cycle.

Developing downstream fish passage guidelines for coastal streams will benefit owners and operators of river infrastructure. It will enable them to prepare for future challenges and risks related to dam and weir upgrades, and to inform cost–benefit decisions regarding the use of mini-hydropower at existing infrastructure.

Field validation of laboratory-generated models (post-assessment)

If there is an expectation that new mini-hydropower facilities attempt to meet certain minimal hydraulic conditions to safeguard against fish injury and mortality, it will be prudent to evaluate fish survival at these facilities. By releasing live fish at structures in parallel to Sensor Fish, laboratory models developed in this report can be validated, and operation ranges put forward at the development stage can be checked.

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APPENDIX 1 – BAROTRAUMA INJURY PHOTOGRAPHS

Some photos have been included for common carp (*Cyprinus carpio*) and carp gudgeon (*Hypseleotris sp.*), because they illustrate the type of injury well. All fish were exposed to simulated infrastructure passage as per the methods outlined in this report.

Figure 30. Skin haemorrhage (juvenile silver perch)

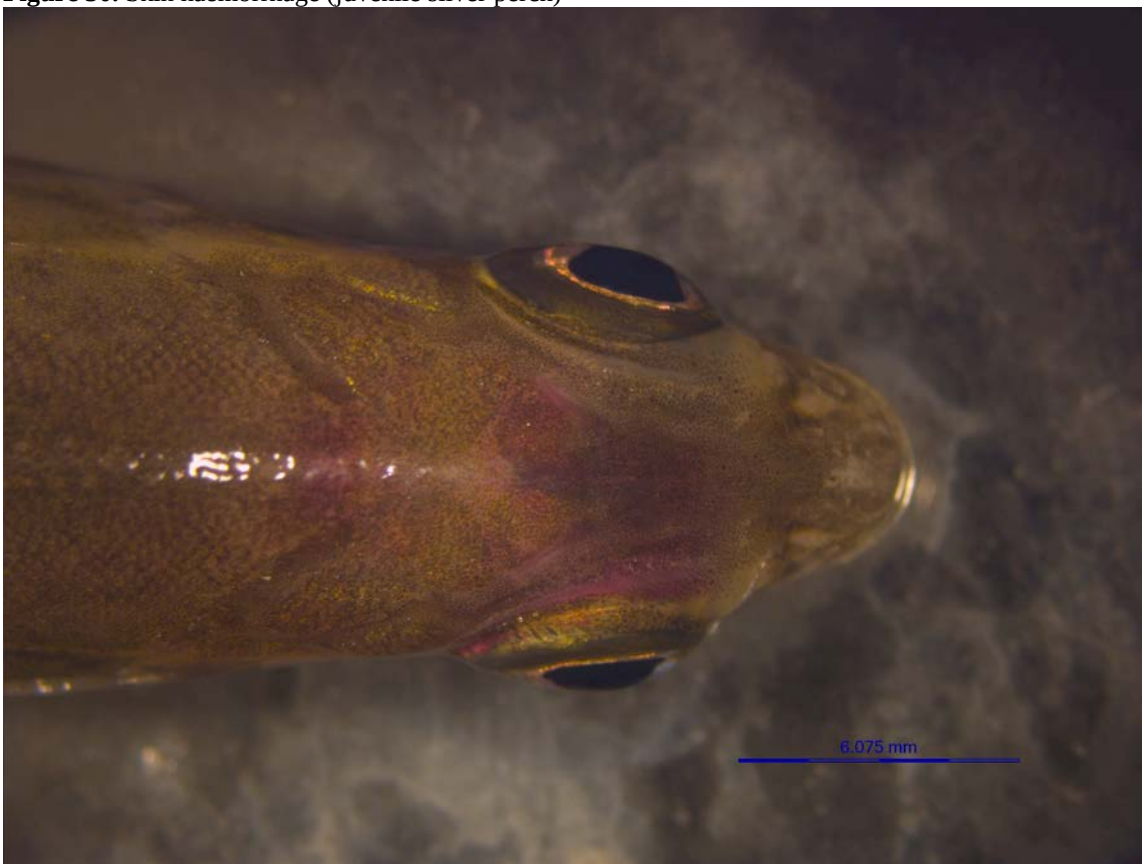


Figure 31. Skin emphysema (shown here in juvenile carp, *Cyprinus carpio*, mirror carp variant)

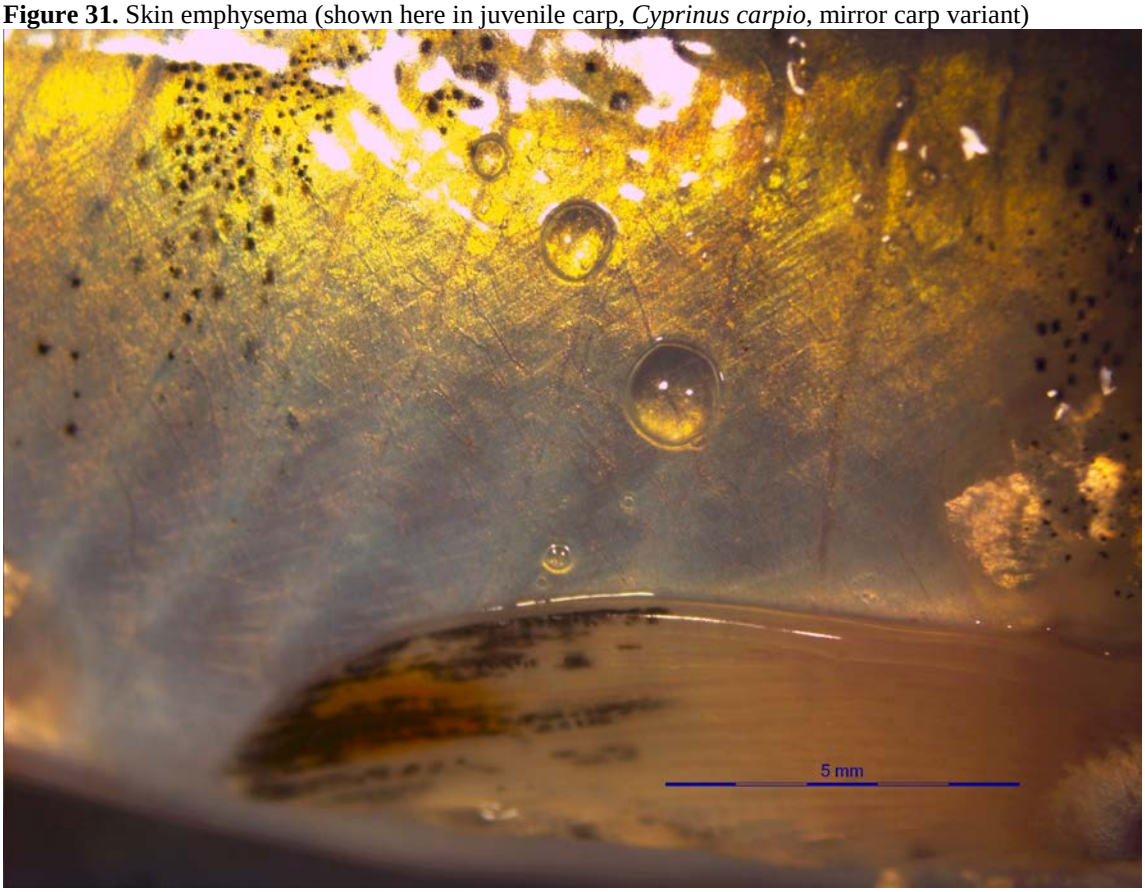


Figure 32. Bloodshot cloaca (juvenile silver perch)

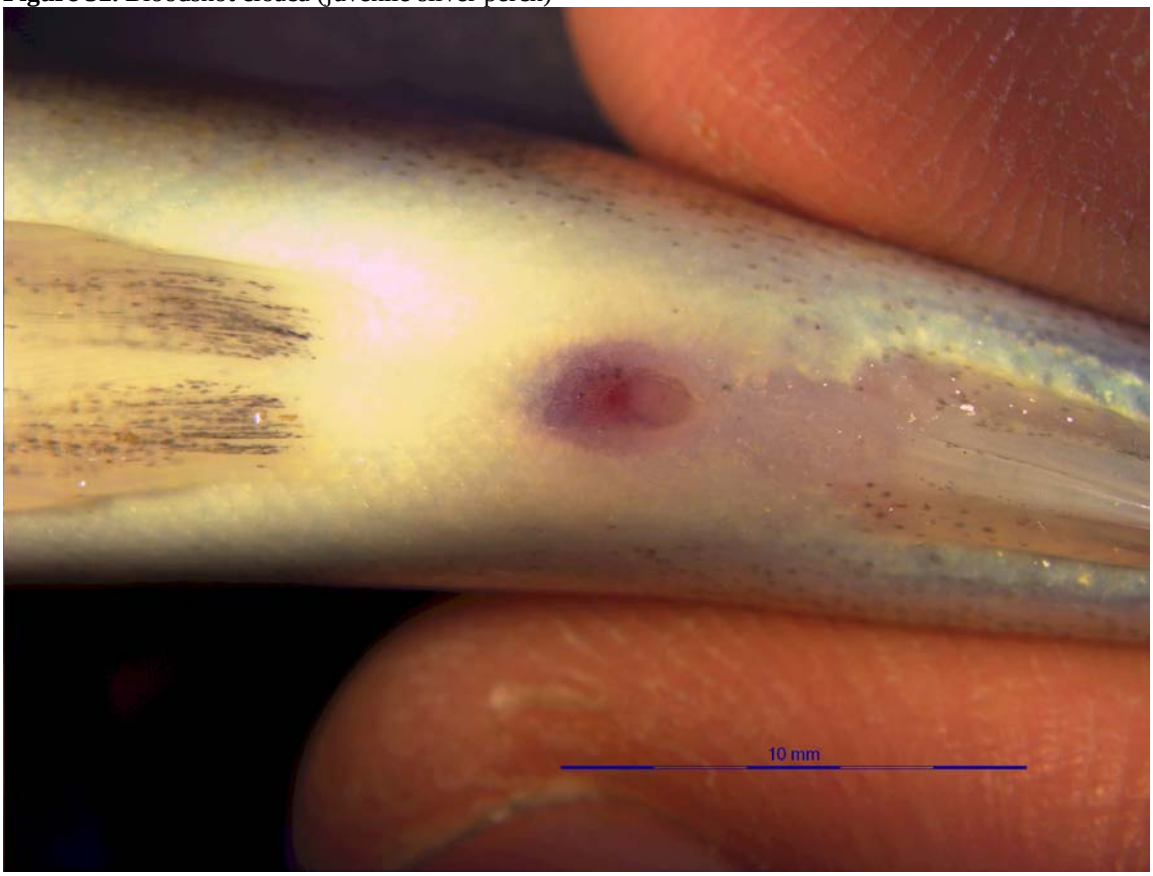


Figure 33. Prolapsed gut (shown here in carp gudgeon, *Hypseleotris spp*.)

Figure 34. Fin haemorrhage (juvenile silver perch)

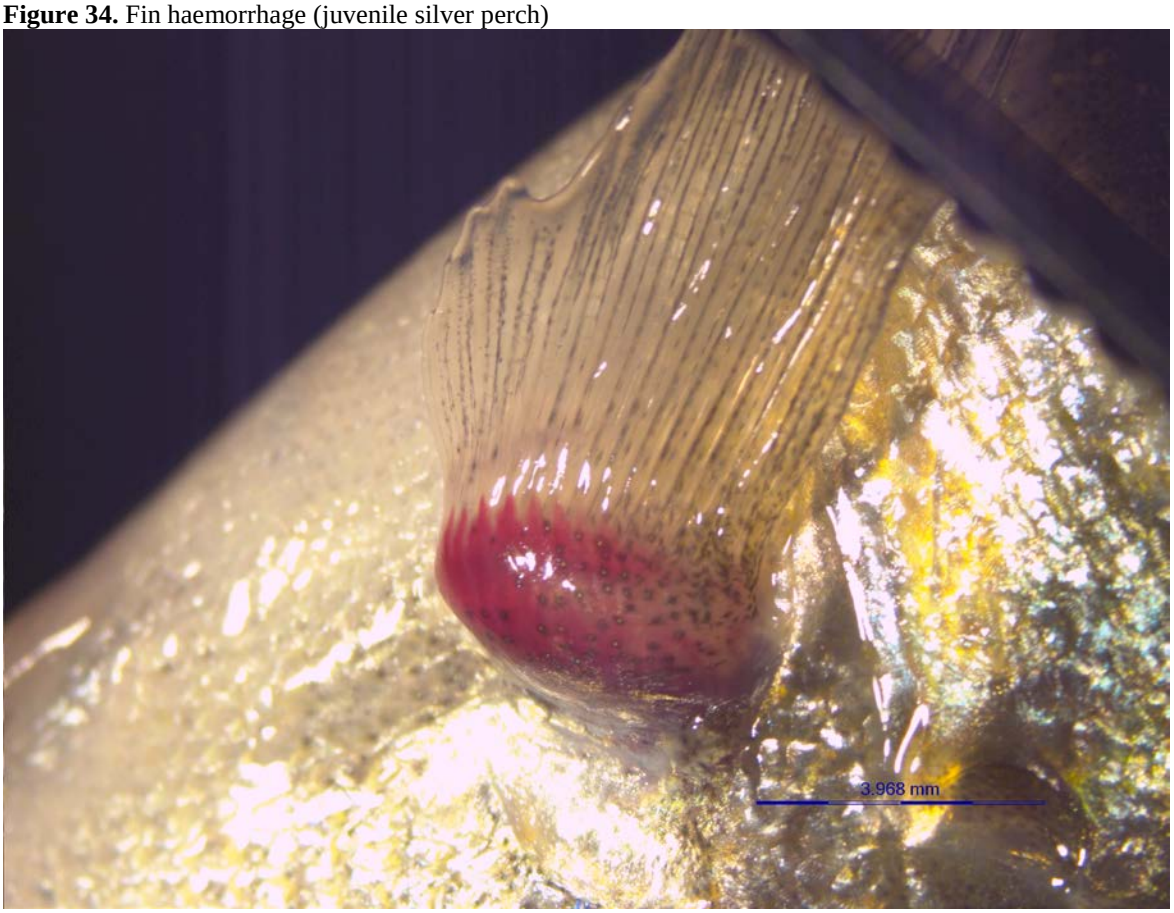


Figure 35. Fin emphysema (juvenile Murray cod)

