A Comparison of Metrics to Evaluate the Effects of Hydro-facility Passage Stressors on Fish

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Complete List of Authors:	Colotelo, Alison; Pacific Northwest National Laboratory Goldman, Amy; Pacific Northwest National Laboratory Wagner, Katie; Pacific Northwest National Laboratory Brown, Richard; Pacific Northwest National Laboratory Deng, Z.; Pacific Northwest National Laboratory Richmond, Marshall; Pacific Northwest National Laboratory
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**A Comparison of Metrics to Evaluate the Effects of Hydro-facility Passage
Stressors on Fish**

Alison H. Colotelo*, Amy E. Goldman, Katie A. Wagner, Richard S. Brown, Z. Daniel Deng, Marshall C.
Richmond

Earth Systems Science Division, Pacific Northwest National Laboratory, Richland, Washington, 99352
USA

*Corresponding Author: 902 Battelle Boulevard, MSIN K7-70, Richland, Washington, 99352; Phone:
509-371-7248; Fax: 509-371-7397; Email: Alison.Colotelo@pnnl.gov

Draft

Abstract

Hydropower is the most common form of renewable energy, and countries worldwide are considering expanding hydropower to new areas. One of the challenges of hydropower deployment is mitigation of the environmental impacts including water quality, habitat alterations, and ecosystem connectivity. For fish species that inhabit river systems with hydropower facilities, passage through the facility to access spawning and rearing habitats can be particularly challenging. Fish moving downstream through a hydro-facility can be exposed to a number of stressors (e.g., rapid decompression, cavitation, blade strike and collision, turbulence, and shear forces), which can affect fish survival in direct and indirect ways. Many studies have investigated the effects of hydro-turbine passage on fish; however, the comparability among studies is limited by variation in the metrics and biological endpoints used. Future studies investigating the effects of hydro-turbine passage should focus on using metrics and endpoints that are easily comparable. This review summarizes four categories of metrics that are used in fisheries research that have application to hydro-turbine passage (i.e., mortality, injury, physiological markers, behavior) and evaluates them based on several criteria (i.e., resources needed, invasiveness, comparability among stressors and species, and diagnostic properties). Additionally, these comparisons are put into context of study setting (i.e., laboratory vs. field). Overall, injury and physiological markers are ideal for studies in which there is a need to understand the mechanisms of effect, whereas behavior and mortality metrics provide information on the whole body response of the fish. The study setting strongly influences the comparability among studies. In laboratory-based studies, stressors can be controlled by both type, intensity, and duration, allowing for easy comparisons among studies. In contrast, field studies expose fish to realistic passage environments but the comparability is limited. Based on these results, future studies, whether lab or field-based, should focus on metrics that relate to mortality for ease of comparison, while some diagnostic capability is important for furthering the science.

Keywords: hydropower, fish, survival, metrics, injury

Introduction

Hydropower is the most heavily relied upon source of renewable energy in the world, accounting for over 16% of total electricity generation (International Energy Agency 2015). The reliance on hydropower is increasing with pushes to develop additional hydro-systems, including in some of the largest rivers in the world (e.g., Amazon River [South America], Mekong River [Asia], Congo [Africa]; Zarfl et al. 2015, Winemiller et al. 2016). With the prospective expansion of hydropower across the globe, it is critical to understand the environmental implications, including the potential for negative effects on fish during downstream hydro-facility passage.

As fish pass downstream through hydro-facilities, they can be exposed to a number of stressors (e.g., rapid decompression, cavitation, blade strike and collision, turbulence, and shear forces [Čada 2001]). The magnitude of the stressor to which a fish may be exposed is dependent upon a number of variables including turbine design and operations. For example, the Electric Power Research Institute (EPRI) found that the ratio of the fish length to blade thickness is an important factor in determining the likelihood of survival for fish struck by a turbine blade and that survival increases as the ratio of fish length to blade thickness decreases (EPRI 2007, 2008, 2011). With regard to turbine operations, higher discharge rates through turbines typically result in lower and more variable nadir pressures (the lowest pressure that a fish experiences during passage) than those recorded in turbines operated using lower discharges (Deng et al. 2010; Trumbo et al. 2014), thus affecting the range of pressures a fish experiences. Once a turbine is installed within the dam, its design cannot be altered, but because turbine operations change depending on the river conditions (e.g., percentage spill, headgate configuration, etc.), the magnitude of stressors can fall across a range.

