Shiau, J., Watson, J.R., Cramp, R.L., Gordos, M.A. & Franklin, C.E. (2020) Interactions between water depth, velocity and body size on fish swimming performance: Implications for culvert hydrodynamics. *Ecological Engineering*, Vol. 156: 105987.

DOI: https://doi.org/10.1016/j.ecoleng.2020.105987

Interactions between water depth, velocity and body size on fish swimming performance: Implications for culvert hydrodynamics

Jenny Shiau[a], Jabin R. Watson[a], Rebecca L. Cramp[a], Matthew A. Gordos[b], Craig E. Franklin [a,c]

[a] School of Biological Sciences, The University of Queensland, Brisbane, Qld 4072, Australia.

[b] Department of Primary Industries Fisheries, Wollongbar, NSW 2477, Australia.

[c] Corresponding author: Craig Franklin c.franklin@uq.edu.au

Abstract

Understanding how fish traverse man-made barriers (e.g. road-crossings and culverts) ensures that engineering and design guidelines achieve positive outcomes for fish communities. Water velocity, depth and fish body size are interrelated factors that influence fish passage through culverts. Velocity barriers have been a major focus of culvert remediation efforts to improve fish passage, however wide culvert designs that aim to limit high water velocities, often create shallow depths in the culvert barrel that can potentially impede fish passage. Here, we quantified how water velocity, depth and body size interact to affect fish swimming performance and their ability to traverse a 12 m culvert-scale swimming channel. Juveniles and sub-adults of three large-bodied native Australian fish species, silver perch (*Bidyanus bidyanus),* Murray cod (*Maccullochella peelii)* and eel-tail catfish (*Tandanus tandanus),* were chosen to represent a range of body shapes, swimming styles and capabilities. Each species was swum in nine treatments, consisting of three velocities at three water depths, with their time to fatigue and traverse success rates over 8 m of flume length quantified. We found that *B. bidyanus* had an exceptional probability of traverse success, with larger individuals traversing faster than smaller sized fish, but they were physically hindered by shallow water depths. The interaction between velocity and depth was non-linear and highly affected the swimming performance and traverse success of *M. peelii* and *T. tandanus*, particularly for fish > 250 mm and < 100 mm. Our results demonstrate the importance of considering size class and species-specific swimming capabilities in culvert design criteria.

Keywords: *Culvert, Maccullochella peelii, Bidyanus bidyanus, eel-tailed catfish, fish passage, anthropogenic barriers, traversability, swimming endurance, Murray cod, Tandanus tandanus, silver perch.*

1.0 Introduction

Freshwater ecosystems support more than 40% of all fish diversity, yet these diverse habitats are seeing large declines in fish populations (Dudgeon *et al.* 2006; Humphries and Walker 2013; Lundberg *et al.* 2000). These declines can be attributed to a range of anthropogenic activities including barriers to fish passage that fragment habitat connectivity (Dudgeon et al. 2006; Humphries and Walker 2013). Barriers such as dams, weirs, barrages, road-crossings and culverts limit the movement of adult and juvenile fish, preventing access to preferred habitats, spawning grounds, forage areas and impacting predator avoidance (Clapp *et al.* 1990; Gowan and Fausch 2002; Harvey 1991; O'Connor *et al.* 2005; Pelicice and Agostinho 2008; Yamamoto *et al.* 2004). While significant research has occurred to improve fish passage at large scale barriers (e.g. weirs and dams), considerably less attention has been focussed towards remediating small scale barriers like road crossings.

Generally, culverts are designed to maximise hydraulic carrying capacity and minimise costs, with little consideration for fish passage requirements; consequently, they account for the majority of road crossing barriers (Erkinaro *et al.* 2017; Gibson *et al.* 2005; Harris *et al.* 2017). Culverts concentrate natural water flows, reducing the hydraulic complexity and causing changes in water velocity, depth, turbulence and light availability (Bouska and Paukert 2010; Kroon and Phillips 2016; Rodgers *et al.* 2014; 2017; Vowles *et al.* 2014; Williams and Watford 1997). The complexity of hydraulic flows in culverts and subsequent fish passage can also be impacted by the size, design and the materials (i.e. box, pipe, single and multiple channels, concrete, plastic and reinforced steel) that these culverts are constructed with (Anderson *et al.* 2012; Goodrich *et al.* 2018; Warren and Pardew 1998). In New South Wales, Australia, the current culvert guidelines recommend a maximum baseline flow water velocity of 0.3 m s^{-1} and minimum depth of 0.2 m (Fairfull and Witheridge 2003). Despite best practises in the design and construction of new culverts, the desired velocity and depth can ultimately be limited by local precipitation and weather extremes. This can be exacerbated in existing culverts with historically poor designs where undesirable features, such as a shallow invert, reduced water depth during baseline and low flows (Harris *et al.* 2017). Both high water velocities and shallow water depths can produce difficult hydraulic conditions for fish passage (Harris *et al.* 2017; Wolter and Arlinghaus 2003). Small-bodied fish have poorer swimming performance than larger fish and may struggle to negotiate higher water velocities (Baker 2014; Nikora *et al.* 2003) while shallow depths disproportionately affect larger fish movements (Bourne *et al.* 2011; Domenici 2001).

Here, we quantified how water velocity, depth and fish body size interact to affect the swimming performance (endurance time) of three native Australian fish species and their ability to traverse a real-scale experimental channel. Understanding the interactive effects of these abiotic and biotic components of fish passage will help inform fisheries management and the iterative improvement of culvert design guidelines. We hypothesized that: (1) a decrease in water depth, coupled with increasing water velocity, will reduce endurance swimming times; and (2) the probability of traverse success of fish through eight metres of the experimental channel will decrease with decreasing water depth and increasing fish size. We predicted that the particular effects of water depth, velocity and fish size on endurance time and traversability will be species-specific. We chose three native Australian fish species, *Maccullochella peelii*, *Tandanus tandanus* and *Bidyanus bidyanus* that have maximum recorded adult sizes of 180, 90 and 40 cm, respectively. Physically, these three species cover a range of different body types and swimming styles. *M. peelii* has a deep and rounded body shape with a rounded caudal fin that is suited to short-burst swimming with juveniles able to swim up to 0.6 m s^{-1} (Ebner 2006; Watson *et al.* 2019; Whiterod 2013). Adult *B. bidyanus* has a deep, laterally compressed body with a slightly forked caudal fin and juveniles capable of swimming up to 0.74 ms^{-1} (Lintermans 2009; Watson *et al.* 2019). *T. tandanus* possess a laterally compressed posterior with an eel-like isocercal caudal fin, with juveniles able to swim up to 0.5 m s^{-1} (Lintermans 2009; Watson *et al.* 2019). Populations of these three species have experienced significant declines of up to 90% in the Murray Darling Basin (MDB) since European settlement (Baumgartner and Harris 2007; Clunie and Koehn 2001; Hill *et al.* 2015; Jerry 2008; Lintermans 2009; Mallen-Cooper and Brand 2007). These declines are in part caused by artificial barriers to movement and extensive regulation of water flow in the MDB (Lintermans 2009; Mallen-Cooper 1994). Understanding how these three species traverse through dynamic hydraulic culvert conditions can aid in population recovery through culvert management, notably in the middle and upper catchments.

2.0 Methods

2.1 Species selection and husbandry

Three native Australian fish species, *M. peelii*; (TL: mean ± se: 161.4 ± 8.66; range: 45 – 330 mm), *B. bidyanus* (TL: mean ± se: 166.75 ± 6.34; range: 60 – 321 mm) and *T. tandanus* (TL: mean ± se: 170.01 ± 6.55; range: 71 – 335 mm), were chosen based on their relatively large adult size, distinct morphological features, and importance both ecologically and to recreational fishing (Haro *et al.*

2004; Lintermans 2009; Zampatti and Leigh 2013). All fish were sourced as juveniles from commercial hatcheries and transported to the Biohydrodynamics Laboratory at The University of Queensland, Australia. The juvenile fish were held in 40 L tanks that formed part of larger 1000 L recirculating aquarium systems. As the fish grew, they were moved to 800 L aquaculture tanks. All holding tanks were maintained at 25°C ± 1°C with fish exposed to a 12:12h – light:dark cycle. Fish were grown to a comparable size across the test species that was appropriate to the experimental design and that fit within the size constraints imposed by the equipment. Fish were fed once daily to satiation using a commercial pellet (Ridley, Brisbane, Australia) and frozen blood worms (*Chironomidae*). Fish were only kept with conspecifics and all fish were fasted for 24 hours to ensure a post-absorptive state prior to each swimming performance trial (Norin *et al.* 2014).