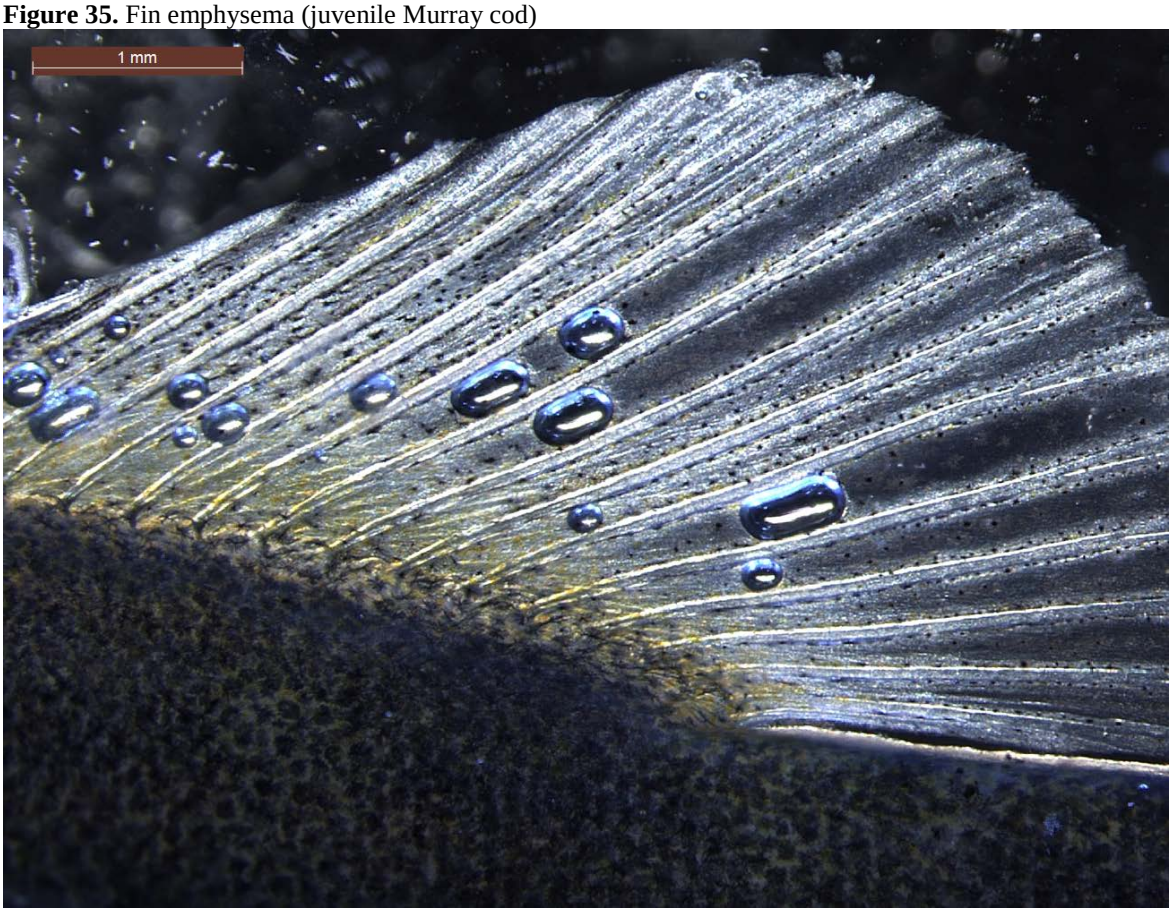


Figure 36. Pharyngo-clitheral membrane emphysema (juvenile Murray cod with operculum removed)

Figure 37. Emphysema of operculum (juvenile silver perch)

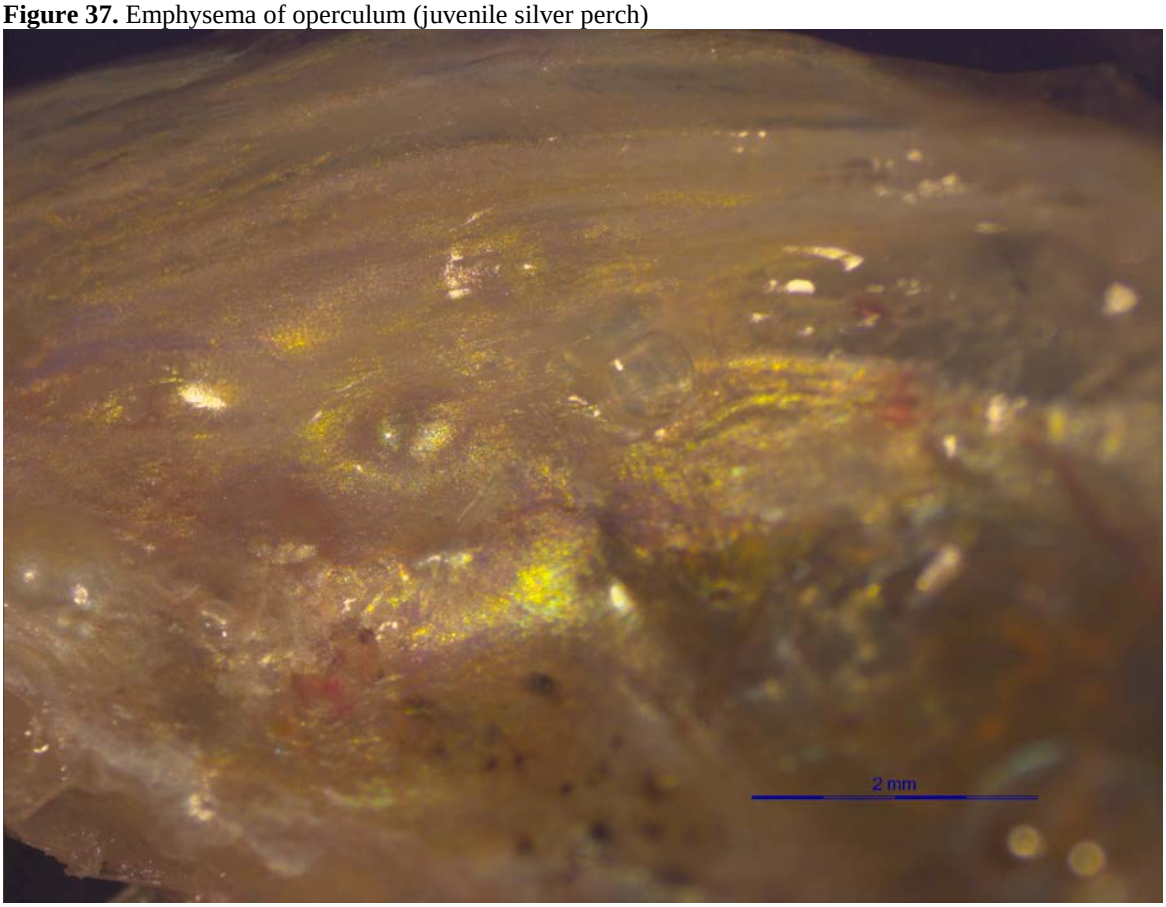
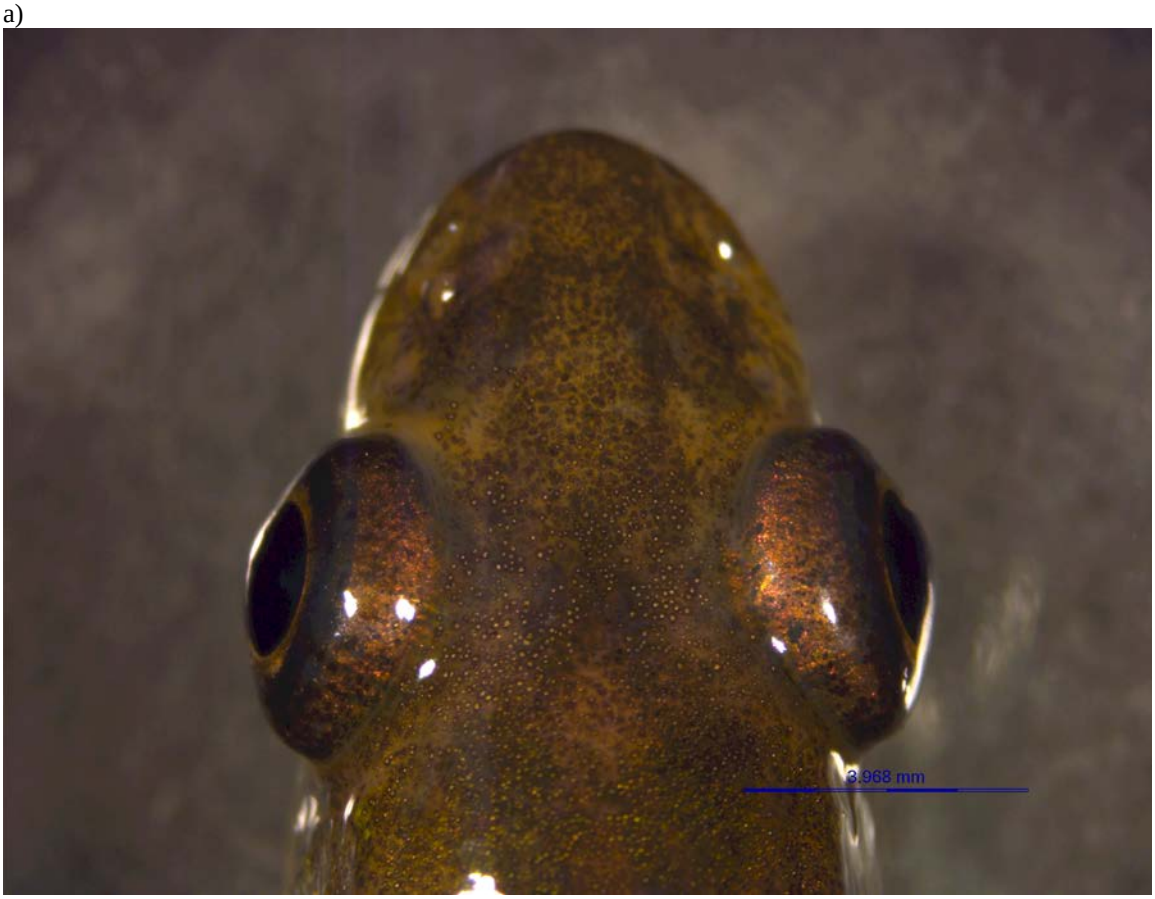


Figure 38. Exophthalmia in juvenile (a) Murray cod and (b) silver perch

a)



b)

Figure 39. Eye haemorrhage (juvenile silver perch)

Figure 40. Eye emphysema (juvenile carp)

Figure 41. Gill haemorrhage (juvenile silver perch)

Figure 42. Gill emphysema (65-day-old Murray cod)