The type of response from an individual fish exposed to a stressor in a hydro-facility environment varies and is dependent upon the type, intensity, and duration of the stressor (defined as an external stimulus or

event that causes a physical, chemical and/or mental response inside of the body) as well as structure of the fish (see Coutant and Whitney 2000 for a summary of fish behavioral responses). For example, Brown et al. (2014) summarized the various traits that can influence the susceptibility of fish to barotrauma due to rapid changes in pressure. Although the presence of an inflated swim bladder (or other undissolved gases in the body) was determined to be the most important variable in predicting the susceptibility of a fish to barotrauma, the type of swim bladder, depth of neutral buoyancy, life stage, migration patterns, and structural integrity can also be important (Brown et al. 2014). In many river systems, a variety of species and life stages of fish may be entrained into hydro-facilities. Therefore, multiple thresholds for injury or mortality may need to be considered in the design of hydro systems to account for the diversity of species and their respective susceptibility to effects from hydro-facility passage.

Many field and laboratory based studies have been conducted to evaluate the effects of exposure to hydro-facility passage (e.g., survival studies) or isolated stressors on fish (e.g., rapid decompression, blade strike). However, methodology and biological endpoints can vary considerably among these studies, which makes it difficult to compare results across stressors and a wide variety of species (Pracheil et al. 2016). Therefore, there is a need to identify a metric(s) that can be used to assess the effects of exposure to the variety of different stressors inside a hydro-facility environment. Use of a standard metric that provides a quantitative value for the physiological state of a fish following exposure to any stressor or combination of stressors will allow for integration of dose-response data across studies. Additionally, few studies (see Brown et al. 2012, Colotelo et al. 2012 for barotrauma relationships to simulated turbine exposures) have evaluated fish along a broad range of exposure doses (i.e., magnitude of the stressor), which limits the transferability of results to other hydro-facilities. For that reason, studies should be conducted to encompass a wide range of doses that fish may encounter during downstream passage through hydro-facilities.

The objective of this manuscript was to compare and evaluate the metrics available to assess the effects of hydro-facility passage on a wide variety of species and life history stages of those species. This review first summarizes the stressors that fish may experience during downstream passage at hydro-facilities, including hydro-turbines, spillways, sluiceways, and other passage routes. Next, is a summary of various metrics (standards for measuring or evaluating) used to assess the health of fish and how these metrics have or could be applied to evaluate the response that fish have to stressors during passage at hydro-facilities. We conclude by discussing the advantages and limitations of the metrics presented based on their cost, invasiveness, diagnostic properties, and comparability among stressors and species. We will also discuss the applicability of the available metrics for use with design tools such as the Biological Performance Assessment (BioPA) tool (Richmond et al. 2014) and evaluation tools such as Hydropower Biological Evaluation Tools (HBET; Hou et al. 2015) and make recommendations on the metrics that should be used for future studies that examine the effects of downstream passage through hydro-facilities.

Stressors

Rapid Decompression

Barotrauma is defined as trauma or injuries to body tissues that result from changes in barometric pressure (Brown et al. 2012). Fish that pass through hydro-turbines or over spillways are at risk for rapid decompression either due to nadir pressures on the backside of the turbine blade or by being quickly brought up to surface pressure in the tailrace. The large decrease in pressure leads to an inversely proportional increase in the volume of any existing gases within the body cavity. This relationship is governed by Boyle's Law, which states that $P_1 \times V_1 = P_2 \times V_2$ (where P_1 and V_1 are the initial pressure and volume and P_2 and V_2 are the resultant pressure and volume; Brown et al. 2012). Based on this relationship, as the ratio of pressure change (acclimation pressure / exposure [nadir] pressure) that a fish experiences during passage increases, the volume of undissolved gases also increases, which leads to greater potential for barotrauma. Common injuries that are observed in fish exposed to rapid

decompression associated with hydro-turbine passage include swim bladder over-inflation or rupture, emboli in the fins and gills, hemorrhaging, and exophthalmia (as reviewed by Brown et al. 2014).

Cavitation

Cavitation is the formation of vapor cavities in a liquid (e.g., river water) and can occur when the liquid is subjected to rapid decompression. These vapor cavities, or bubbles, can collapse violently, resulting in a shock wave that can damage both fish and turbine structures. In the past, it was believed that cavitation was the most important source of injury and mortality for juvenile fish that passed through hydro-turbines (Muir 1959). However, further research has shown that cavitation occurs when the pressures drop below the vapor pressure of water (approximately 2 kPa), which is rare for many turbines (Abernethy et al. 2001). While turbines are typically designed to minimize the likelihood of cavitation, fish may still be exposed to cavitation. Muir (1959) documented that cavitation can result in hemorrhaging of the eyes and gill plates and even death, but the response of fish to these forces has been largely unstudied (Pracheil et al. 2016).