2.2 Experimental setup & design

A nested experimental design was used with three water depths, 5, 10 and 15 cm. These were kept constant across the test species, while the three test velocities at each depth were species-specific and reflective of their different swimming capabilities. For reference, the hydraulic parameter for each depth-velocity treatment expressed as Froude and Reynolds number, based on the hydraulic diameter of the open channel flume as the characteristic length, is calculated in Table 1. The respective Froude and Reynolds number for each treatment were deemed to be subcritical ($Fr < 1$) and turbulent flow ($Re > 2300$). See Table 1 for details of the nine depth-velocity treatments for each species. The fish were divided into arbitrary small and large size classes to ensure inclusion of both size classes in each treatment: small fish had a total length (TL) less than 200 mm, and large fish had TL greater than 200 mm (see Table 1 for details; NB. Statistical analysis for fish length remains continuous and not based on categorical size class). Sample sizes for each species and treatment are detailed in Table 1. Swimming endurance tests (Kern *et al.* 2017) were conducted in a 12 m flume (dimensions: 12.0 x 0.49 x 0.30 m; L x W x H; Fig. 1) that formed part of a 40,000 L recirculating fish swimming facility. The flume was constantly supplied by a header tank system with the inlet fitted with flow straighteners and stainless-steel mesh screen to prevent fish entering. The flume outlet was fitted with wire mesh to prevent fish from exiting the flume when fatigued. A constant water temperature was maintained at 25°C ± 1°C using an industrial heater/chiller (Oasis C58T- Vb, New Zealand). The inside of the flumes glass walls were lined with grey PVC sheeting (4.5 mm thickness) to prevent external influences on fish swimming behaviour during the trials. Constant lighting was used during each trial and the water depth was set using a depth gauge at the middle of the flume (at 6 m; Fig. 1). We did not detail how the point velocities changed along the entire vertical and horizontal profile of the 12 m experimental channel, but we expect that the conditions did vary subtly as a result of boundary layer effects and small differences in water depth (<1 cm across 12 m). The depths reported were measured at the middle point of the channel (6 m mark) and used to calculate an average velocity from a known discharge rate. As fishes in each treatment were subjected to the same flume configuration, any effect of differences in water velocity experienced by the fish over the length of the channel were likely the same across trials within a treatment.

2.3 Swimming performance and channel traversability

For *M. peelii* and *T. tandanus,* we quantified swimming performance by measuring the time taken for the fish to fatigue at a constant water velocity. Fatigue was defined as the fish resting on the wire mesh at the downstream end of the flume for three seconds. During each trial, we also recorded whether or not the individual fish traversed up 8 m of the flume without encouragement from the point of release to quantify as traverse success. The traverse distance was set at 8 m as this is the most common culvert length in NSW, Australia (Watson *et al.* 2018). For each trial, we set a maximum time limit of 60 min, with trials ≥ 60 min treated as censored or unobservable data (Rodgers *et al.* 2014). Once fatigue was observed, fish were removed from the flume and total length (mm) and body mass (g) were recorded.

For *B. bidyanus*, we quantified swimming performance as the time taken for the fish to traverse up 8 m of the flume from the point of release. This method of quantifying swimming performance was used due to the exceptionally strong performance of adult *B. bidyanus* that have been reported burst swimming against 1.8 m s^{-1} water velocities (Mallen-Cooper 1994), and the physical limitations of the flume. There was a maximum water velocity that the flume could produce at each of the test depths, any further increase in discharge rate would cause the depth to increase. During each trial, individual *B. bidyanus* that did not traverse up 8 m of the flume had their time to fatigue recorded and was subsequently treated as censored data with trials capped at 60 min. For individual fish that successfully traversed up 8 m of the flume, the trial was completed, and time recorded along with the total length (mm) and mass (g).

For each depth-velocity treatment, individual fish were netted from their holding tanks and placed into the flume at the 10 m downstream mark (Fig. 1). Each fish was released at the centre of the flume and the swimming trial began from the time the fish volitionally swam out of the net. If a fish did not swim out of the net within 30 s, the trial was stopped, and the fish was replaced and not included. Individual *B. bidyanus* and *T. tandanus* were swum no more than twice to prevent training effects (Davidson 1997). Juvenile *M. peelii* individuals were given seven days between trial periods for resting period due to their high growth rates and order of treatments randomised. In all cases, no individual fish was swum in the same treatment more than once.

2.4 Statistical analysis

2.4.1 Traverse success

All statistical analysis were performed using *R* version 3.4.4 (R Development Core Team, 2018), in the *RStudio* environment (version 1.1.442). A binomial generalised linear model with a 'logit' link function was used to analyse the effects of depth, velocity and size affecting the traverse success between the three species. A full model with interaction terms was initially used and followed by a stepwise model simplification to include only significant interactions between species, depth and velocity. Finally, a type II ANOVA was used to perform a chi-square test on the simplified stepwise model. For any significant interaction effects, a Tukey's post hoc honest significant differences test was used to determine where the significance resides within the interactions.

2.4.2 Endurance analysis for M. peelii and T. tandanus

M. peelii and *T. tandanus* were analysed separately to *B. bidyanus*. Swimming performance was analysed using classic survival analysis with right censoring of unobservable data. The time to event data is the time taken for the fish to fatigue, and any fish taking ≥ 60 min to fatigue was treated as censored data. We used an extended Cox Proportional Hazard (CPH) model with time-varying coefficients of covariates to analyse the effects of depth, velocity and size between the two species.

In order to satisfy the assumptions of the CPH model, assumptions of proportional hazards and linearity of continuous covariates must be met (Keele 2010). First, we identified the linearity assumption on the continuous covariate of fish size by inspecting the martingale residuals of the null model (classical model) against fish size. Non-linearity of fish size was determined and subsequently corrected for by transforming fish size using penalized-spline transformation (Malloy *et al.* 2017). The penalized spline function (P-spline) for fish size is equal to the sum of all $\beta_i \cdot f_i$(size), where β_i is the vector of partial coefficients for each *i*th spline term function, f_i(size). Here, f_i(size), is computationally determined using the *pspline* function in the '*pspline*' package in *R* using the default number of terms and knots, penalized to four degrees of freedom (Malloy *et al.* 2017; Ramsey and Ripley 2017). Second, proportional hazards of species, depth, velocity and size were tested both graphically via examining Schoenfeld residuals against time and using Grambsch & Therneau's (1994) test of proportional hazards. For factors exhibiting non-proportional hazards, such as depth and velocity, we incorporated an interaction term with time, accounting for time-varying coefficients. However, due to the two species exhibiting severe violation of proportional hazards, stratification of the species was used in the model. A CPH regression analysis was then performed and the predicted hazard ratios graphed using *R* version 3.4.4.

The full model of the hazard function was:

$$h(t, \boldsymbol{X}_{ij}(t))=h_{0i}(t)\exp\{\beta(t)\cdot\mathrm{Depth}_i+\beta(t)\cdot\mathrm{Velocity}_i+\boldsymbol{\beta}\cdot\mathrm{Depth}_i + \boldsymbol{\beta}\cdot\mathrm{Velocity}_i+ \boldsymbol{\beta}\cdot P\text{-}spline(\mathrm{Size})\}$$

where $h(t, \boldsymbol{X}ij(t))$ is the hazard function at time t to the jth factor of $Xi(t)$ with ith levels of the factor. Due to the time-varying coefficient for each covariate, each iteration of $\beta(t)$ is the equivalent to the function of $\beta(t) = \boldsymbol{\beta}+b_i\mathrm{Ln}(t)$, where bi is the ith coefficient for each level of X and $\mathrm{Ln}(t)$ is the linear transformation on time. Standard to all CPH models, $h_{0i}(t)$ is the baseline hazard function at when all covariates equal 0. However, in this model, the baseline hazard is allowed to vary between the species of *M. peelii* and *T. tandanus*, whilst keeping the effect of our predictors the same for each species. Here, $\boldsymbol{\beta}$ represents the fixed time coefficient for depth, velocity and each β_i vector for each penalized spline term for size. Finally, a backwards elimination process was used to identify any significant interaction terms of factors. The final model based on an analysis of deviance using a log-likelihood test only determined a significant three-way interaction between depth, velocity and size with a chi-square value of 352.29 on 98 degrees of freedom with a p-value of <0.001. Lastly, the interpretation of the interaction of covariates was qualitatively examined (see Fig. 5). Figures for endurance probability and hazard ratios were graphed in *R* version 3.4.4 using the '*survival*' and '*survminer*' package (Bewick *et al.* 2004; Kassambara *et al.* 2017; Therneau and Lumley 2014).

2.4.3 Time to traverse for B. bidyanus

B. bidyanus were analysed based on the time taken to successfully traverse through 8 m of the flume without fatiguing. The censored observations for *B. bidyanus* represent the time during which fish fatigued without successfully traversing through 8 m. We used a classical CPH model with Penalized-spline transformation on size to analyse the effects of depth, velocity and size for *B. bidyanus*.

Assumptions for the CPH model were validated with Grambsch & Therneau (1994)'s test of proportional hazards and linearity was tested using penalized-splines. Both linear and non-linear models were significant, however, a penalized-spline transformation on size was still used to satisfy both proportional hazards and linearity assumptions of the CPH model.

The full model for *B. bidyanus* is as below where β_i represents the fixed time coefficient for each covariate.

$$h(t, \boldsymbol{X}_{ij}(t))=h_0(t)\exp\{\beta_i \cdot \mathrm{Depth}_i+\beta_i \cdot \mathrm{Velocity}_i+\beta_i \cdot \textit{P-spline}(\mathrm{Size})\}$$

3.0 Results

3.1 Traverse success

Traverse success significantly varied between species ($\chi^2_2 = 90.14, p < 0.001$) with *B. bidyanus* having the highest traverse success probability of 90.5%, regardless of fish size, depth and velocity (Fig. 2). Overall, traverse success increased with increasing water depth ($\chi^2_2 = 11.44, p = 0.003$) and decreasing velocities ($\chi^2_5 = 52.70, p < 0.001$). The effect of fish length on traverse success was non-significant ($\chi^2_1 = 2.66, p = 0.103$) with data from all species pooled. However, the traverse success between species was significantly dependent on the effects of water velocity but independent of water depth (ANOVA: Species x Velocity $\chi^2_7 = 24.83$, p = <0.001; Fig. 2). The effects of water depth did not significantly influence the traverse success between species (ANOVA: Species x Depth $\chi^2_4 = 9.03$, p = 0.06). Latter post hoc analysis revealed that *B. bidyanus* had roughly equivalent traverse successes at all velocities, with greater traverse success probability than both *M. peelii* (β = 2.9, p = <0.01) and *T. tandanus* (β = 2.47, p = 0.015) at the common water velocity of 0.37 m s^{-1}.