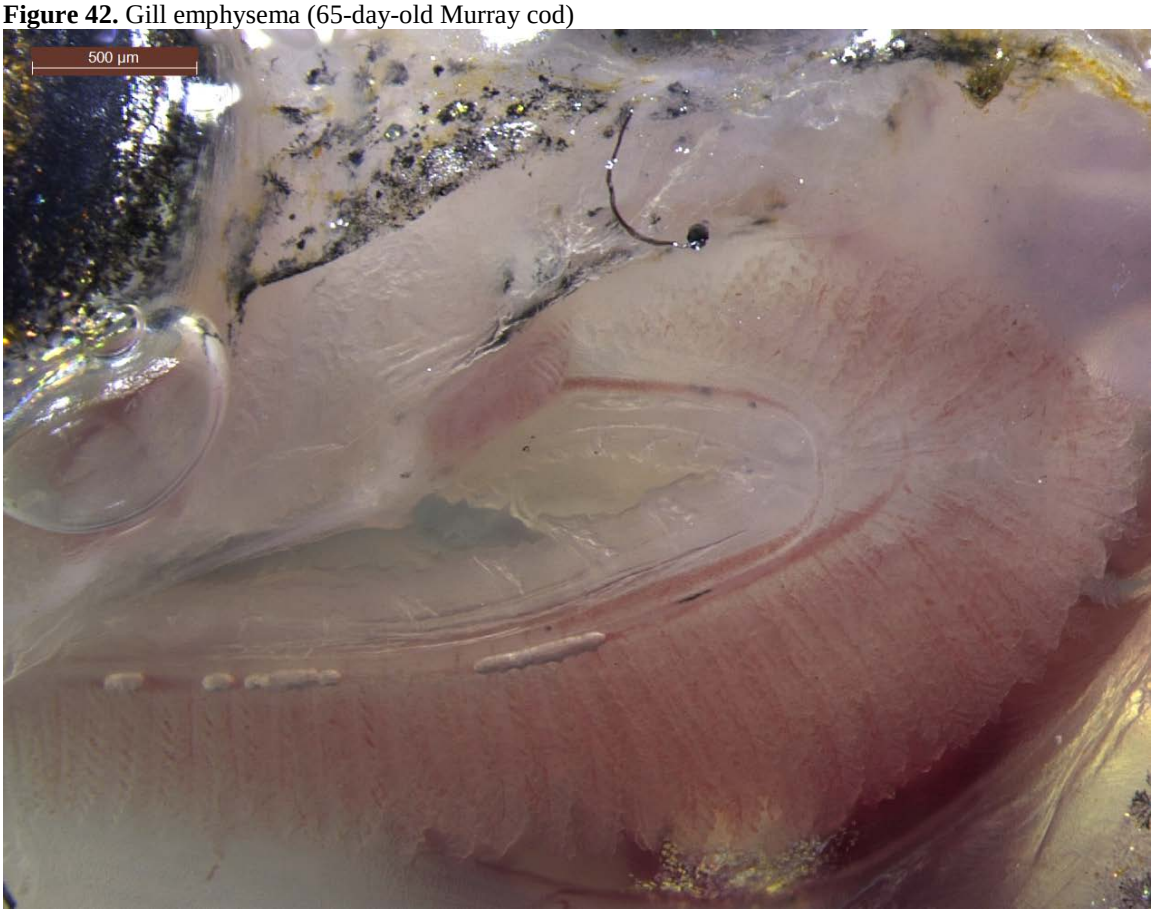


Figure 43. Mouth haemorrhage (juvenile Murray cod)

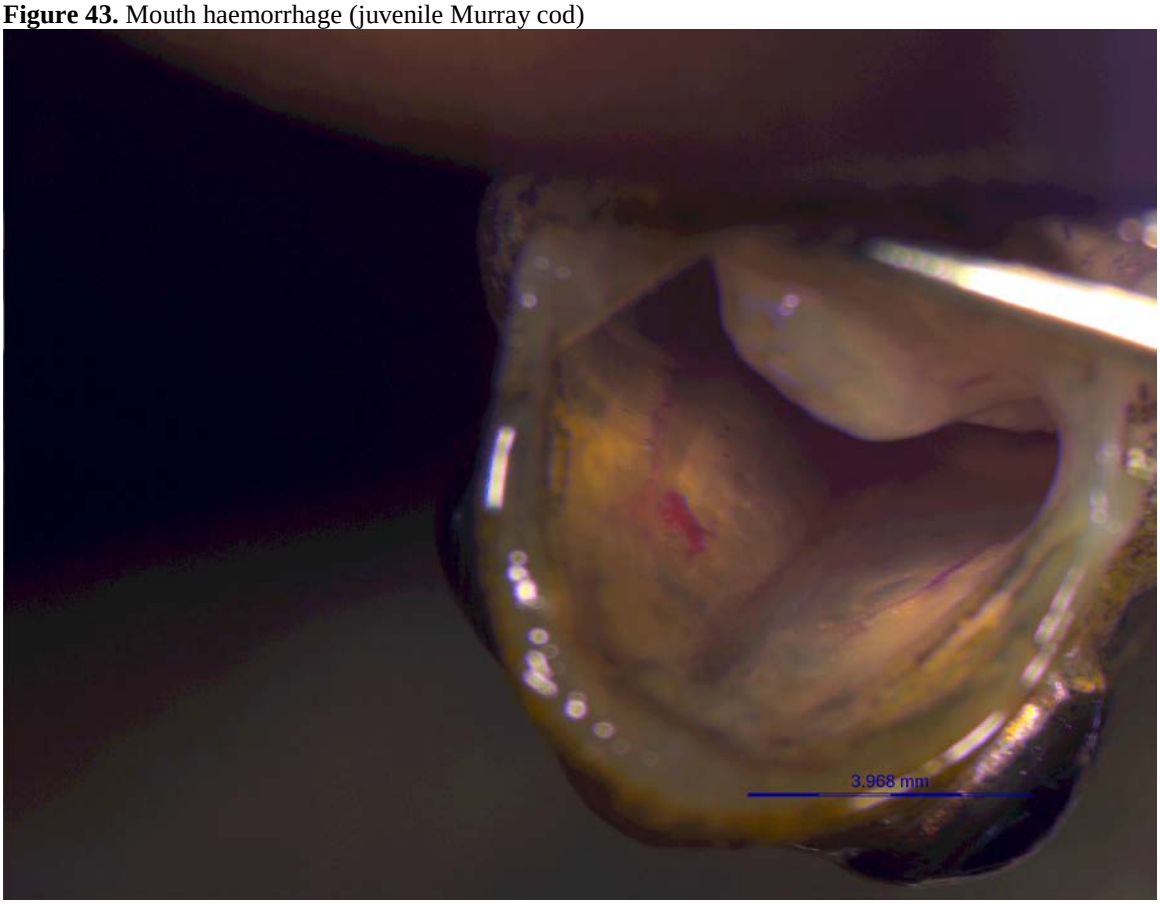


Figure 44. Mouth emphysema (juvenile carp)

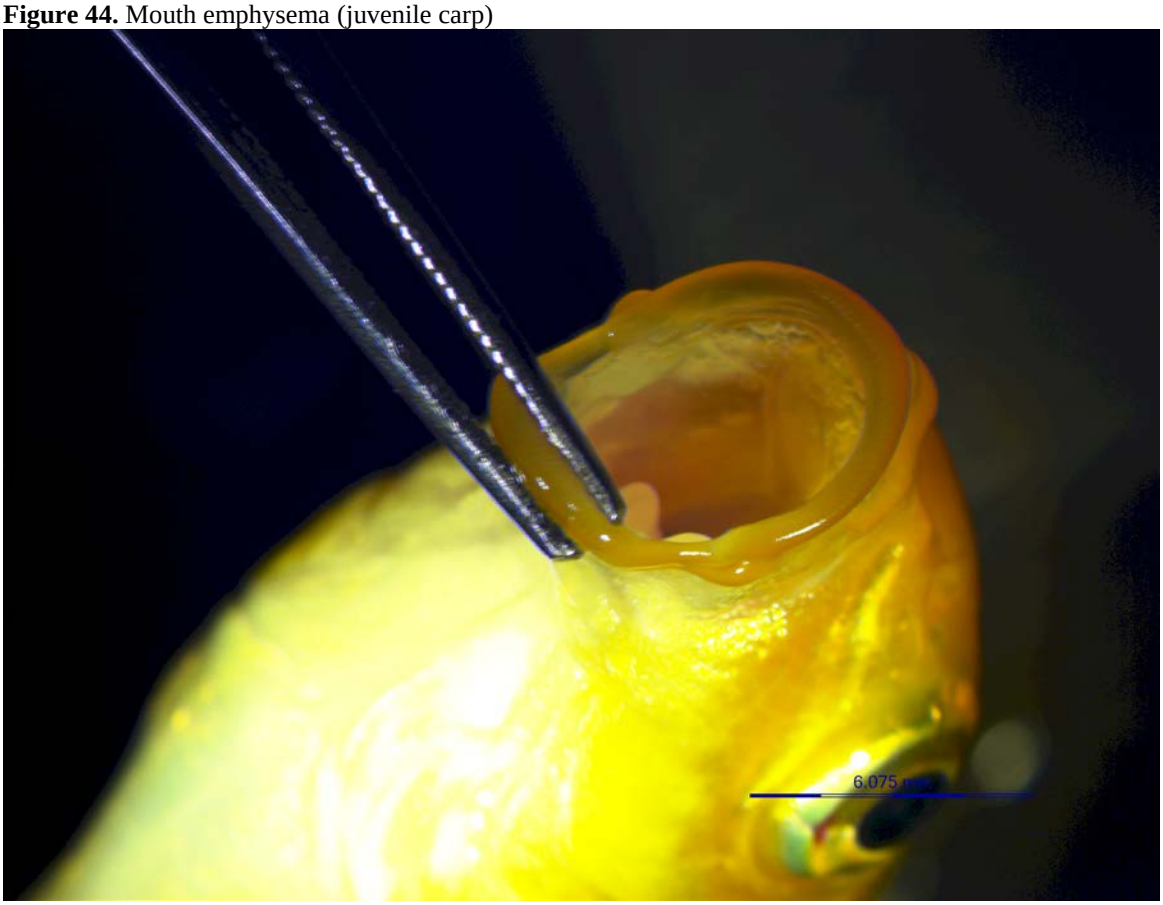


Figure 45. Viscera haemorrhage (juvenile silver perch)

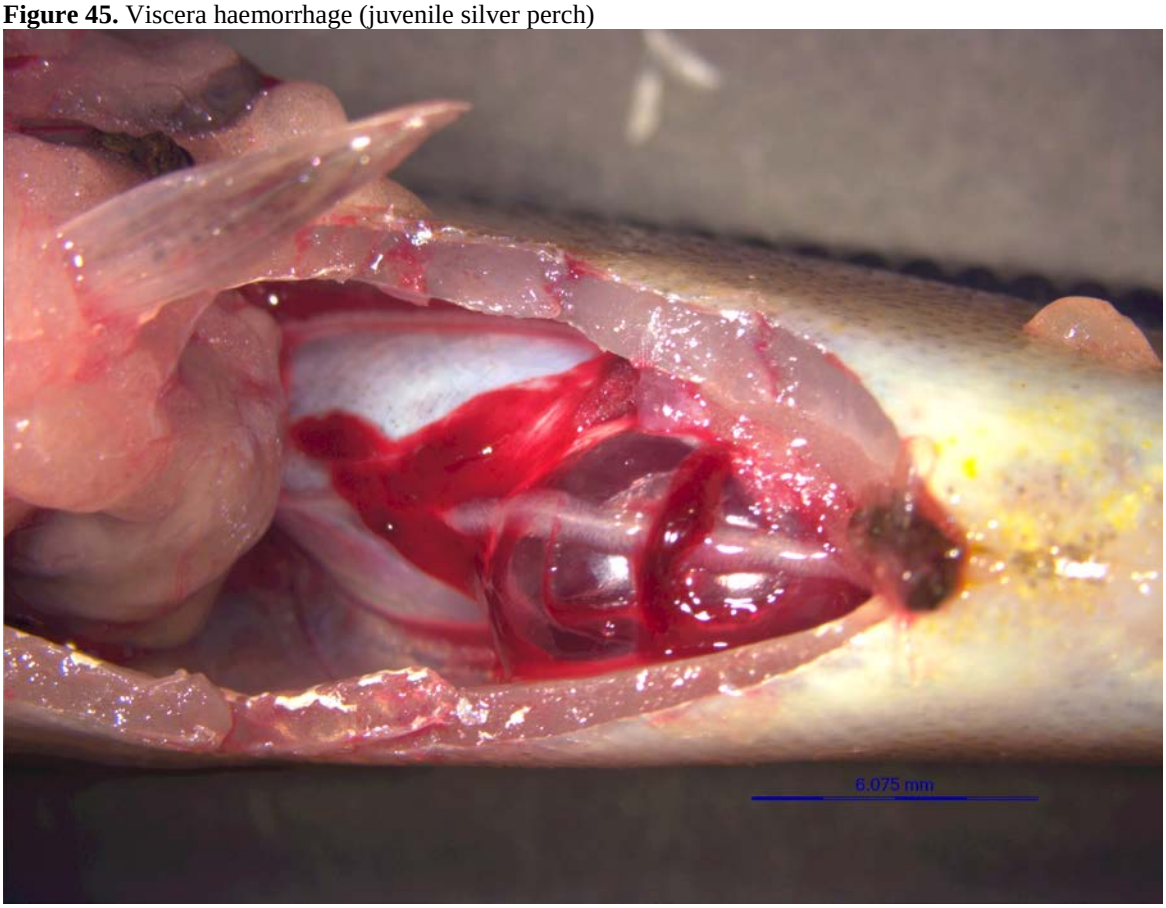


Figure 46. Viscera embolism (juvenile carp)