Blade Strike and Collision

Fish that pass through hydro-turbines must pass by turbine blades as the turbines rotate to produce electricity, and thus are at risk of being struck by the leading edge of the turbine blade. The likelihood of blade strike is dependent upon many physical and biological factors including turbine type, the number of blades, blade length, blade width relative to fish length, turbine runner rotational speed, force of the impact, fish length, and fish tissue sensitivity and flexibility (summarized in Deng et al. 2007). The orientation of fish that are struck by turbine blades is largely unknown; however, many laboratory studies have focused on blade impact with the broad side of the fish as it has the greatest surface area and so the highest likelihood of occurrence (Cook et al. 2003). Injuries typically observed in fish exposed to blade strike are scale loss, lacerations, abrasions, hemorrhaging, bruising, and eye damage (Turnpenny 1998).

In addition to coming into contact with the turbine blades, fish may collide with other hydro-facility structures during downstream passage. Structures include the turbine runner, wicket gates, stay vanes, draft tubes, and spillways. Collision may occur due to disorientation of the fish or the inability of the fish to swim against the current and being pushed into structures. Due to the similarities between injuries from blade strike and collision, many studies that have investigated the effects of blade strike on turbine-passed fish have likely included injuries due to collision with other structures in their analyses. As such, many of the injuries typically observed in fish exposed to blade strike are relevant to those that collide with other structures in the hydro-turbine environment (Cook et al. 2003).

Turbulence

Turbulence during hydro-facility passage can influence the behavior of a fish, potentially resulting in disorientation. This disorientation may increase the likelihood of the fish colliding with structures in the turbine, and may also influence their ability to survive the tailrace environment (Čada 2001). Specifically, disorientation of a fish may result in increased susceptibility to predation in the tailrace (Blackwell and Juanes 1998; Neitzel et al. 2000). Although the direct effects of turbulence may not be lethal, there is little known about turbulence intensity (Gordon et al. 1992) and how that relates to a fish's ability to navigate through the hydro-turbine and tailrace environments.

Shear Forces

Shear forces during downstream passage at hydro-facilities are created when two masses of water, that are moving at different velocities, intersect or when water slows near a solid structure, a turbine blade, blade runners, or wicket gates (Čada et al. 2007). The effects of shear forces on turbine-passed fish have not been extensively studied for many species; however, effects are species-dependent and seem to vary with the orientation of the fish (Neitzel et al., 2004). Injuries can include descaling, lacerations, bruising, damage to eyes and operculum, and decapitation (Deng et al. 2005; Čada et al. 2007).

Metrics

Mortality

Mortality is one of the most common and simplest metrics used to assess how fish respond to downstream passage through hydro-facilities. Depending on the requirements of the study, mortality can be assessed immediately to determine the direct effects of exposure or after a prescribed period of time to include any delayed mortality. Monitoring mortality can be minimally invasive to the fish, if fish that survive are permitted to continue their natural life after the experiment is over, and can be easily assessed. However, measuring mortality alone has a low diagnostic capacity because causes of mortality are rarely identified.

Mortality assessments can be conducted in both laboratory and field environments. Researchers conducting laboratory studies have exposed fish to stressors that simulate specific conditions that are found in the large Kaplan turbine environment (e.g., Abernethy et al. 2001, Neitzel et al. 2004, Brown et al. 2012, Deng et al. 2012) or have passed fish through reduced scale turbines to encompass multiple stressors (e.g., Cook et al. 2003). Following controlled exposures in test apparatuses, researchers have either released the fish into a natural river environment (e.g., Brown et al. 2013) or have held fish in a laboratory setting and monitored immediate and delayed mortality (e.g., Davis and Ottmar 2006; Čada et al. 2007; Colotelo et al. 2012). Laboratory studies are controlled, allowing researchers to isolate specific stressors and sources of injury and mortality; however, holding conditions may not be representative of what the fish experience in the wild (e.g., predation).