3.2 Endurance performance for M. peelii and T. tandanus

3.2.1 Water depth (independent of velocity and fish size)

There was a significant reduction in the likelihood of fish fatiguing and exhibiting longer swimming endurance times with an increase in water depth (Fig. 3). We also found evidence of a time-dependent effect of water depth on the endurance duration and risk of fish fatigue (i.e. the longer the fish spent swimming, the greater the risk of fatigue; refer to Supplementary Table 1 for hazard ratios at fixed 5 min intervals). When compared to the 5 cm baseline water depth, fish swum at 10 cm water depths initially had an 89 % lower risk of fatigue (at time = 1), but the risk of fatigue was significantly higher than that of the fish at the 5 cm depth if they sustained swimming for more than 45 minutes at 10 cm water depth (Fixed: $\chi^2_1 = 36.09$, p = <0.001, Time-varying: $\chi^2_1 = 18.42$, p = <0.001; $\beta(t) = -2.20+0.76*\mathrm{Ln}(t)$; $HR_1 = 0.11$(0.05-0.23 CI); $HR_{45} = 2.02$(0.26-15.55 CI)). Conversely, fish swum at 15 cm depths had a significantly lower risk of fatiguing than fish swum at 5 cm depths and on average were 37% less likely to fatigue over 60 minutes with greater average endurance times (Fixed: $\chi^2_1 = 46.25$, p = <0.001, Time-varying: $\chi^2_1 = 10.26$, p = 0.001; $\beta(t) = -2.77+0.67*\mathrm{Ln}(t)$; HR = 0.63 (0.07-6.34 CI)).

3.2.2 Water velocity (independent of depth and fish size)

Increasing water velocities significantly increased the likelihood of fish fatiguing (Fig. 3). Overall, water velocity significantly influenced endurance times and risk of fish fatigue but the direction and magnitude of the effects were not constant over time (Refer to Supplementary Table 1 for hazard ratios at fixed 5 minute intervals). Independent of fish size, fish swum at 0.30 m s^{-1} were on average 68% less likely to fatigue within 1 hour than fish swum at 0.20 m s^{-1}; however, the risk of fatigue at 0.3 m s^{-1} was much higher (71%) that at 0.2 m s^{-1} in the early stages of swimming trials (8 min) (Fixed: $\chi^2_1 = 7.01$, p = 0.008, Time-varying: $\chi^2_1 = 7.28$, p = 0.007; $\beta(t) = 1.22-0.58*\mathrm{Ln}(t)$; $HR_{60} = 0.32$(0.02-4.39 CI)). Likewise, fish swum at 0.37 m s^{-1} were on average 60% less likely to fatigue than fish swum at 0.20 m s^{-1} over the duration of the trial; however, this effect was also outweighed by the initial 116% increase of fatigue risk in the first 16 minutes (Fixed: $\chi^2_1 = 18.87$, p = <0.001, Time-varying: $\chi^2_1 = 9.99$, p = 0.001; $\beta(t) = 1.95-0.70*\mathrm{Ln}(t)$; $HR_{60} = 0.4$(0.03-5.68 CI)). On average across 60 minutes, fish swum at 0.47 m s^{-1} were approximately three times more likely to fatigue than 0.20 m s^{-1} (Fixed: $\chi^2_1 = 30.26$, p = <0.001, Time-varying: $\chi^2_1 = 6.21$, p = 0.013; $\beta(t) = 3.36-0.73*\mathrm{Ln}(t)$; $HR_{avg} = 3.84$(0.18-7.56)); and fish swum at 0.56 m s^{-1} were approximately 13 times more likely to fatigue than 0.20 m s^{-1} (Fixed: $\chi^2_1 = 70.79$, p=<0.001, Time-varying: $\chi^2_1 = 6.36$, p = 0.016; $\beta(t) = 4.89-0.85*\mathrm{Ln}(t)$; $HR_{avg} = 13.90$(1.66-194)).

3.2.3 Fish size (independent of water velocity and depth)

When examining the main effects of fish size, we found a significant non-linear relationship between fish length and the risk of fatigue ($\chi^2_{3.05} = 32.59$, p = <0.001). For *M. peelii*, individual fish that were under 85 mm, and between 182 and 288 mm in length, were on average 1.5 to 4.6 times more likely to fatigue than fish that had a total length of 161 mm (mean baseline in our data; Fig. 4a). When comparing between size classes of small (<200 mm) and large (>200 mm) sized fish, large sized fish were on average 37% more likely to fatigue than small sized fish.

For *T. tandanus*, fish that were < 150 mm or between 272 and 305 mm in length were on average 1.5 to 2.2 times less likely to fatigue than fish that were 170 mm in length (mean fish length in our data; Fig. 4b). The comparison between small and large sized *T. tandanus* revealed that large sized fish were on average 23% more likely to fatigue than small sized fish.

3.3 Interactive effects of depth, velocity and fish size

The effects of fish size on endurance time were significantly influenced by the effects of water depth and velocity (χ^2_{98} = 352.29, p = <0.001; Fig 5). At 0.37 m s^{-1}, small (< 200 mm) *M. peelii* swum in 5 cm deep water were 39.8% less likely to fatigue than similarly sized fish swum in 15 cm deep water (Fig. 5a,c); conversely, large *M. peelii* (>200 mm) were 5.2 times more likely to fatigue when swum at 5 cm depth compared with at 15cm depth. However, the negative effect of shallow water depth (5 cm) on the risk of fatigue was greatly reduced (5-fold) when large (>200 mm) *M. peelii* were swum lower water velocities (0.20 ms^{-1}; NB. this does not include fish that were observed to be physically unable to swim in the shallow depth). Independent of body size, in 15 cm deep water, fish swum at the fastest water velocity (0.56 m s^{-1}) were on average 452% more likely to fatigue than fish swum at 0.37 m s^{-1} (Fig. 3a, 5c). Overall, large *M. peelii* swum for longer in deep water than small fish, but smaller fish performed better than large fish in shallow water depth flowing at higher velocity.

For *T. tandanus*, fish < 200 mm were on average 79.9% less likely to fatigue than large sized fish when swum at 15 cm depth and at 0.37 m s^{-1} velocity (Fig. 5f); however, small fish were more likely to fatigue than larger fish in 15 cm deep water at higher water velocities (0.47 and 0.56 m s^{-1}). At shallow water depths, however, large *T. tandanus* were more at risk of fatiguing earlier than small sized fish when swimming at high water velocities (0.37 m s^{-1}).

3.4 Traverse time for B. bidyanus

For *B. bidyanus*, we found that only fish length was a significant predictor of traverse time but the effect was not linear ($\chi^2_{3.07}$ = 13.033, p = 0.005). Individual fish that were > 100 mm in total length had, on average, a faster traverse time and were 52% more likely to successfully traverse the channel than fish that were < 100 mm (Fig. 6). Water velocity and water depth had no significant effect on the traverse time (p > 0.22; Fig. 7).

4.0 Discussion

Our data shows that decreasing water depth and increasing water velocity can interact to reduce fish endurance swim times and decrease the likelihood of successfully traversing 8 m of an experimental channel. As hypothesised, swimming endurance times decreased with decreasing water depth and increasing water velocities, with larger sized *M. peelii* and *T. tandanus* more heavily affected than smaller sized conspecifics (Fig. 3, 5). On the contrary, the traverse time for *B. bidyanus* was not influenced by water depth or velocity, only fish size. The probability of *B. bidyanus* successfully traversing the channel was greater than both *M. peelii* and *T. tandanus* irrespective of body size (Fig. 2). Our results show that elevated water velocities remain a strong predictor of poor fish passage success for *M. peelii* and *T. tandanus*, but that bigger fish performed better than smaller fish at higher water velocities. However, the performance of large fish decreased with decreasing water depth, suggesting that shallow water depths can surpass velocity as the main challenge to their swimming performance and traverse success rates.

Fish size was a strong predictor of reduced swimming performance at shallow water depths and this relationship was exacerbated by elevated water velocities. This is congruent with Warren & Pardew (1998) who also observed that the relationship between water depth and velocity on fish swimming performance was non-linear and that it negatively affected fish movement through culverts. For *M. peelii* and *B. bidyanus*, our results are consistent with the allometric scaling of fish size and swimming performance (Domenici 2001; Nikora *et al.* 2003; Webb *et al.* 1991; Wolter and Arlinghaus 2003), where smaller sized fish have an increased risk of fatigue and shorter endurance times, compared to larger sized fish swimming at the same water velocities and depths, due to their higher metabolic demands. Despite the observation that *M. peelii* and *T. tandanus* performed better, on average, at velocities below 0.37 m s^{-1} and at depths greater than 10 cm, fish had to be able to sustain performance for longer than eight minutes for an improved chance of traverse success due to the time-varying effects of velocity and depth (Fig. 2, 3). Simply put, the risk of fatigue was greatest in the first 8 min of the swimming trial if they had not already traversed the full length of the flume/culvert, and this was exacerbated if the fish were smaller than 150 mm. Despite this general trend, the effects of depth, velocity and fish size on fish performance were species specific and so caution must be taken to consider fish species when applying generalised data to understanding issues with existing culverts or their design.