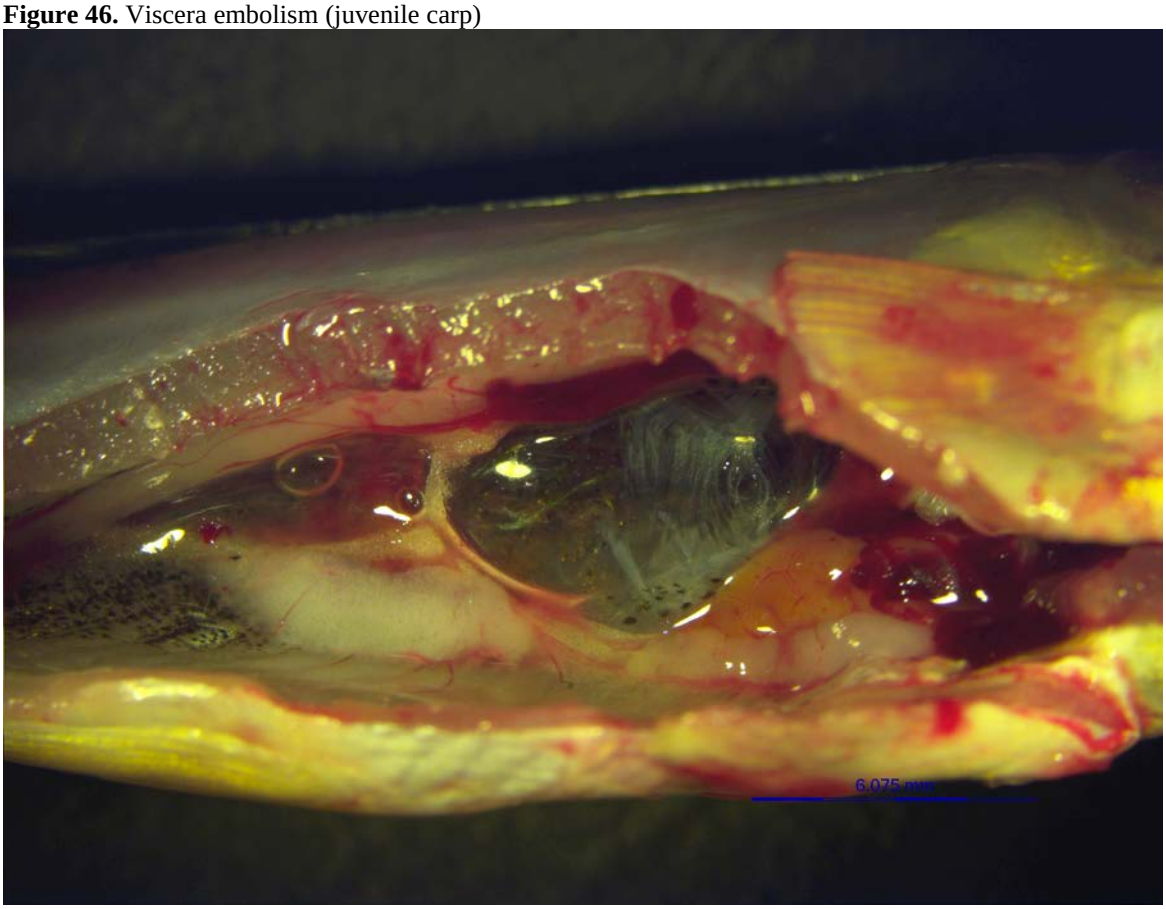


Figure 47. Heart emphysema (juvenile carp)

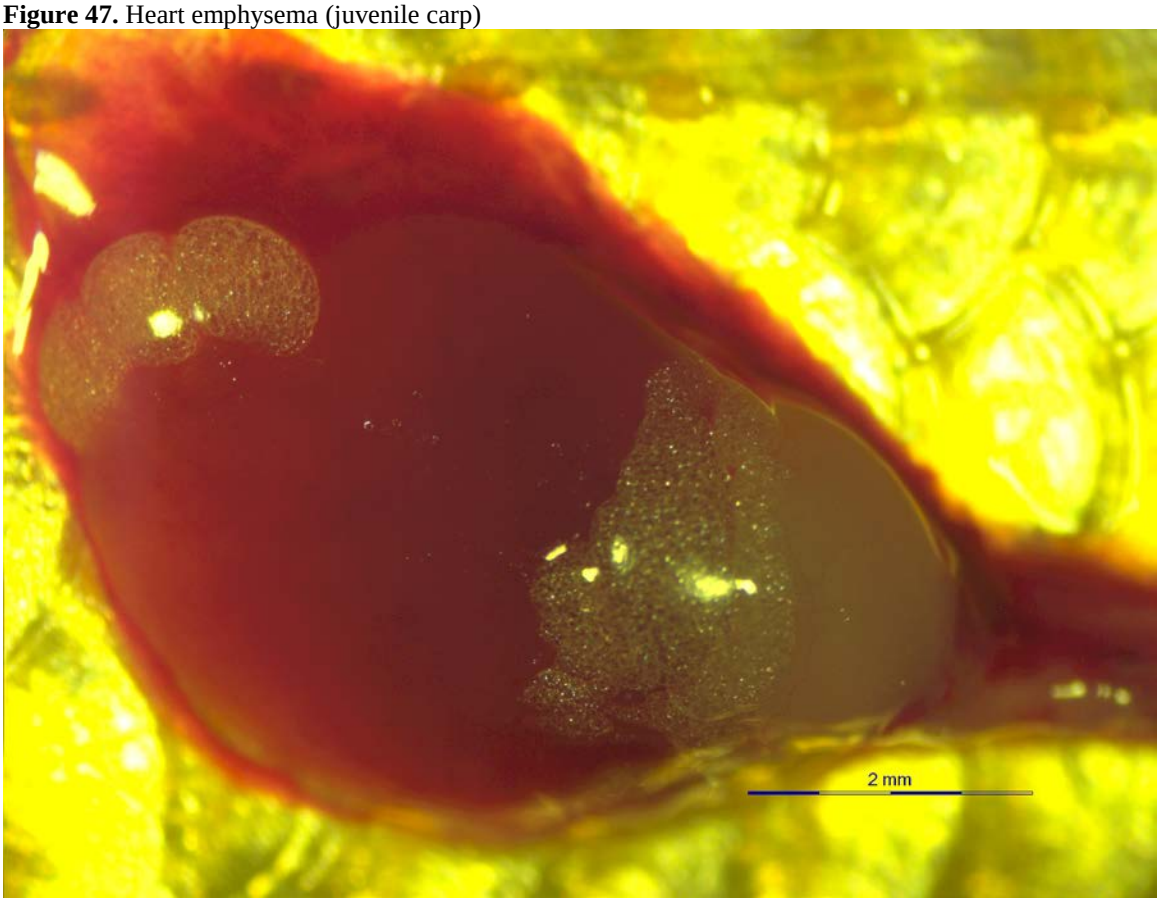


Figure 48. Heart haemorrhage (juvenile silver perch)

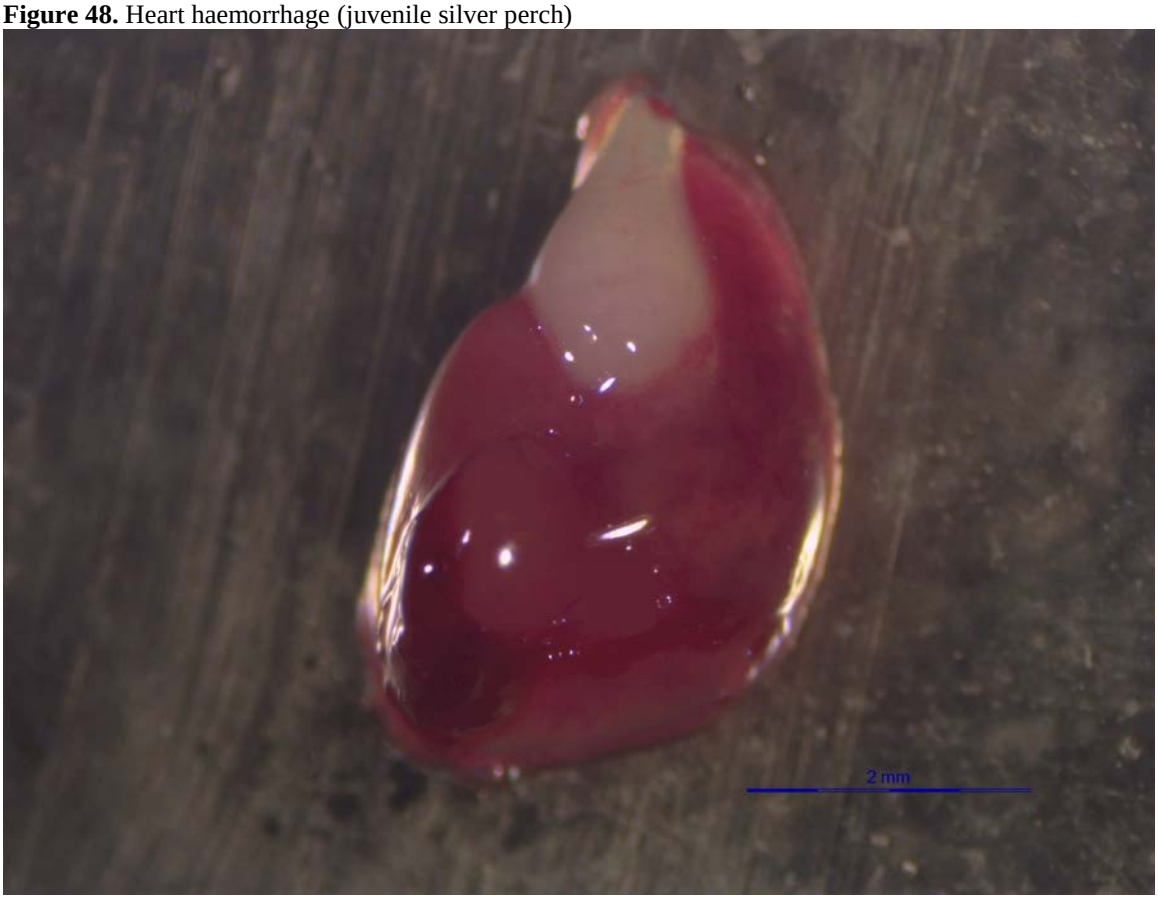


Figure 49. Liver haemorrhage (juvenile Murray cod)

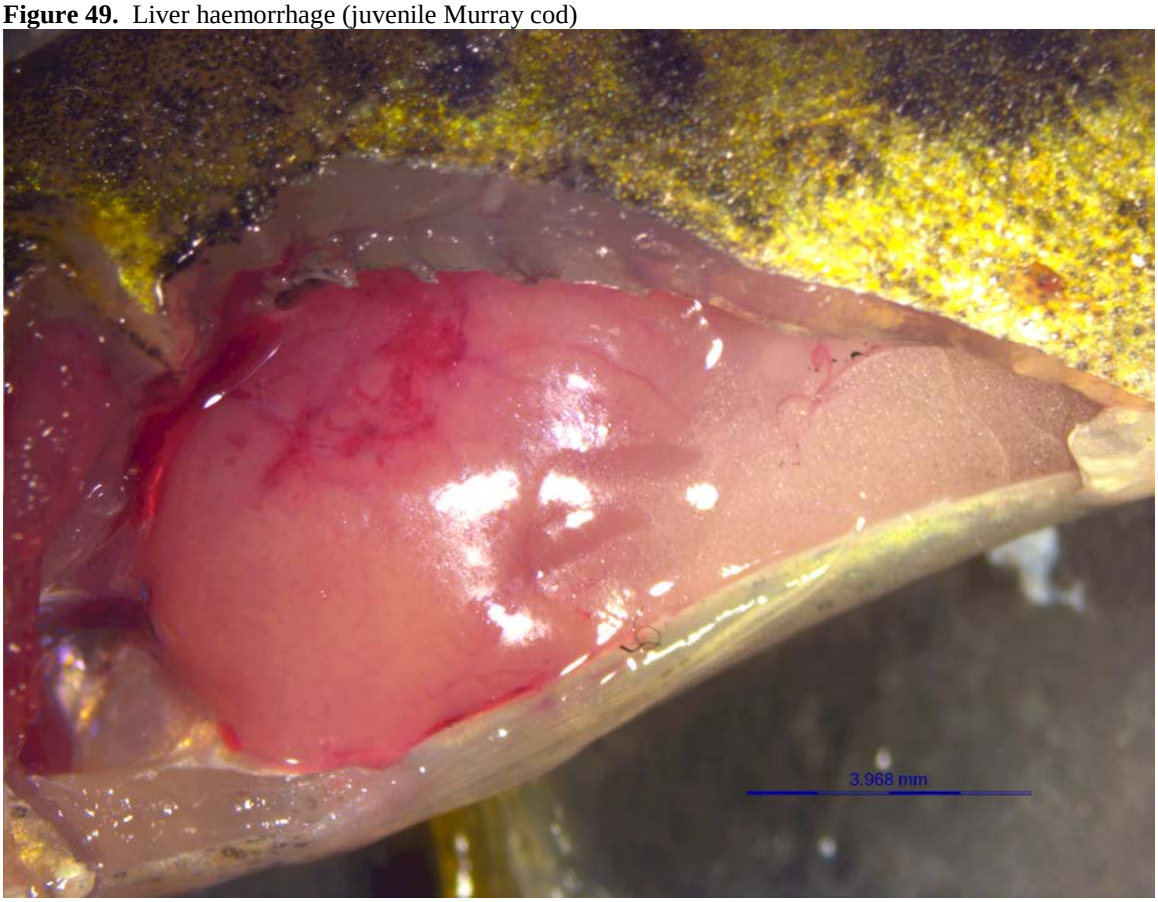


Figure 50. Liver emphysema (juvenile carp)