Field studies require the ability to recapture or remotely monitor tagged fish to determine their fate post-passage. The development of tags that inflate post-passage, such as the Hi-Z Turb'N Tag (Heisey et al. 1992), allows researchers to release fish directly into specific passage structures in the dam and recover those individuals in the tailrace of the dam (e.g., Mathur et al. 1996). These fish can then be examined for the presence of injuries and held to monitor mortality (typically up to 48 hours). Similarly, some studies

have collected fish in the tailrace environment using nets to assess injuries and mortality (e.g., Taylor and Kynard 1985; Navarro et al. 1996). The advantages of recapture studies include the ability to expose fish to multiple stressors and to examine individuals immediately after exposure.

Run-of-the-river fish bearing transmitters can also be used to assess hydro-facility mortality rates. In these studies, fish are typically released several kilometers upstream of a dam to allow them to become acclimated to the river, and are allowed to pass volitionally through the dam, including all available passage routes (known as a virtual release; Skalski et al. 2010). Active telemetry systems (i.e., acoustic or radio) are used in these studies to determine the route of passage and estimate the mortality rates of fish (e.g., Stier and Kynard 1986; Metcalfe et al. 1997; Wertheimer 2007). This method is advantageous as fish are more likely to approach and pass the hydro-facility in similar ways to untagged individuals; limiting bias with study results. However, it has the disadvantage that it is sometimes difficult to parse effects of damage incurred by a particular stressor.

Injury Assessment

In its simplest form, injury assessment after exposure to a stressor is an external and/or internal visual assessment of any injuries present, which frequently includes necropsy, and imaging techniques such as x-rays, magnetic resonance imaging (MRI) and computed tomography (CT) common in medical applications as well as standard photography. Many forms of visual assessment do not require costly instrumentation or analyses and so it is widely used as a way to determine the impacts of different stressors, particularly in studies of barotrauma (e.g., Neitzel et al. 2000, Abernathy et al. 2001, Guensch et al. 2002, Deng et al. 2011, Casper et al. 2012, Brown et al. 2013, Brown et al. 2014); however, results can be difficult to compare across studies, stressors, or species. These difficulties are especially true when a visual assessment is broken down into an intensity model, such as “no injury, minor injury, or major injury,” which can be highly subjective, although strict guidelines can aid evaluation (Guensch et al. 2002, Morrissey et al. 2005, Gravel and Cooke 2008). Additional analytical tools for injury assessment

include fluorescence of damaged epithelial tissue (Noga and Udomkusionsri 2002, Davis and Ottmar 2006) and histological examination (Elston et al. 1997, Brown et al. 2009, Brown et al. 2012), but use of these tools is limited in comparison to the methods described below.

Injury assessment indices can give context to visual assessments and allow for standardization across studies. For example, McKinstry et al. (2007) created a classification system to predict survival outcomes for juvenile Chinook salmon exposed to rapid decompression simulating turbine passage. In accordance with the metric the fish is categorized as mortally injured if it dies immediately or any one of eight injuries are observed: ruptured swim bladder; exophthalmia; blood or bile secretions from the vent; emboli in the gills; emboli in the pelvic fins; or hemorrhaging in the pericardium, liver, or kidney (McKinstry et al. 2007). The eight mortal injuries were shown to be associated with mortality prior to inclusion in the mortal injury metric. Mortal injuries can be identified for a species of interest by systematic exposure to a broad range of the stressor (e.g., range of shear forces that a fish may be exposed to during hydro-facility passage). Exposed fish can be held for a prescribed period of time to determine delayed mortality rates and to determine the types of injuries observed for fish that survived and died during the post-treatment holding period. Once the mortal injuries are validated for the species of interest, the need for prolonged observation to determine delayed mortality is eliminated (Brown et al. 2012).

Another classification system for identifying damage to fish due to rapid decompression was derived by Halvorsen et al. (2012). Their main model (the Fish Index of Trauma [FIT]) was used to quantify damage associated with pile driving and has similar endpoints to the McKinstry et al. (2007) mortal injury metric. The FIT model attaches weighted numerical scoring to the presence or absence of 22 different injuries (e.g., hematomas, hemorrhages, swim bladder rupture; Halvorsen et al. 2012). The FIT model then combines fish damage into a single value, referred to as the Response Weighted Index (RWI), which is the weighted sum of the injuries, with weighting based on the severity of the injury type (Halvorsen et al. 2012). Similar to the McKinstry et al. (2007) mortal injury metric, the FIT model was created using

juvenile Chinook salmon exposed to rapid decompression, and its applicability to other life stages, species, and stressors has not been investigated (Halvorsen et al. 2012).