The effects of depth and velocity on *M. peelii*, *T. tandanus* and *B. bidyanus* can be related back to their different physical characteristics and ecology. Haro *et al.* (2004) also noted that increases in velocity reduced the distance fish could traverse, and suggested that the interspecific differences may be dependent on physical and behavioural traits. Although *M. peelii* may undertake seasonal upstream migrations of up to 130 km, they and *T. tandanus* generally inhabit slower moving waters and move < 10 km from their home range (Koehn *et al.* 2009; Lintermans 2009; Reynolds 1983). In contrast, *B. bidyanus* are excellent swimmers that prefer fast-flowing waters and migrate at the macro scale (> 500 km upstream) (Lintermans 2009; Reynolds 1983). This was reflected in their swimming performance (traverse time) and traverse success probability that exceeded both *M. peelii* and *T. tandanus*. Despite adult *B. bidyanus* having exceptional swimming capabilities, their large physical size (body height) restricted their ability to swim in shallow water depths, with some of the largest individuals observed resorting to swimming on their sides. This would have markedly increased drag and dramatically reduced their ability to produce thrust, resulting in much higher energetic costs to swim (Hughes 2004; Webb *et al.* 1991). While our sample size range for *M. peelii* and *T. tandanus* did not include their maximal adult size ranges, it can be reasonably assumed that individuals larger than those examined would also be impaired by very shallow water. This could have significant implications for migrating adult animals. This was reflected in our results showing *B. bidyanus* that were longer than 250 mm were on average less likely to traverse the full length of the flume at the shallowest water depth than smaller conspecifics.

T. tandanus displayed some unexpected variations in swimming performance with changes in depth and velocity and across size classes. Although deeper water did reduce overall fatigue risks, small fish (< 127 mm) were more likely to be negatively affected by deep water at velocities greater than 0.47 m s^{-1}, while larger fish were more affected by deep water at ≤ 0.37 m s^{-1}. During the swimming trials, some individual *T. tandanus* exhibited negative rheotaxis, which could have had a negative influence on the probability of fatigue (Boys *et al.* 2013). The negative rheotaxis displayed by *T. tandanus* was possibly avoidance behaviour of excessive velocities, or individuals seeking lower velocity waters similar to their preferred habitat. These behaviours can cause them to be more readily displaced downstream by high velocity flood events (Lintermans 2009; Stuart and Berghuis 2002).

While individual behavioural responses to water depth and velocity, and the energetic cost of swimming were not specifically examined in the present study, they likely contribute markedly to overall fish traverse success rates in natural settings. It is also likely that although an individual fish may successfully traverse through a culvert, the energy exerted could be high enough to impact their subsequent survival prospects, particularly for small fish (Nikora *et al.* 2003; Rodgers *et al.* 2014). Ultimately it is not just traversing a single in-stream barrier, but the cumulative impacts of multiple biotic and abiotic stressors such as successive barriers, other anthropogenic waterway changes, inter- and intra-specific interactions and life stage-specific pressures acting simultaneously on an individual fish's fitness that will determine the recruitment rates of juvenile fish in modified water ways (Wolter and Arlinghaus 2003).

The MDB relies on flow events to stimulate fish to migrate and spawn. Under natural conditions, baseline flows can be highly restrictive to fish movement, particularly in the upper catchments where culverts are more numerous. This makes the optimisation of culvert design and construction to fit both environmental and civil requirements difficult, particularly if minimum water depths and maximum velocities during baseline flows need to pass the entire fish community. The complexity is amplified by the effects of climate change on flow regimes will continue to be a major challenge for fish passage management in the MDB. With reduced precipitation and prolonged drought periods extending the dry season (Balcombe *et al.* 2011), the negative effects of shallow water in culverts and causeways (< 10 cm) will likely contribute to reduced migratory spawning movements of large-bodied *B. bidyanus* and *M. peelii*.

With increasing efforts to mitigate the impacts of in-stream barriers on fish movement, our understanding of the requirements of fish to effect successful passage through culverts is improving. Significant knowledge gaps still remain, particularly for culverts with poor water flow conditions in Australia's highly variable environment (Harris *et al.* 2017; Lintermans 2009). The management of fish movement through culverts can be improved by increasing our understanding of the cause and effect relationships underpinning the physiological responses that fish have to specific culvert conditions (Rodgers *et al.* 2017). This data can, in turn, improve our understanding of how existing in-stream fish passage structures are performing, and improve the design guidelines and regulations for new structures in sensitive environments.

5.0 Acknowledgements

The Research was funded by a National Environmental Science Program grant from the Threatened Species Recovery Hub to CEF [3.3.7] and an Australian Research Council Linkage Grant to CEF and

MG [LP140100225]. This work was conducted with the approval of The University of Queensland Ethics Committee (SBS/312/15/ARC). We would like to thank Dr Simone Blomberg for statistical advice.

The data that support the findings of this study are openly available in CloudStor at https://cloudstor.aarnet.edu.au/plus/s/DRfVVzlen6CXQXG.

We declare no conflicts of interest.

6.0 References

Anderson, G.B., Freeman, M.C., Freeman, B.J., Straight, C.A., Hagler, M.M., Peterson, J.T., 2012. Dealing with uncertainty when assessing fish passage through culvert road crossings. Environ. Manage. 50, 462-477.

Balcombe, S.R., Sheldon, F., Capon, S.J., Bond, N.R., Hadwen, W.L., March, N., Bernays, S.J., 2011. Climate-change threats to native fish in degraded rivers and floodplains of the Murray-Darling Basin, Australia. Mar. Freshw. Res. 62, 1099-1114.

Baker, C.F., 2014. Effect of ramp length and slope on the efficacy of a baffled fish pass. J. Fish Biol. 84, 491-502.

Baumgartner, L.J., and Harris, J.H., 2007. Passage of non-salmonid fish through a deelder lock on a lowland river. River Res. Appl. 23, 1058-1069.

Bewick, V., Cheek, L., Ball, J., 2004. Statistics review 12: Survival analysis. Crit. Care. 8, 389-394.

Bourne, C.M., Kehler, D.G., Wiersma, Y.F., Cote, D., 2011. Barriers to fish passage and barriers to fish passage assessments: The impact of assessment methods and assumptions on barrier identification and quantification of watershed connectivity. Aquat. Ecol. 45, 389-403.

Bouska, W.W., Paukert, C.P., 2010. Road crossing designs and their impact on fish assemblages of great plains streams. T. Am. Fish. Soc. 139, 214-222.

Boys, C.A., Robinson, W., Baumgartner, L.J., Rampano, B., Lowry, M., 2013. Influence of approach velocity and mesh size on the entrainment and contact of a lowland river fish assemblage at a screened irrigation pump. PLoS ONE. 8, 1-13. https://doi.org/10.1371/journal.pone.0067026

Clapp, D.F., Clark, R.D., Diana, J.S., 1990. Range, activity, and habitat of large, free-ranging brown trout in a Michigan stream. T. Am. Fish. Soc. 119, 1022-1034.

Clunie, P., Koehn, J., 2001. Freshwater catfish: A resource document. Murray-Darling Basin Comission, Canberra.

Davidson, W., 1997. The effects of exercise training on teleost fish, a review of recent literature. Comp. Biochem. Phys. A. 117, 67-75.

Domenici, P., 2001. The scaling of locomotor performance in predator–prey encounters: From fish to killer whales. Comp. Biochem. Phys. A. 131, 169-182.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L.J., Sullivan, C.A., 2006. Freshwater biodiversity: Importance, threats, status and conservation challenges. Biol. Rev. 81, 163-182.

Ebner, B., 2006. Murray cod an apex predator in the Murray River, Australia. Ecol. Freshw. Fish. 15, 510-520.

Erkinaro, J., Erkinaro, H., Niemelä, E., 2017. Road culvert restoration expands the habitat connectivity and production area of juvenile Atlantic salmon in a large subarctic river system. Fisheries Manag. Ecol. 24, 73-81.

Fairfull, S., Witheridge, G., 2003. Why do fish need to cross the road? Fish Passage Requirements for Waterway Crossings. NSW Fisheries, Cronulla, NSW.

Gibson, R.J., Haedrich, R.L., Wernerheim, C.M., 2005. Loss of fish habitat as a consequence of inappropriately constructed stream crossings. Fisheries. 30, 10-17.

Goodrich, H. R., Watson, J. R., Cramp, R. L., Gordos, M. A., and Franklin, C. E. (2018). Making culverts great again. Efficacy of a common culvert remediation strategy across sympatric fish species. Ecological Engineering 116, 143-153.

Gowan, C., Fausch, K.D., 2002. Why do foraging stream salmonids move during summer? Environ. Biol. Fish. 64, 139-153.

Grambsch, P.M., Therneau, T.M., 1994. Proportional hazards tests and diagnostics based on weighted residuals. Biometrika. 81, 515-526.

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

Harris, J.H., Kingsford, R.T., Peirson, W., Baumgartner, L.J., 2017. Mitigating the effects of barriers to freshwater fish migrations: The Australian experience. Mar. Freshw. Res. 68, 614-628.

Harvey, B.C., 1991. Interactions among stream fishes: Predator-induced habitat shifts and larval survival. Oecologia. 87, 29-36.

Hill, E., Ingram, B.A., Rourke, M., Mitchell, J., Strugnell, J. M., 2015. Genetic diversity and population structure of the threatened freshwater catfish, *Tandanus tandanus*, in Victoria, Australia. Conserv. Genet. 16, 317-329.

Hughes, N.F., 2004. The wave-drag hypothesis: An explanation for size-based lateral segregation during the upstream migration of salmonids. Can. J. Fish. Aquat. Sci. 61,103-109.