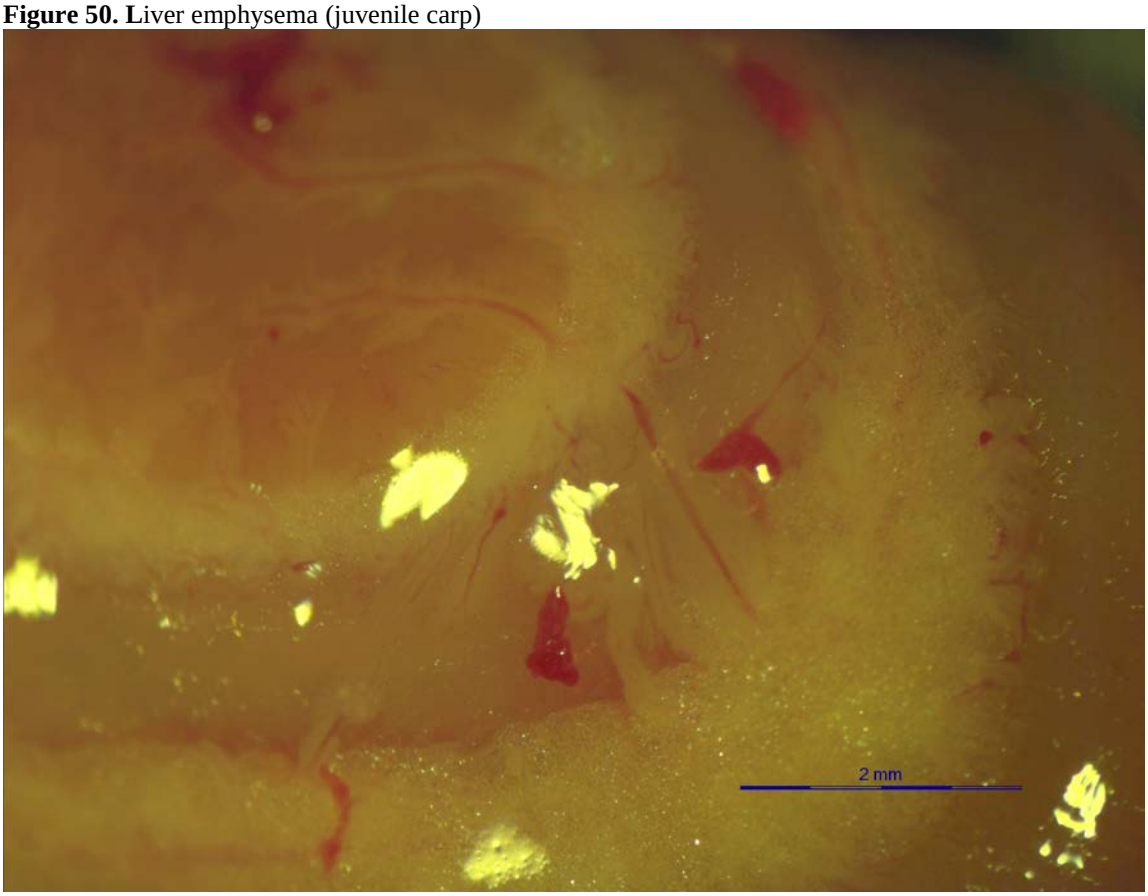


Figure 51. Kidney haemorrhage and emphysema (juvenile carp)

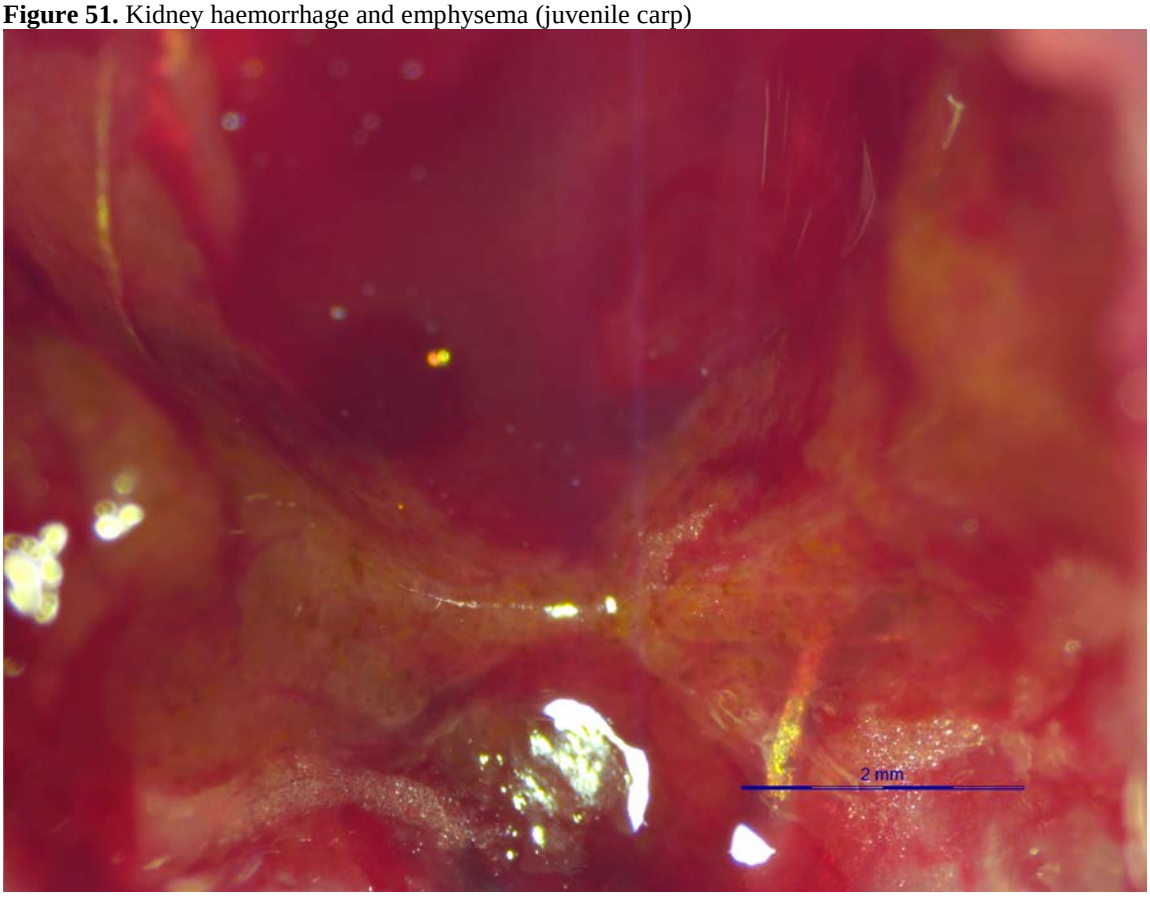


Figure 52. Kidney emphysema (juvenile silver perch)

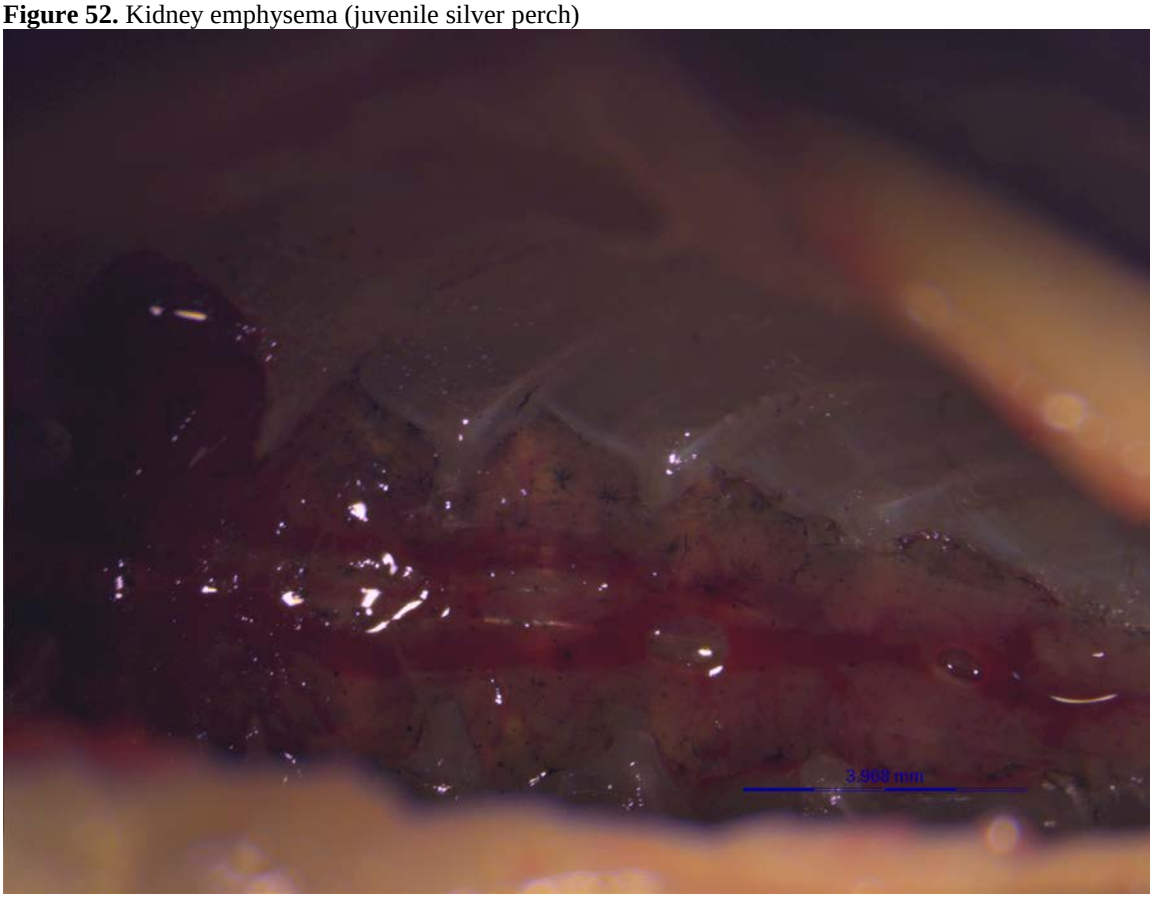
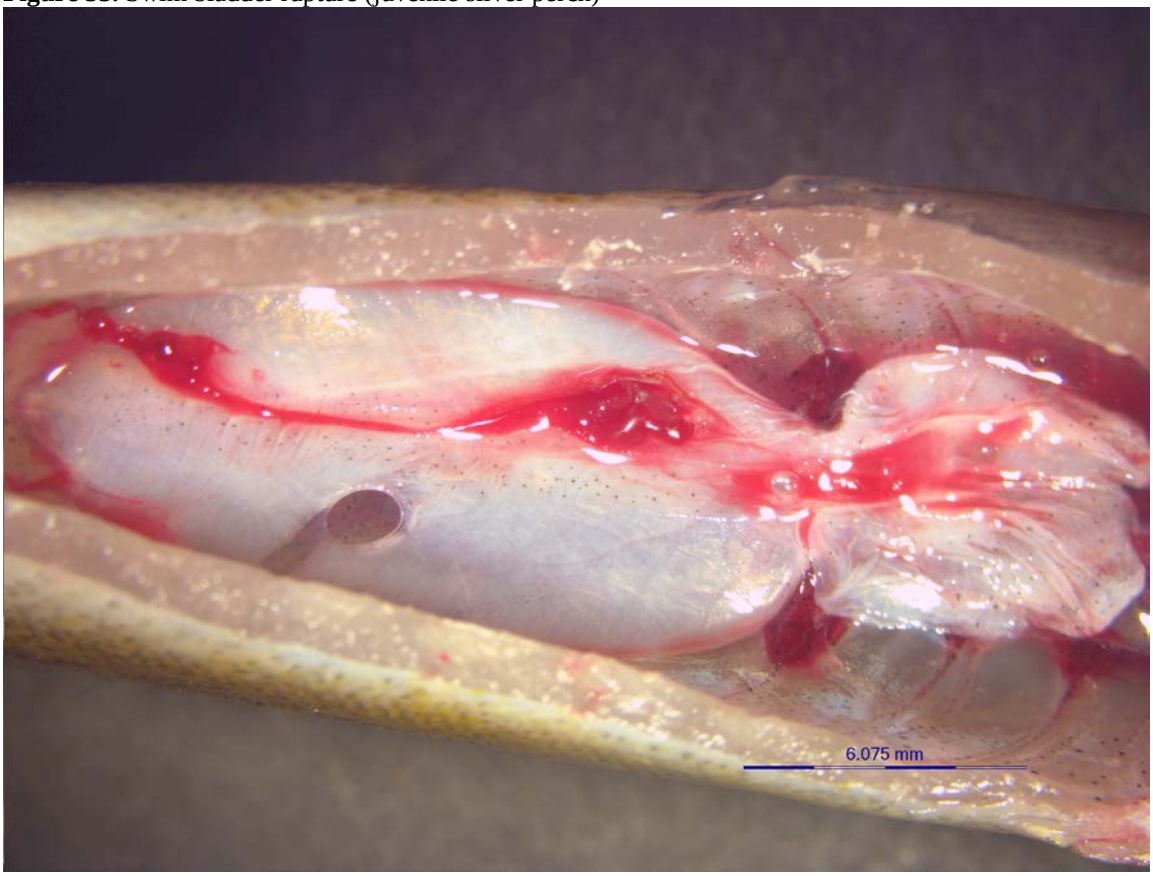
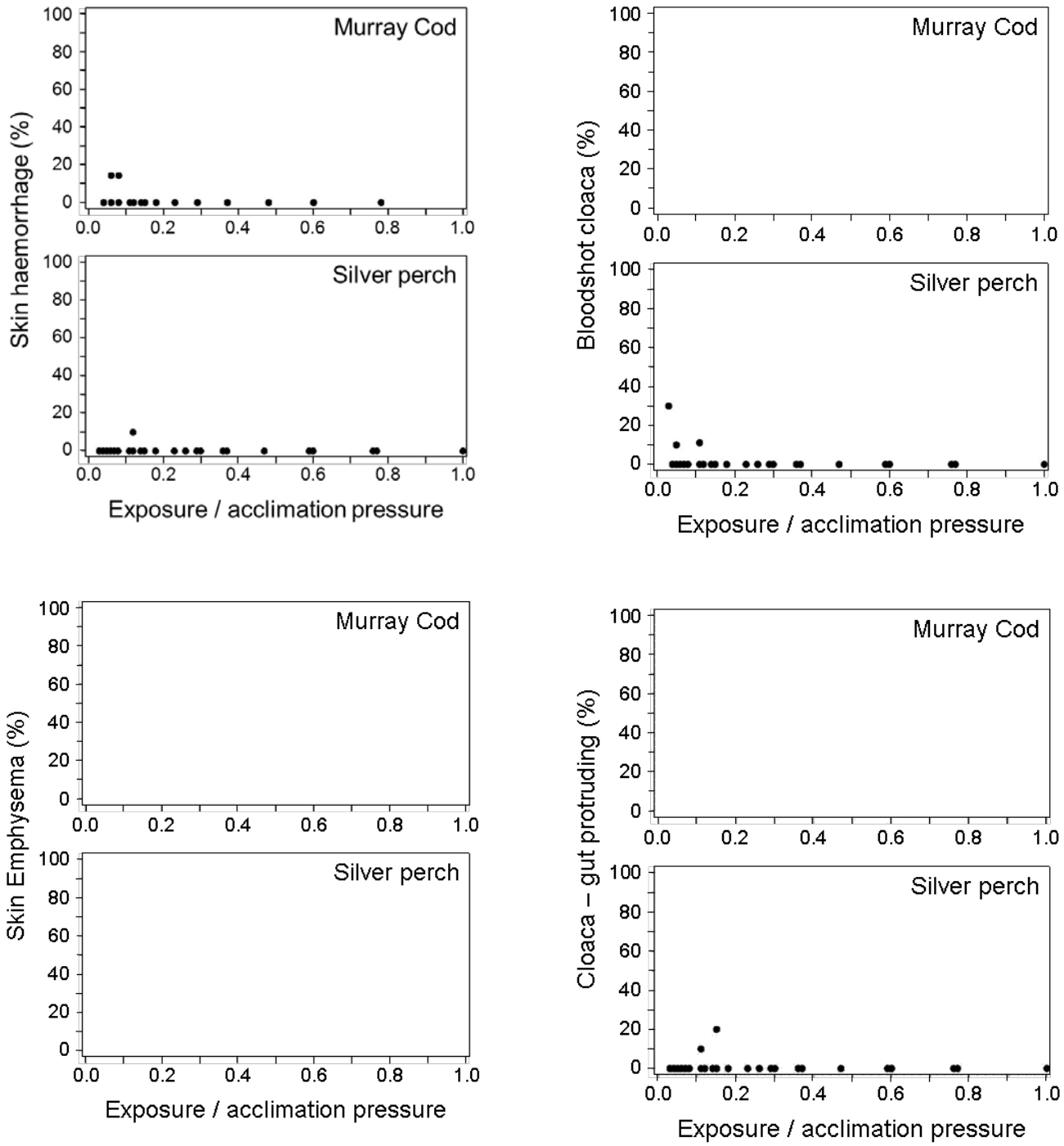


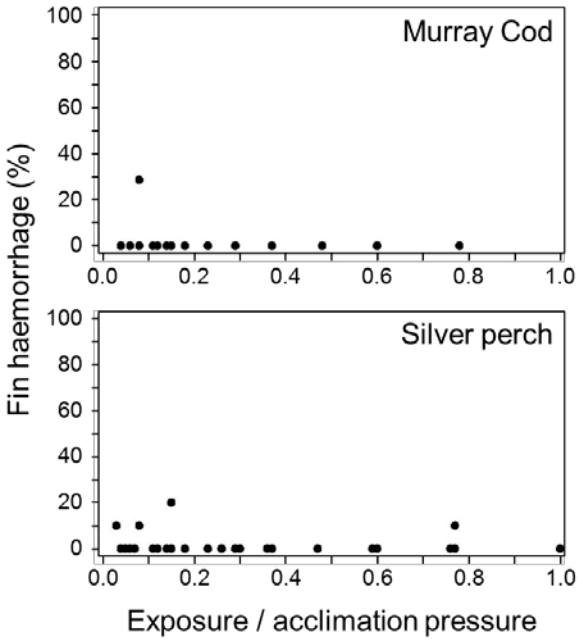
Figure 53. Swim bladder rupture (juvenile silver perch)

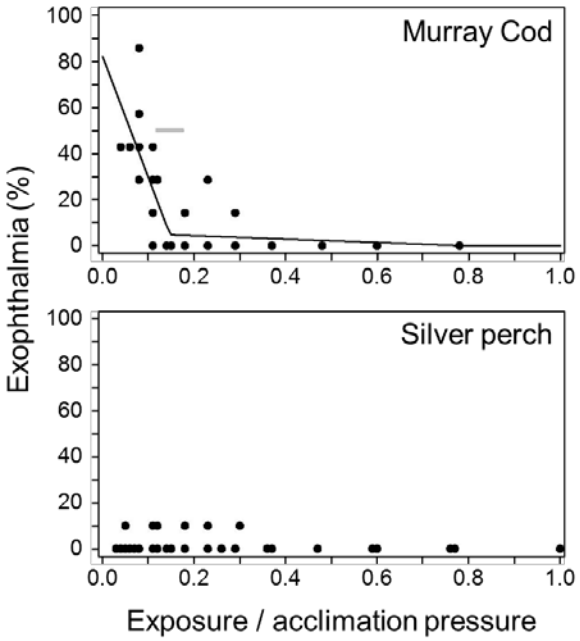


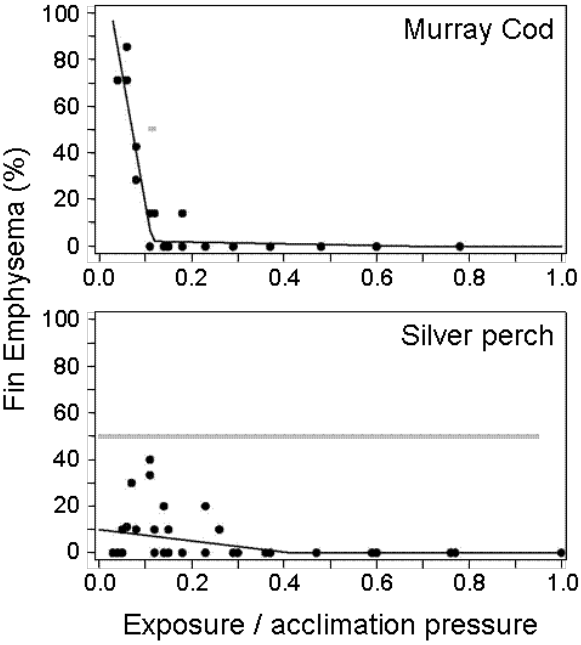
APPENDIX 2 – COMPLETE COLLECTION OF BAROTRAUMA INJURY MODELS

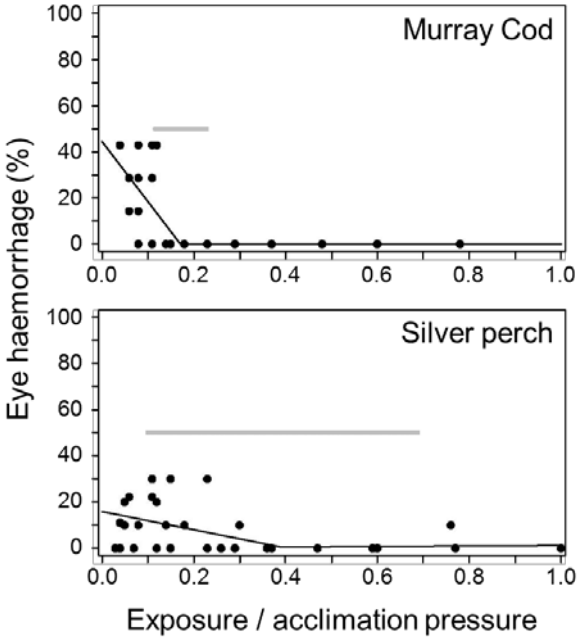
Figure 54. The complete collection of barotrauma injury plots showing the percentage of Murray cod (top) and silver perch (bottom) that displayed certain injuries following simulated infrastructure passage over a range of ratio of pressure changes (exposure/acclimation pressure). Lines are shown if there was convergence in the piecewise linear regression model and if a significant relationship was found. The grey line shows the band between the 95% confidence intervals of the breakpoint