Humphries, P., Walker, K., 2013. Ecology of Australian Freshwater Fishes. Victoria, Australia.

Jerry, D.R., 2008. Phylogeography of the freshwater catfish *Tandanus tandanus* (Plotosidae): A model species to understand evolution of the eastern Australian freshwater fish fauna. Mar. Freshw. Res. 59, 351-360.

Kassambara, A., Kosinski, M., Biecek, P., Fabian, S., 2017. Package 'survminer'. *Drawing Survival Curves using 'ggplot2'*. (R package version 0.3.1.).

Keele, L., 2010. Proportionally difficult: Testing for nonproportional hazards in cox models. Polit. Anal. 18, 189-205.

Kern P., Cramp R.L., Gordos M.A., Watson J.R. and Franklin C. E. (2017). Measuring *U*crit and endurance: equipment choice influences estimates of fish swimming performance. Journal of Fish Biology 92**,** 237-247.

Koehn, J.D., Mckenzie, J.A., O'Mahony, D.J., Nicol, S.J., O'Connor, J.P., O'Connor, W.G., 2009. Movements of Murray Cod (*Maccullochella peelii peelii*) in a large Australian lowland river. Ecol. Freshw. Fish. 18, 594-602.

Kroon, F.J., Phillips, S., 2016. Identification of human-made physical barriers to fish passage in the wet tropics region, Australia. Mar. Freshw. Res. 67, 677-681.

Lintermans, M., 2009. Fishes of the Murray-Darling Basin. An introductory guide. Canberra, Australia.

Lundberg, J.G., Kottelat, M., Smith, G.R., Stiassny, M.L.J., Gill, A.C., 2000. So many fishes, so little time: An overview of recent ichthyological discovery in continental waters. Ann. Mo. Bot. Gard. 87, 26-62.

Mallen-Cooper, M., 1994. Swimming ability of adult golden perch, *Macquaria ambigua* (Percichthyidae), and adult silver perch, *Bidyanus bidyanus* (Teraponidae), in an experimental vertical-slot fishway. Mar. Freshw. Res. 45, 191-198.

Mallen-Cooper, M., Brand, D.A., 2007. Non-salmonids in a salmonid fishway: What do 50 years of data tell us about past and future fish passage? Fisheries Manag. Ecol. 14, 319-332.

Malloy, E.J., Kapellusch, J.M., Garg, A., 2017. Estimating and Interpreting Effects from Nonlinear Exposure-Response Curves in Occupational Cohorts Using Truncated Power Basis Expansions and Penalized Splines. Comput. Math. Method. M. 2017, 1-16.

Nikora, V.I., Aberle, J., Biggs, B.J.F., Jowett, I.G., Sykes, J.R.E., 2003. Effects of fish size, time-to-fatigue and turbulence on swimming performance: A case study of *Galaxias maculatus*. J. Fish Biol. 63, 1365-1382.

Norin, T., Malte, H., Clark, T.D., 2014. Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. J. Exp. Biol. 217, 244-251.

O'Connor, J.P., O'Mahony, D.J., O'Mahony, J.M., 2005. Movements of *Macquaria ambigua*, in the Murray River, south-eastern Australia. J. Fish Biol 66, 392-403.

Pelicice, F.M., Agostinho, A.A., 2008. Fish-passage facilities as ecological traps in large neotropical rivers. Conserv. Biol. 22, 180-188.

Ramsey, J., Ripley, B., 2017. Package 'pspline'. *Penalized Smoothing Splines*. (R package version 1.0-18).

Reynolds, L.F., 1983. Migration patterns of five fish species in the Murray-Darling river system. Mar. Freshw. Res. 34, 857-871.

Rodgers, E.M., Cramp, R.L., Gordos, M., Weier, A., Fairfall, S., Riches, M., Franklin, C.E., 2014. Facilitating upstream passage of small-bodied fishes: Linking the thermal dependence of swimming ability to culvert design. Mar. Freshw. Res. 65, 710-719.

Rodgers E.M., Heaslip, B.M., Cramp, R.L., Riches, M., Gordos, M.A., Franklin, C.E. (2017). Substrate roughening improves swimming performance in two small-bodied riverine fishes: implications for culvert remediation and design. Conservation Physiology 5 DOI: 10.1093/conphys/cox034

Stuart, I.G., Berghuis, A.P., 2002. Upstream passage of fish through a vertical-slot fishway in an Australian subtropical river. Fisheries Manag. Ecol. 9, 111-122.

Therneau, T.M., Lumley, T., 2014. Package 'survival'. *Survival Analysis.* (R package version 2.37-7).

Vowles, A.S., Anderson, J.J., Gessel, M.H., Williams, J.G., Kemp, P.S., 2014. Effects of avoidance behaviour on downstream fish passage through areas of accelerating flow when light and dark. Anim. Behav. 92, 101-109.

Warren, M.L., Pardew, M.G., 1998. Road crossings as barriers to small-stream fish movement. T. Am. Fish. Soc. 127, 637-644.

Watson, J.R., Goodrich, H.R., Cramp, R.L., Gordos, M.A., Franklin, C.E., 2018. Utilising the boundary layer to help restore the connectivity of fish habitats and populations. Ecol. Eng. 122, 286-294.

Watson, J.R., Goodrich, H.R., Cramp, R.L., Gordos, M.A., Franklin, C.E., 2019. Assessment of the effects of microPIT tags on the swimming performance of small-boded and juvenile fish. Fish. Res. 218, 22-28.

Webb, P.W., Sims, D., Schultz, W.W., 1991. The effects of an air/water surface on the fast-start performance of rainbow trout (*Oncorhynchus mykiss*). J. Exp. Biol. 155, 219-226.

Whiterod, N.S., 2013. The swimming capacity of juvenile Murray cod (Maccullochella peelii): An ambush predator endemic to the Murray-Darling Basin, Australia. Ecol. Freshw. Fish. 22, 117-126.

Williams, R.J., Watford, F.A., 1997. Identification of structures restricting tidal flow in New South Wales, Australia. Wetl. Ecol. Manag. 5, 87-97.

Wolter, C., Arlinghaus, R., 2003. Navigation impacts on freshwater fish assemblages: The ecological relevance of swimming performance. Rev. Fish Biol. Fisher. 13, 63-89.

Yamamoto, S., Morita, K., Koizumi, I., Maekawa, K., 2004. Genetic differentiation of white-spotted charr (*Salvelinus leucomaenis*) populations after habitat fragmentation: Spatial–temporal changes in gene frequencies. Conserv. Genet. 5, 529-538.

Zampatti, B.P., Leigh, S.J., 2013. Within-channel flows promote spawning and recruitment of golden perch, *Macquaria ambigua ambigua* – implications for environmental flow management in the River Murray, Australia. Mar. Freshw. Res. 64, 618-630.

Table 1. Experimental design including fish size (total length, mm) for each velocity-depth combination. Fish were divided into two size classes within each velocity-depth combination.

Species	Treatment	Sample Size (n)	Froude number	Reynolds number	Water Depth (cm)	Velocity (ms^{-1})	Small (<200 mm) body size (cm mean ± SD [range])	Large (>200 mm) body size (cm mean ± SD [range])
Maccullochella peelii (Murray Cod)	1	15	0.29	20449	5	0.20	82.5 ± 21 [45 – 115]	291.4 ± 23.5 [264 – 324]
	2	15	0.43	30674	5	0.30	74.1 ± 17.6 [50 – 93]	306.2 ± 18.4 [80 – 324]
	3	15	0.53	37831	5	0.37	155.3 ± 22.7 [130 – 189]	261.9 ± 33.3 [205 – 295]
	4	15	0.30	55955	10	0.30	76.3 ± 17.5 [57 – 97]	298.6 ± 20.6 [268 – 320]
	5	15	0.37	69011	10	0.37	156.6 ± 15 [132 – 181]	239.9 ± 31.8 [200 – 282]
	6	15	0.47	87663	10	0.47	84.0 ± 18.2 [63 – 109]	307.2 ± 17.2 [283 – 322]
	7	15	0.31	95618	15	0.37	64.1 ± 14.1 [48 – 81]	309.6 ± 17.9 [294 – 330]
	8	15	0.39	121461	15	0.47	72 ± 17.1 [53 – 92]	286.6 ± 15 [271 – 305]
	9	15	0.46	144719	15	0.56	75.5 ± 17.7 [57 – 97]	301 ± 13.9 [287 – 319]
Tandanus tandanus (Eel-tail Catfish)	1	16	0.29	20449	5	0.20	111.4 ± 28.4 [88 – 180]	216.3 ± 13.7 [200 – 230]
	2	16	0.43	30674	5	0.30	107.2 ± 31.1 [72 – 185]	244.4 ± 45.6 [203 – 335]
	3	16	0.53	37831	5	0.37	118.5 ± 43.3 [73 – 194]	222.8 ± 14.9 [205 – 244]
	4	16	0.30	55955	10	0.30	109.1 ± 33.8 [73 – 190]	245.9 ± 44.4 [205 – 315]
	5	16	0.37	69011	10	0.37	93.8 ± 19.3 [72 – 122]	262.4 ± 21.6 [223 – 290]
	6	16	0.57	104449	10	0.56	96.6 ± 7.1 [87 – 106]	254.8 ± 31.8 [221 – 309]
	7	16	0.31	95618	15	0.37	91.9 ± 16.5 [71 – 125]	258.5 ± 25.6 [232 – 302]
	8	16	0.39	121461	15	0.47	106.8 ± 19.1 [85 – 145]	285.3 ± 19 [265 – 322]
	9	16	0.46	144719	15	0.56	132.5 ± 46.9 [85 – 198]	289.3 ± 9.6 [279 – 302]
Bidyanus bidyanus (Silver Perch)	1	20	0.29	20449	5	0.20	101 ± 40 [68 – 188]	242 ± 36.5 [200 – 300]
	2	20	0.43	30674	5	0.30	96.6 ± 35.6 [69 – 199]	226.8 ± 26 [205 – 289]
	3	20	0.53	37831	5	0.37	99.1 ± 29.4 [70 – 180]	256.6 ± 31.4 [229 – 314]
	4	20	0.30	55955	10	0.30	83.6 ± 16.2 [63 – 120]	245.6 ± 30.7 [205 – 302]
	5	20	0.37	69011	10	0.37	101.7 ± 49 [61 – 197]	229.9 ± 17.9 [205 – 255]
	6	20	0.57	104449	10	0.56	94.5 ± 38.0 [60 – 199]	267.6 ± 25.3 [234 – 321]
	7	20	0.31	95618	15	0.37	84.6 ± 13.9 [67 – 116]	271.1 ± 27.2 [235 – 307]
	8	20	0.46	144719	15	0.56	89.6 ± 17.9 [60 – 119]	270.5 ± 19.9 [245 – 310]
	9	20	0.57	178315	15	0.69	94.5 ± 18.8 [62 – 135]	258.7 ± 19.71 [220 – 287]

Figures

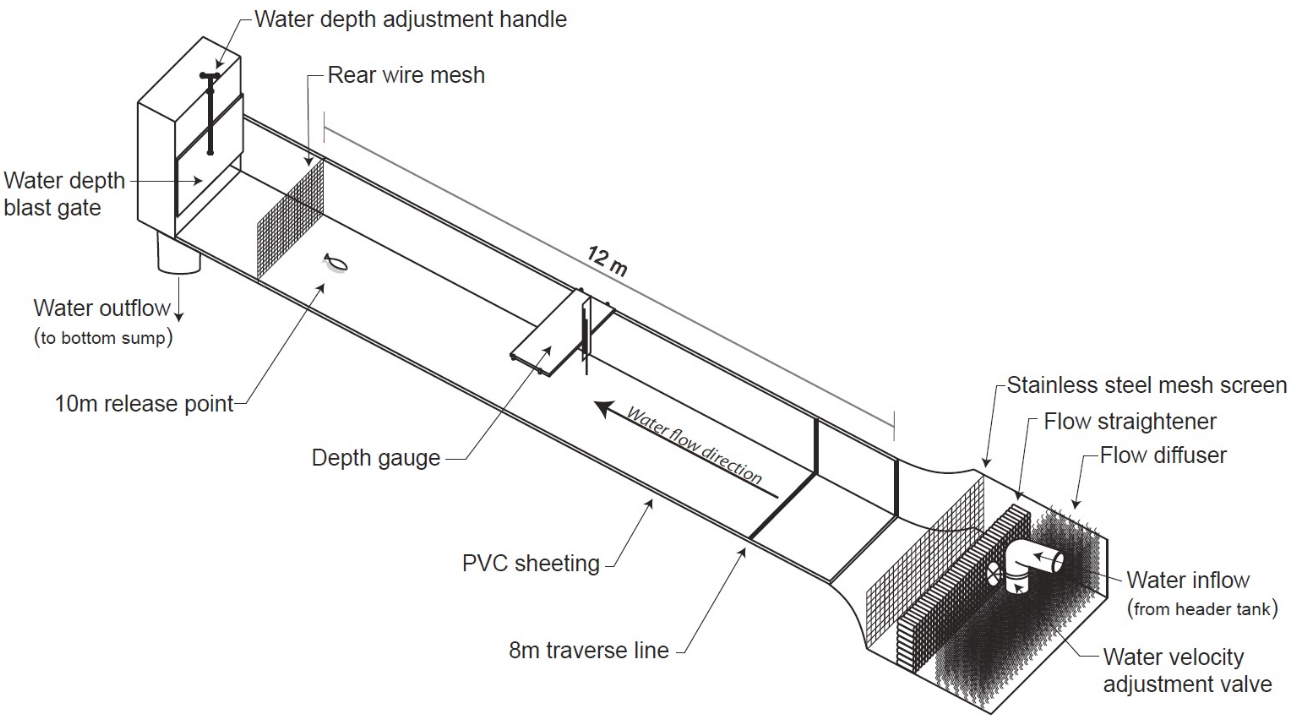

Figure 1. Cross sectional diagram of the 12m flume design. *Note: figure is not to scale.*

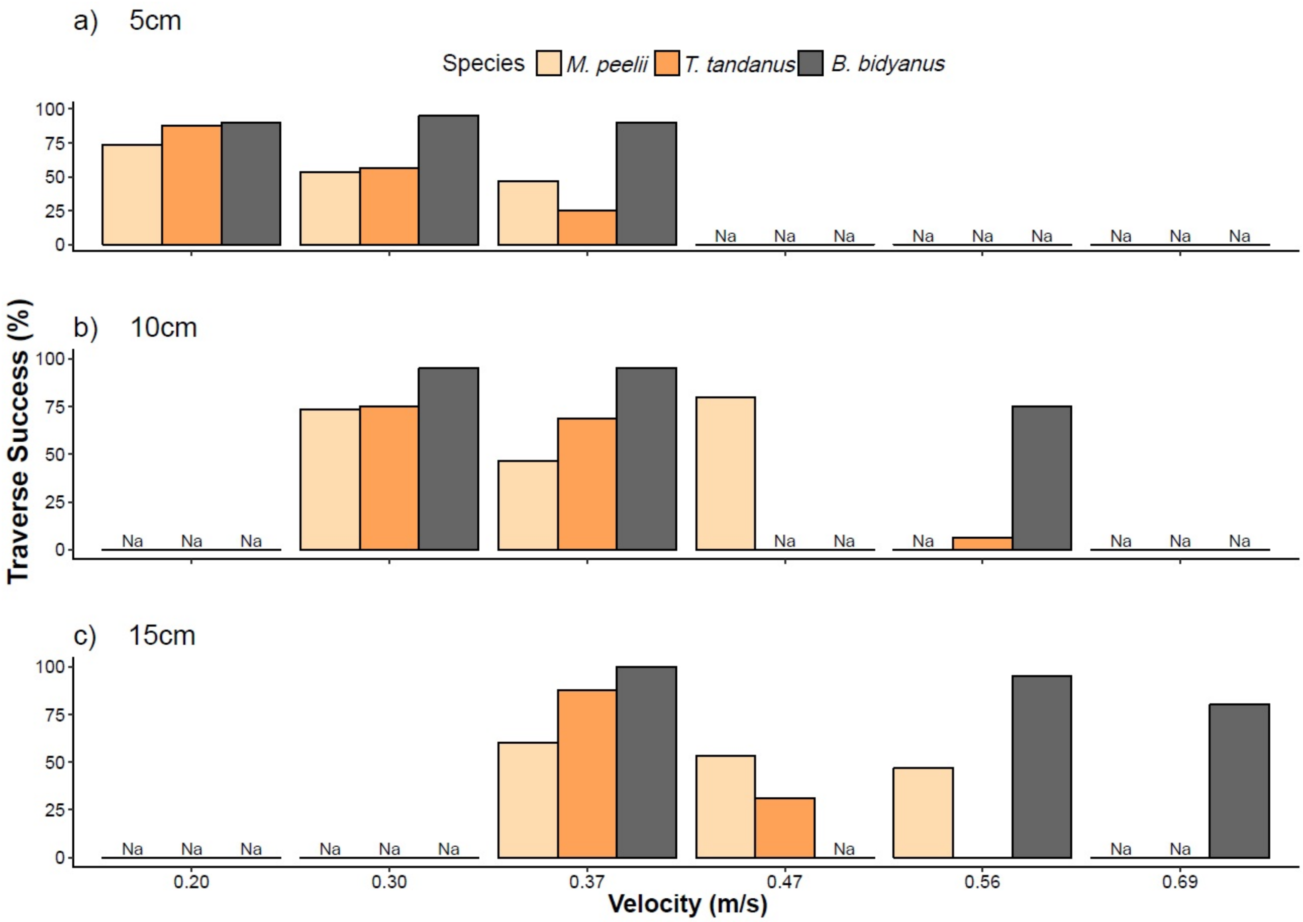

Figure 2. The traverse success of *M. peelii*, *T. tandanus* and *B. bidyanus* with respect to each individual water velocity treatment at a) 5 cm, b) 10 cm and c) 15 cm water depths. *Note: Froude number for all treatment trials was deemed to be subcritical, Fr <1.*