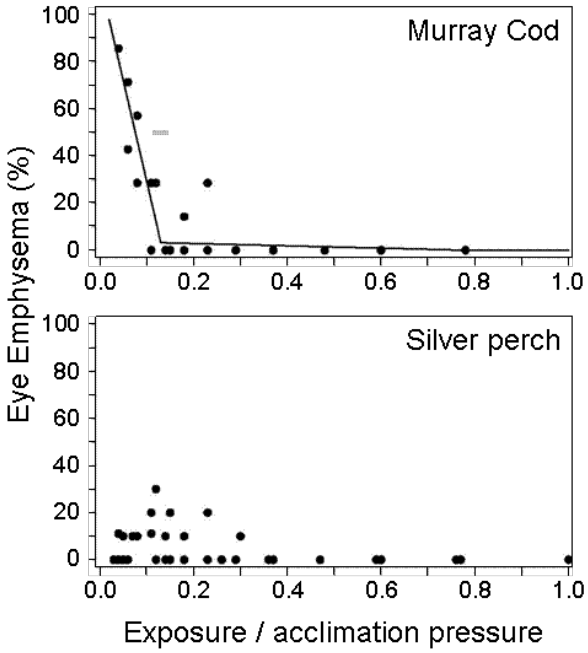


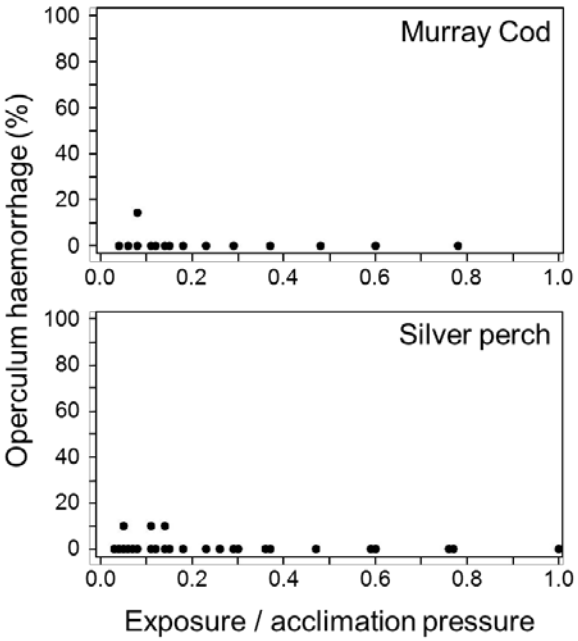

Murray Cod
Silver perch
Fin haemorrhage (%)
Exposure / acclimation pressure

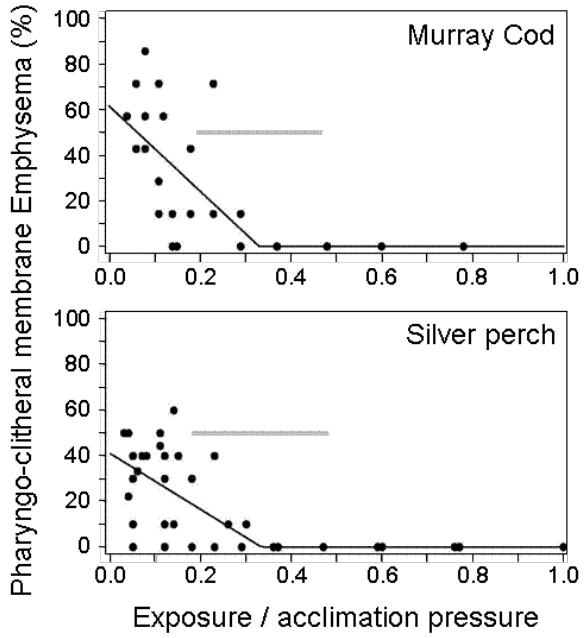

Murray Cod
Silver perch
Exophthalmia (%)
Exposure / acclimation pressure

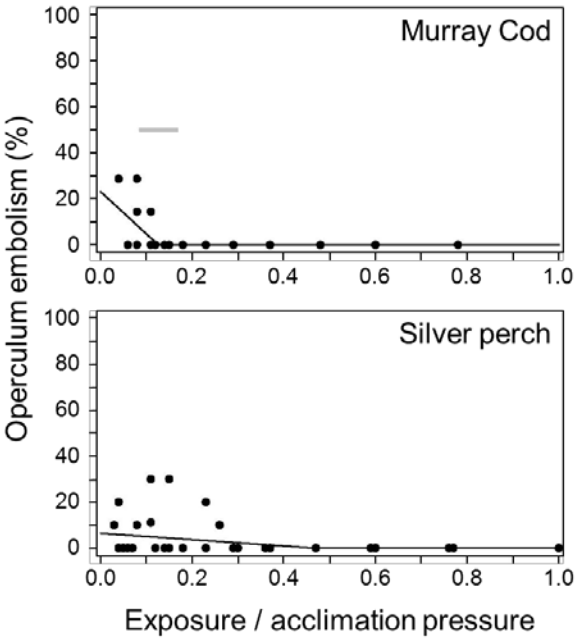

Murray Cod
Silver perch
Fin Emphysema (%)
Exposure / acclimation pressure

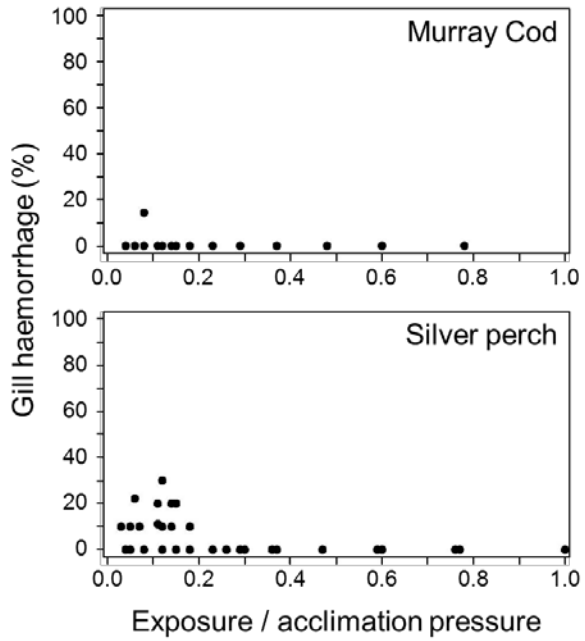

Murray Cod
Silver perch
Eye haemorrhage (%)
Exposure / acclimation pressure

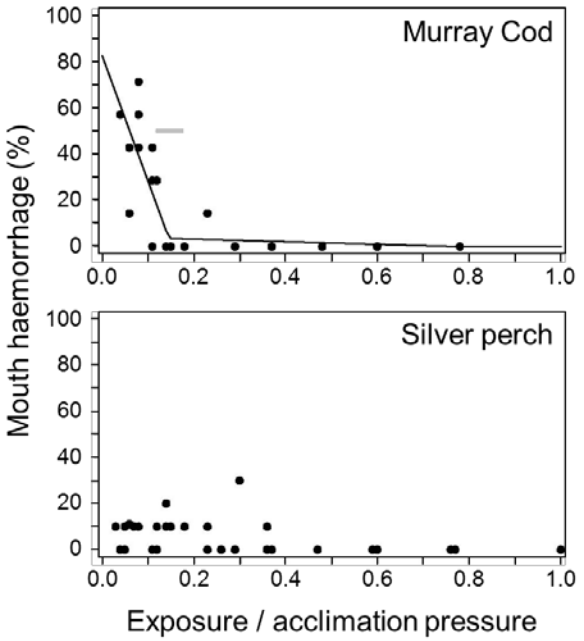

Murray Cod
Silver perch
Eye Emphysema (%)
Exposure / acclimation pressure

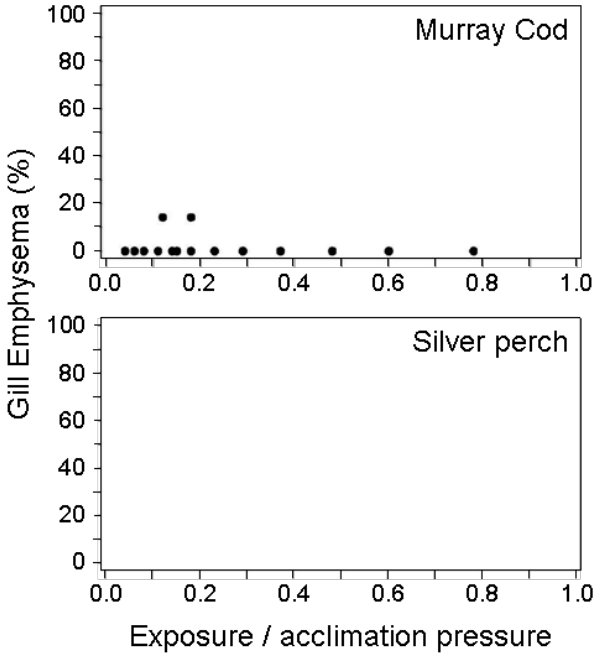

Murray Cod
Silver perch
Operculum haemorrhage (%)
Exposure / acclimation pressure

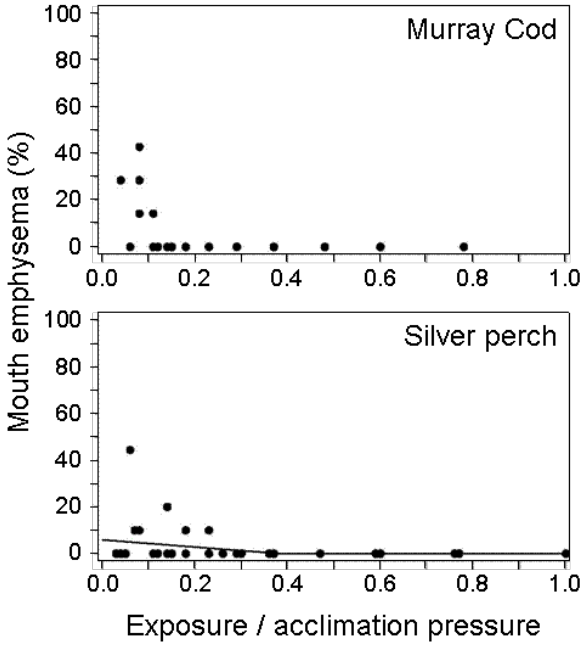

Murray Cod
Silver perch
Pharyngo-clitheral membrane Emphysema (%)
Exposure / acclimation pressure

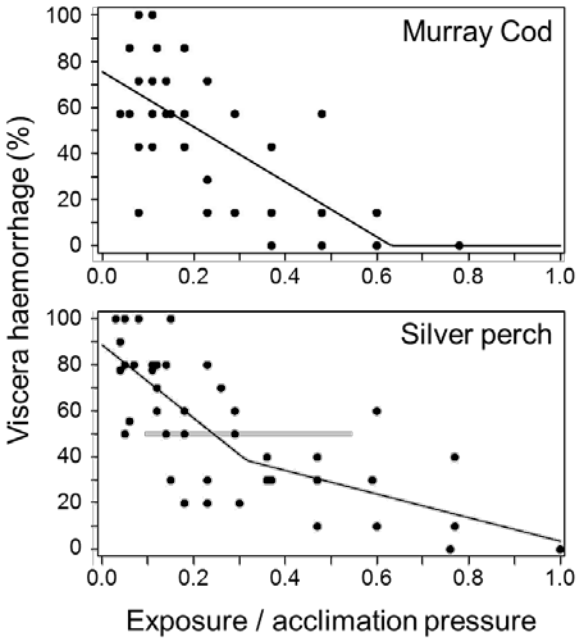

Murray Cod
Silver perch
Operculum embolism (%)
Exposure / acclimation pressure

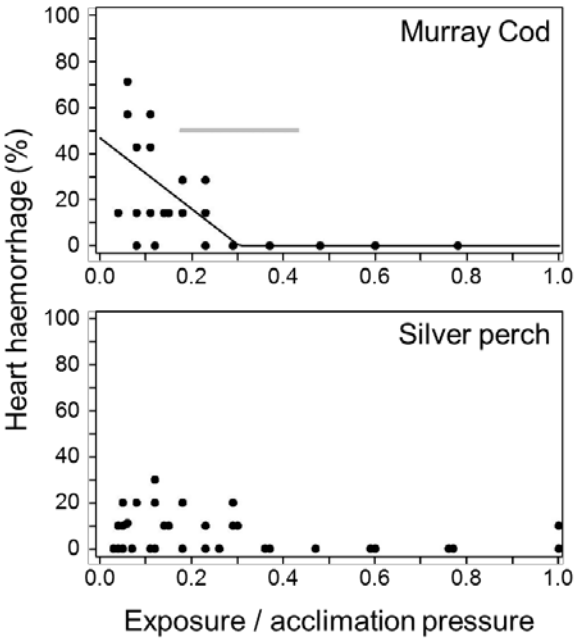

Murray Cod
Silver perch
Gill haemorrhage (%)
Exposure / acclimation pressure

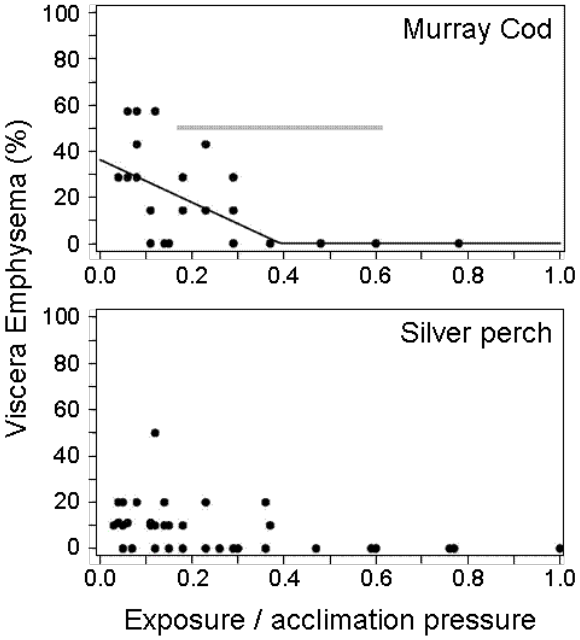

Murray Cod
Silver perch
Mouth haemorrhage (%)
Exposure / acclimation pressure

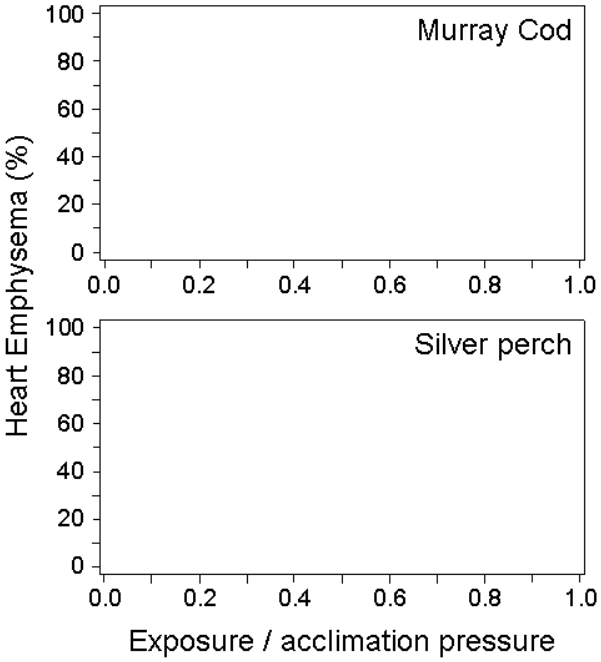

Murray Cod
Silver perch
Gill Emphysema (%)
Exposure / acclimation pressure

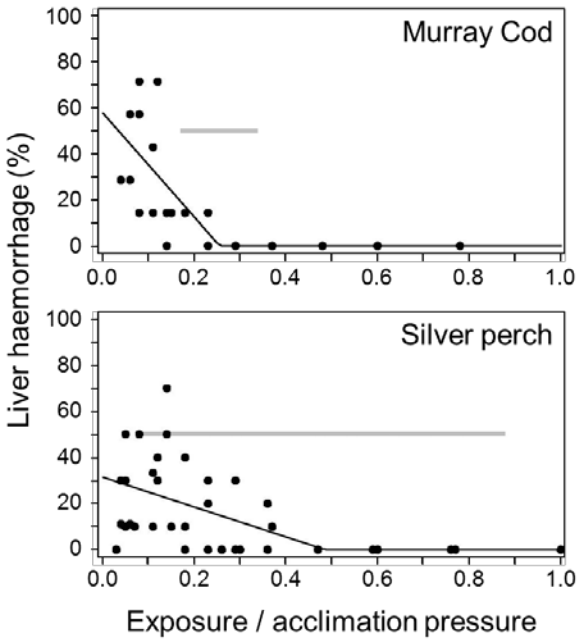

Murray Cod
Silver perch
Mouth emphysema (%)
Exposure / acclimation pressure

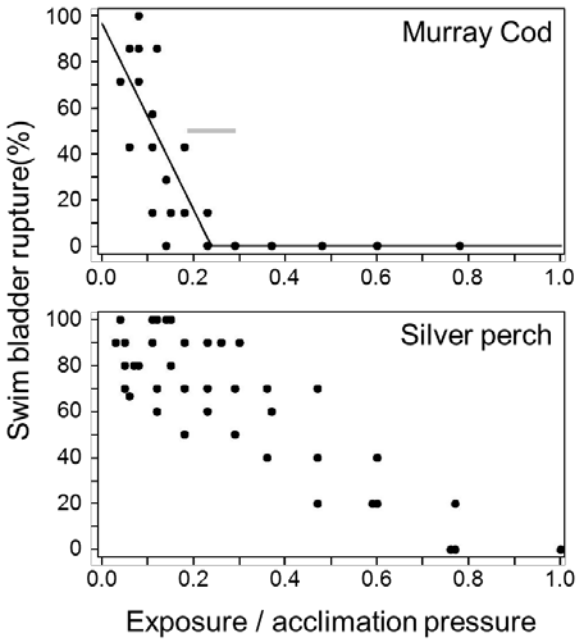

Murray Cod
Silver perch
Viscera haemorrhage (%)
Exposure / acclimation pressure

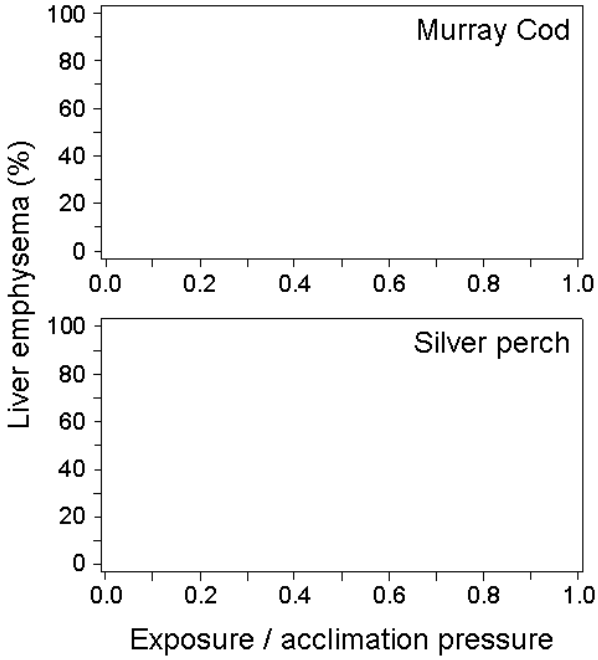

Murray Cod
Silver perch
Heart haemorrhage (%)
Exposure / acclimation pressure

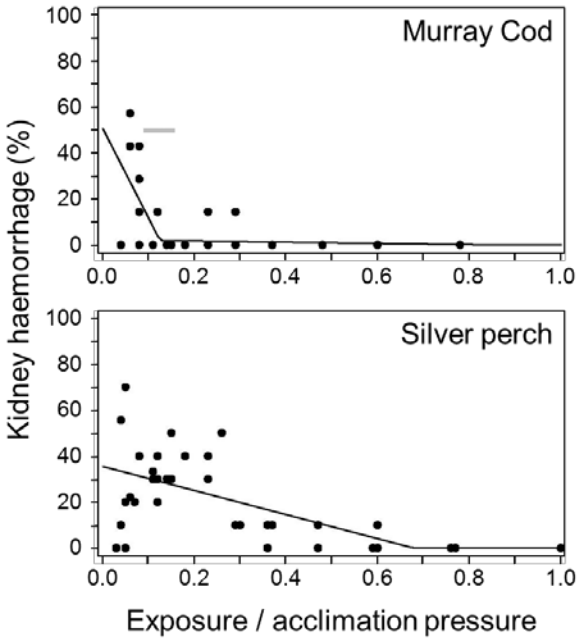

Murray Cod
Silver perch
Viscera Emphysema (%)
Exposure / acclimation pressure

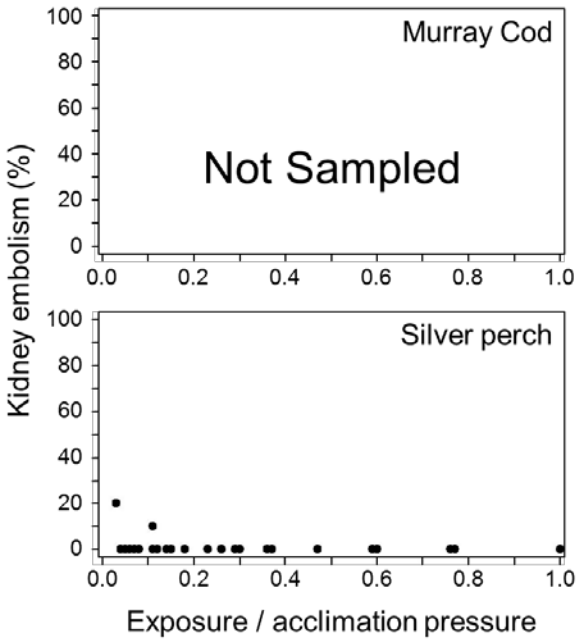

Murray Cod
Silver perch
Heart Emphysema (%)
Exposure / acclimation pressure

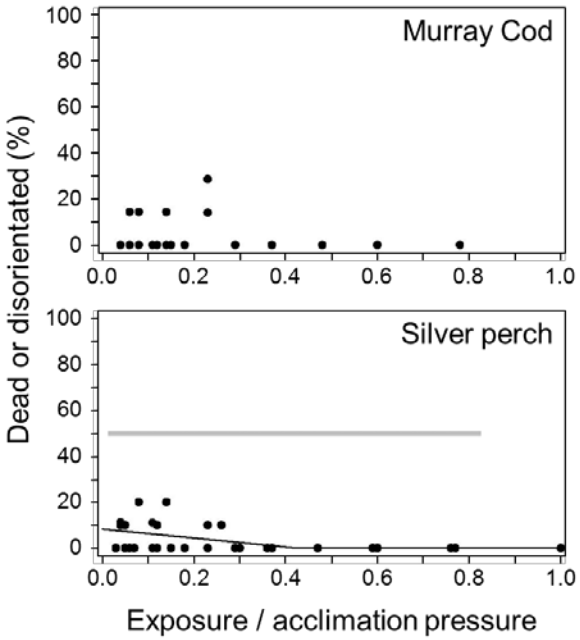

Murray Cod
Silver perch
Liver haemorrhage (%)
Exposure / acclimation pressure


Murray Cod
Silver perch
Swim bladder rupture(%)
Exposure / acclimation pressure


Murray Cod
Silver perch
Liver emphysema (%)
Exposure / acclimation pressure

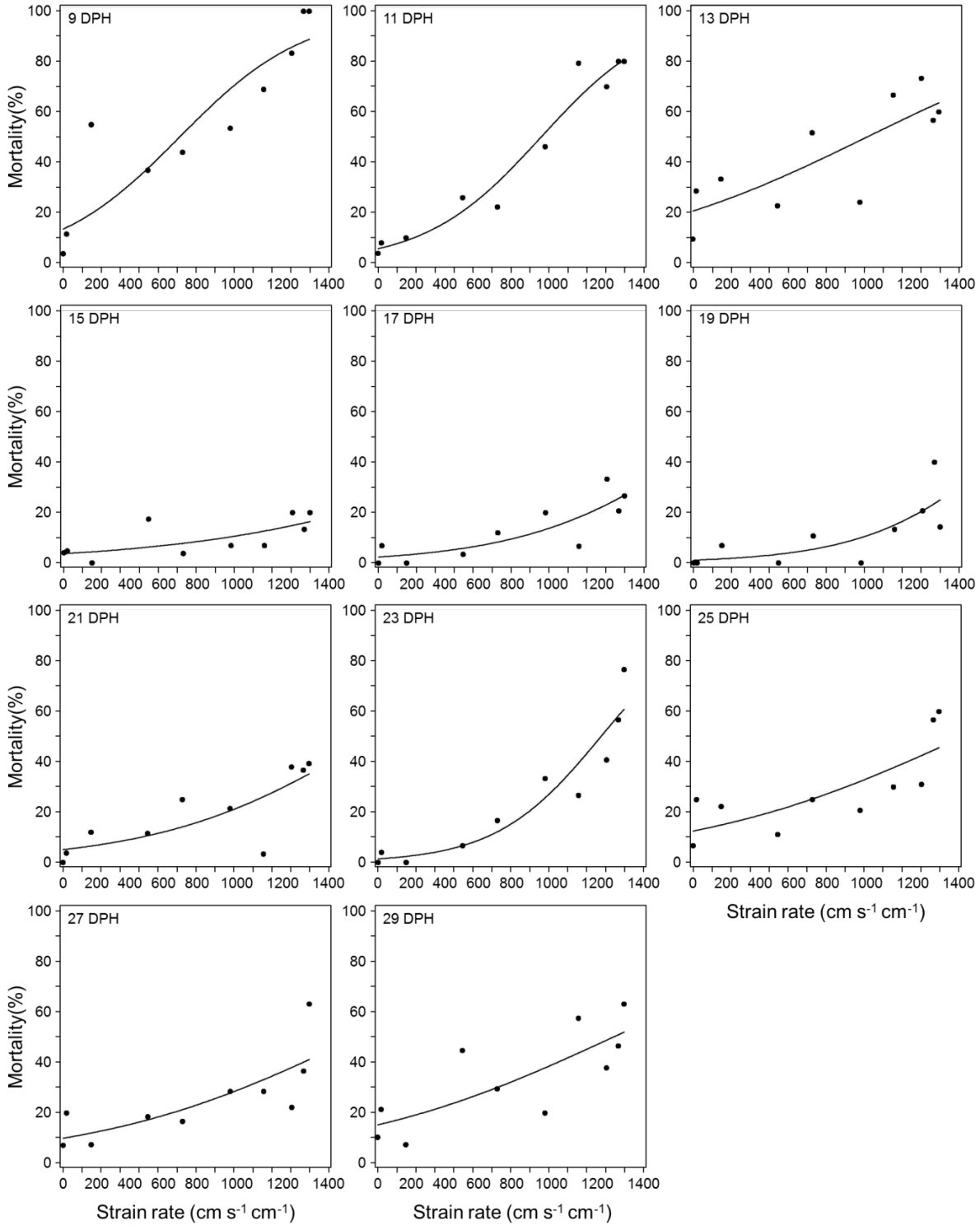

Murray Cod
Silver perch
Kidney haemorrhage (%)
Exposure / acclimation pressure


Murray Cod
Not Sampled
Silver perch
Kidney embolism (%)
Exposure / acclimation pressure


Murray Cod
Silver perch
Dead or disorientated (%)
Exposure / acclimation pressure

APPENDIX 3 – COMPLETE COLLECTION OF MURRAY COD FLUID SHEAR MODELS

Average percentage mortality (immediate and delayed) of Murray cod at all ages (days post hatch DPH) after exposure to various level of shear (strain rate). The line defines the probability of mortality calculated by the logistic regression model.



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