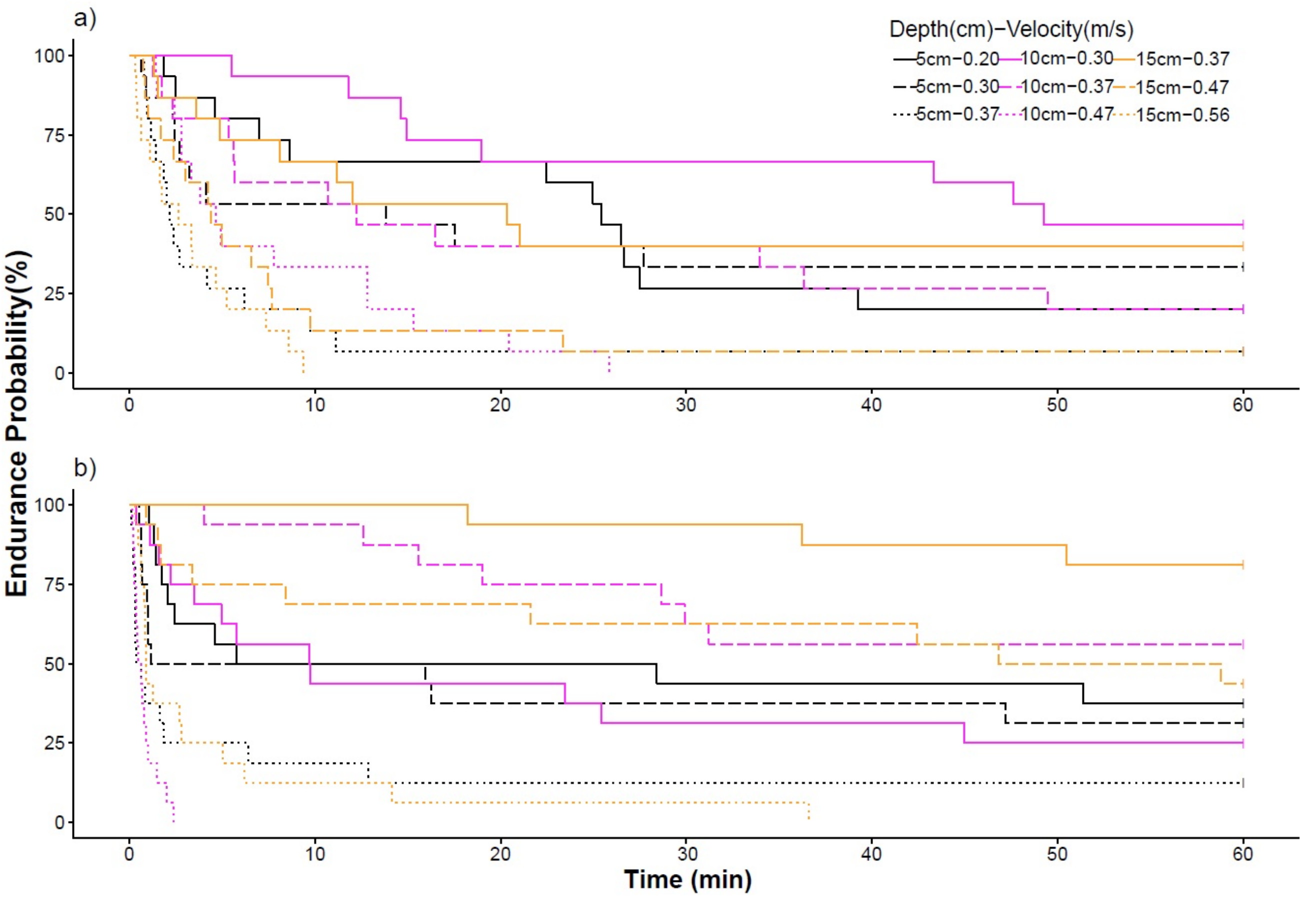

Figure 3. Endurance probability curve over time for a) *M. peelii* and b) *T. tandanus* with respects to depth and velocity. Here, censored observations are marked as "|" and represents fish that have swum for > 60 minutes without fatiguing. Sample size for each depth and velocity treatment for *M. peelii* is n = 15 and n = 16 for *T. tandanus*. The endurance probability was calculated using the Kaplan-Meier method (Bewick *et al.* 2004) and can be defined as the probability of enduring a certain time period based on the proportional of individuals having already endured up to that certain time period without fatigue. NB: the 95% confidence intervals were intentionally left out from the figure for graphical clarity.

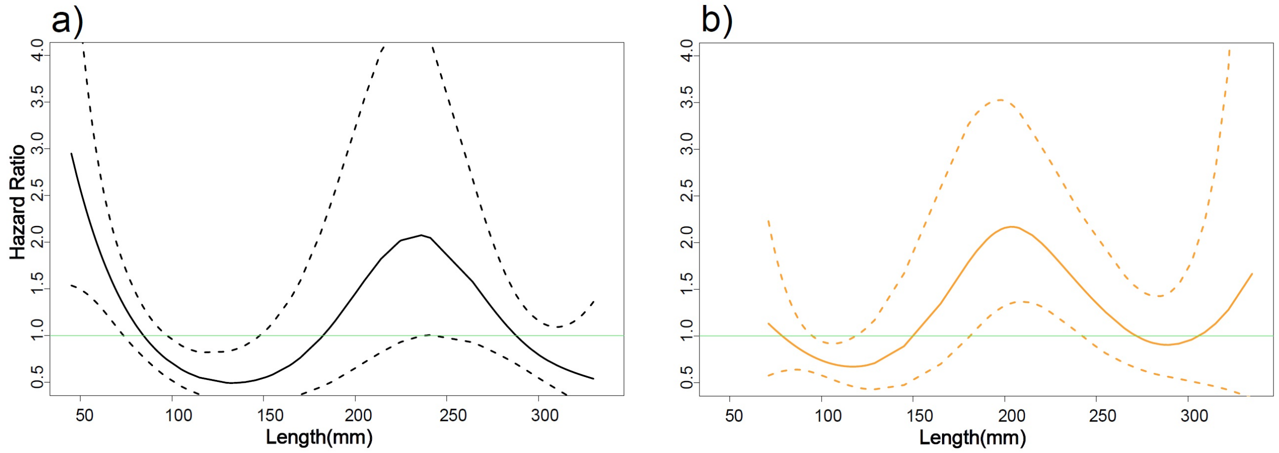

Figure 4. The overall effect of fish size (total length) on the likelihood of fish fatigue of a) *M. peelii* and b) *T. tandanus* when adjusted for the effects of water depth and velocity. Shown are the estimated hazard ratio values ± 95% confidence interval. Any value above the green reference line represents an increase to the risk of a fish fatiguing when compared to the mean baseline fish length, any value below the green line is a reduced risk of a fish fatiguing. The distance from the green line represents the magnitude of increase or decrease in risk of fatigue. The hazard ratio can be calculated as: $HR = exp\{p\text{-}spline(\text{size})\}$, where $p\text{-}spline(\text{size}) = \sum^{I}_{i=1} \beta_{i} \cdot f_{i}(\text{size})$.

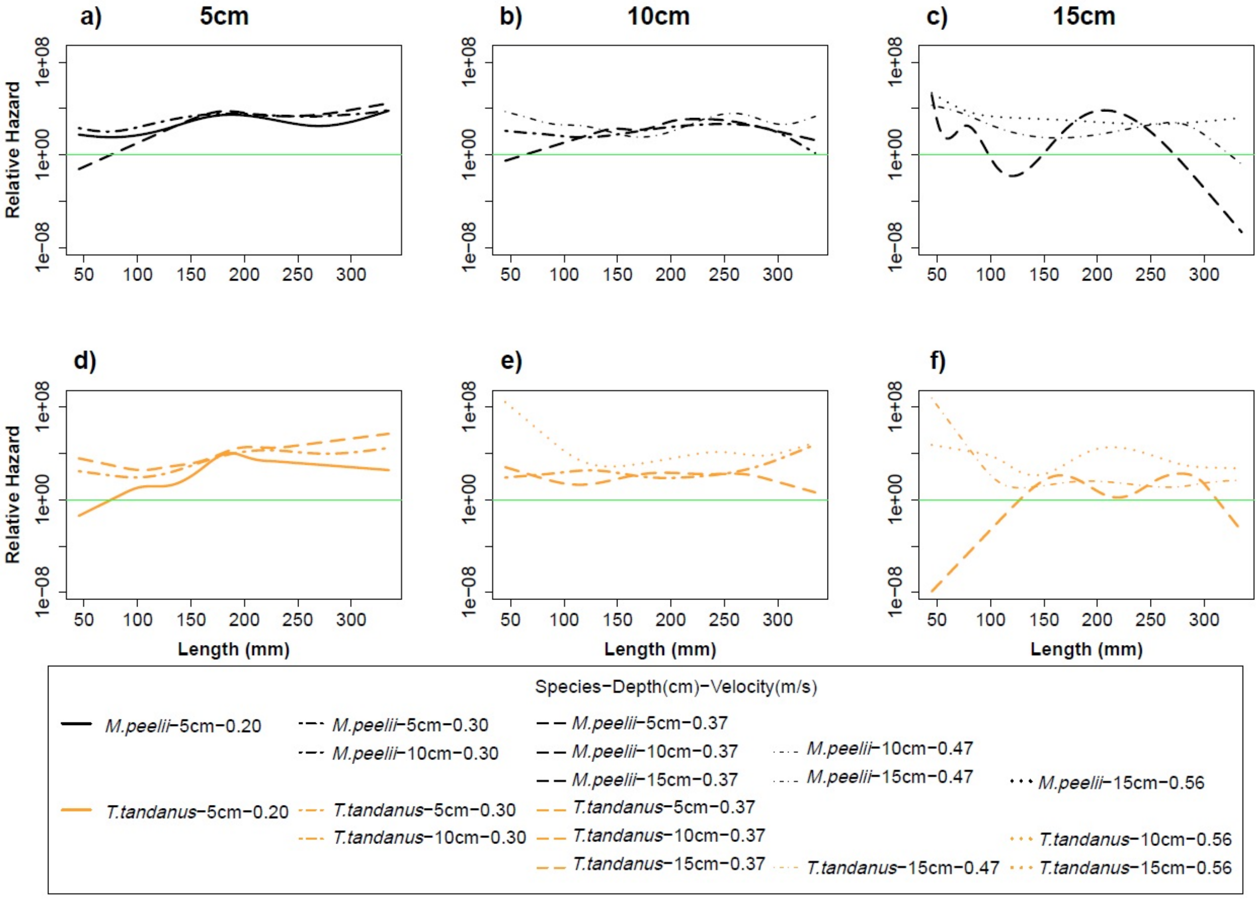

Figure 5. The effect of fish size (body length, mm) and water velocity on the relative hazard (risk) of fatiguing for *M. peelii* (black) in a) 5 cm, b) 10 cm and c) 15 cm deep water. The relative hazard (risk) of fatigue for *T. tandanus* in d) 5 cm, e) 10 cm and f) 15 cm deep water is marked in orange. Different water velocities/ depth configurations are denoted by line type. Here, any value above the green reference line represents an increase relative hazard of fish fatiguing when compared to the mean baseline fish length, any value below the green line is a reduced risk of a fish fatiguing. NB: the term relative hazard (calculated as HR) is used here instead of hazard ratio to denote the qualitative interpretation of the interactive effects and the direction of the risk. The 95% confidence intervals were intentionally left out from the figure for graphical clarity.

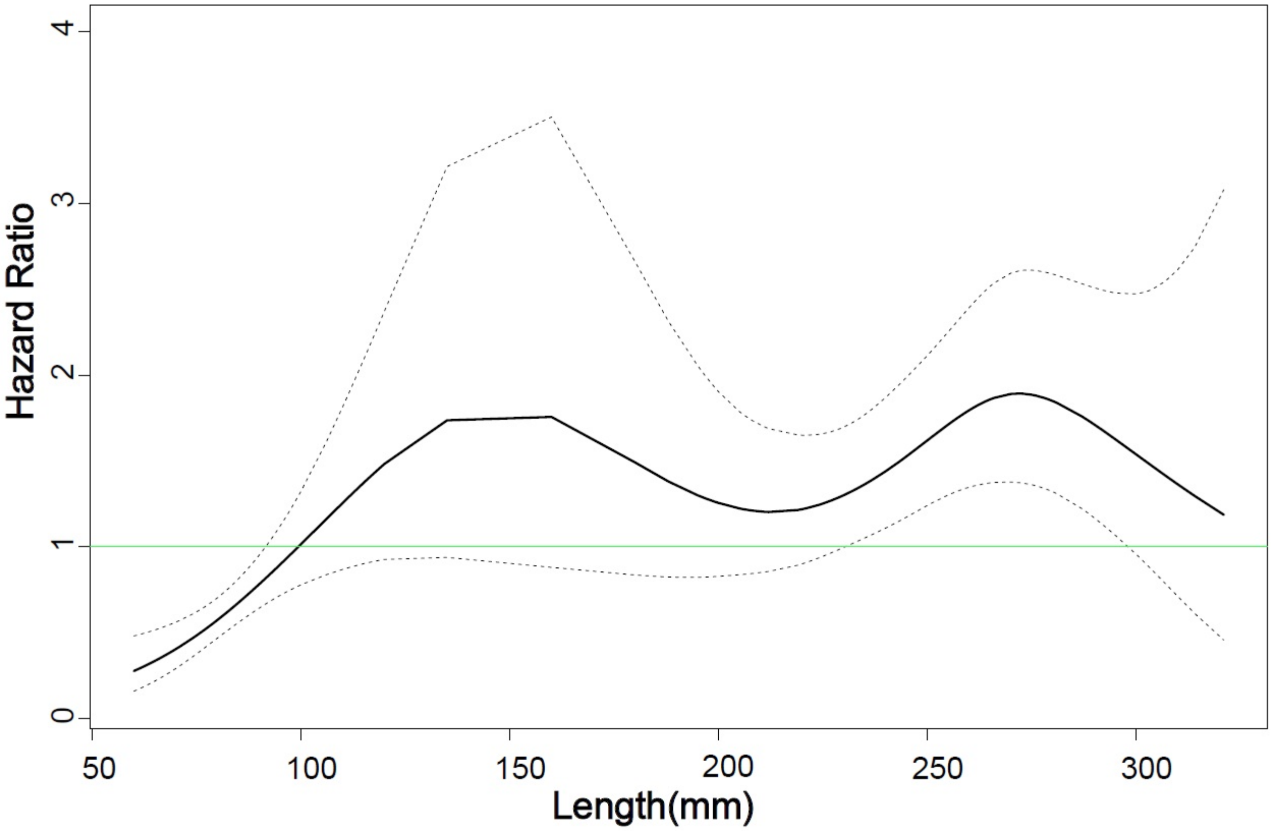

Figure 6. The average effect of fish size (total length) on the likelihood of *B. bidyanus* traversing up 8 m of the channel. Shown are the estimated hazard ratio values ± 95% confidence interval. Any value above the green reference line represents an increase to the risk of a fish fatiguing before traversing the channel when compared to the mean fish length, any value below the green line is a reduced risk of a fish fatiguing. The distance from the green line represents the magnitude of increase or decrease in risk of fatigue.

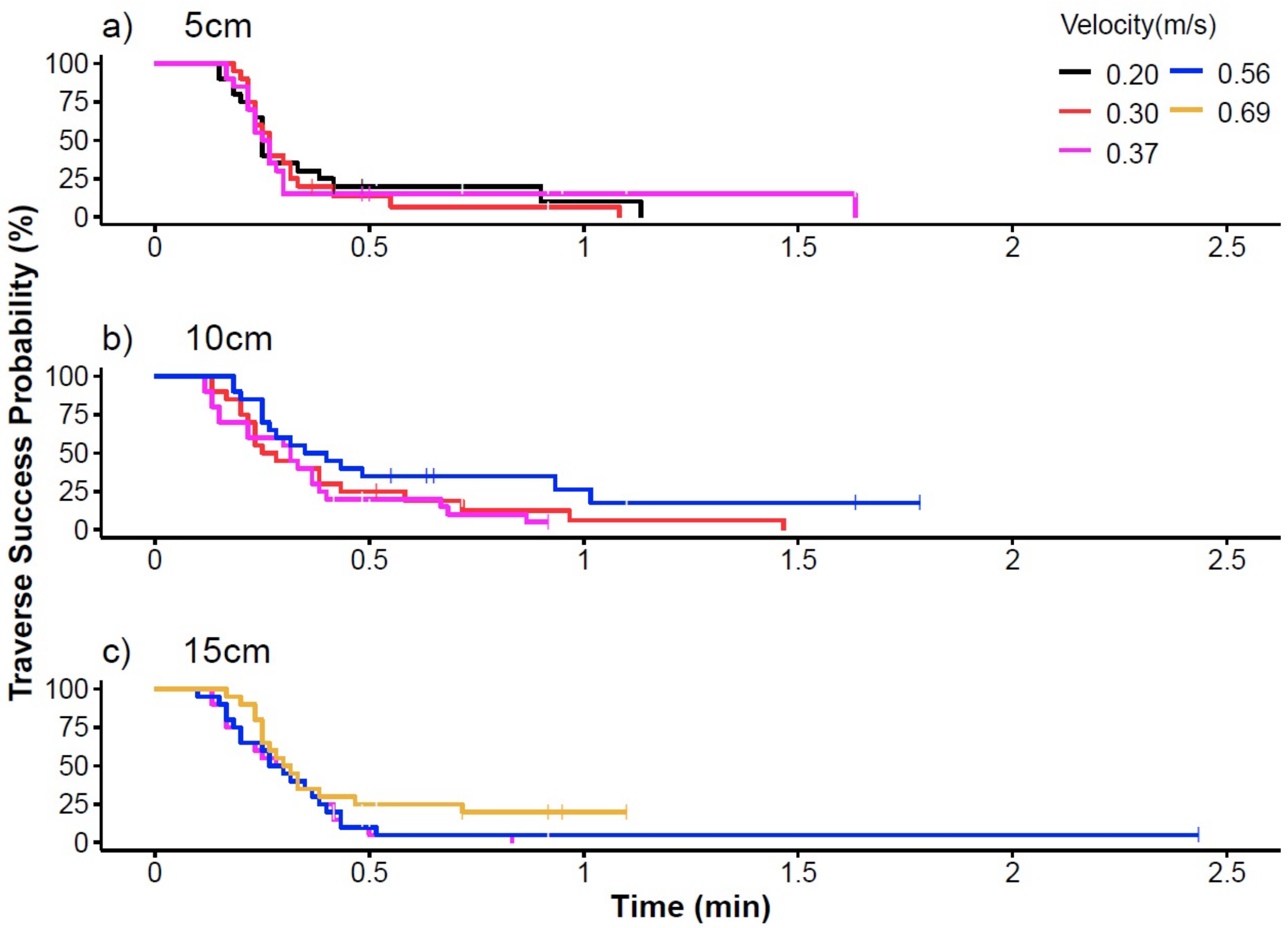

Figure 7. Traverse success probability curve over time for *B. bidyanus* with respect to water depth and velocity. Here, censored observations, denoted by "|", represent fish that have failed to traverse the channel and subsequently fatigued. Sample size for each depth-velocity treatment is n =20. The traverse success probability was calculated using the Kaplan-Meier method (Bewick *et al.* 2004) and can be defined as the probability of 'successfully traversing 8 m' in a certain time period based on the proportional of individuals having already 'successfully traversed 8 m' up to that certain time period without fatigue. NB: the 95% confidence intervals were intentionally left out from the figure for graphical clarity.