Injury assessments provide a number of benefits over other methods used to evaluate the effects of hydro-facility passage on fish. In part, this is because visual assessments can be done with limited resources; although in field studies, visual assessment does necessitate recapture (e.g., Stokesbury and Dadswell 1991; Mathur et al. 1996). With strict guidelines to reduce subjectivity and with further development and validation of indices such as the McKinstry et al. (2007) mortal injury metric and Halvorsen et al. (2012) FIT model to a variety of species and stressors, visual assessment indices can provide the widespread comparability needed to be a key metric in assessing the impacts of downstream hydro-facility passage on fish. Injury assessments, however, have the potential to be misleading when multiple sub-lethal injuries compound (as can occur during hydroturbine passage in a field setting) to create a larger impact on a fish, such as mortality, and this fact needs to be kept in mind during the creation and validation of injury indices.

Physiological Markers

Physiological markers can provide insight into both near and long term influences of hydro facility passage on fish. The range of available physiological markers is vast, as outlined by Hasler et al. (2009), and includes measures of endocrine concentration, gene expression, enzymatic activity, protein breakdown products, and blood chemistry, among others (e.g., Matsche and Gibbons 2012). The most common physiological markers are stress indicators derived from blood chemistry, and include measures of osmolality, cortisol, lactate, and glucose (Wendelaar Bonga 1997). Stress indicators, such as plasma cortisol, have been used to compare the responses of fish through different passage structures (e.g., Weber et al., 2002, Mesa et al., 2013). These indicators are advantageous as they can be assessed from a non-lethal blood sample, are quantitative and less subjective than injury assessments, and some can be measured in the field using handheld meters (e.g., glucose and lactate; Stoot et al. 2014).

259
260 Genomic tools, such as polymerase chain reaction (PCR) and microarrays, have been used more widely in
261 recent years to explore the consequences of environmental stressors on individual organisms and
262 populations (, Gerwick et al. 2007, Gonzalez et al. 2007, Talbot et al. 2009, Ingerslev et al. 2010, Qi et al.
263 2011, Pribyl et al. 2012, Andrews et al. 2014). While not used extensively in fish passage research,
264 genomic tools offer the benefit of being able to separate specific responses of interest such as those
265 pertaining to a single organ (e.g., the liver [Gerwick et al. 2007, Talbot et al. 2009, Pribyl et al. 2012]) or
266 a single type of response (e.g., immune system [Ingerslev et al. 2010]). In fish passage research, genomic
267 tools could be used as indicators to determine extent of injury and time to recovery in response to
268 exposure to hydro facility related stressors. For example, Pribyl et al. (2012) quantified expression of
269 genes involved in innate immune responses for black rockfish (*Sebastes melanops*) in response to
270 barotrauma exposure and recovery following exposure.

271
272 Assessments of enzymatic activity and associated breakdown products have similar advantages and
273 disadvantages as genomic tools and can also be used to focus on specific types of responses and injuries
274 (e.g., nutrition, tissue damage, and lipid metabolism [Wagner and Congleton 2004]; traumatic brain injury
275 [Miracle et al. 2009]). Additionally, physiological tools can be used in predicting survival of individuals
276 and populations (Cooke and O'Connor 2010); however, physiological indicators need to be ecologically
277 relevant and linked to fitness. One reason that physiological tools have been underutilized in fish passage
278 research may be the substantial investments required in both time and instrumentation to develop the
279 cause-and-effect relationships.

280
281 The diversity of physiological markers used to assess stress responses highlights the versatility of
282 physiological tools. In addition, with the high emphasis on human research, the utility of physiological
283 tools and markers is growing rapidly. Many of these tools have the potential to be applied to fish-related
284 research. For example, human traumatic brain injury research has identified several diagnostic protein

markers, one of which is alpha II-spectrin. This marker was then used in fish to evaluate traumatic brain injury in fish that passed through a hydro-facility (Miracle et al. 2009). For many of the physiological markers, however, their specificity and high cost to measure makes them less useful in considering a metric that can be applied across numerous studies for a wide variety of species. A single marker may not provide sufficient diagnostic information; instead a suite of physiological markers may be necessary. It is necessary to consider what factors, aside from stress, can alter each physiological marker and influence the signal. The blood chemistry stress indices described above are the most widely applicable, low-cost physiological markers and can be used across species. The direct comparability of different values derived from blood chemistry would need to be validated across species and ages before widespread use could occur. An effort to categorize degrees of stress using ranges of values for each species and age group or linking the responses to an ecologically relevant endpoint, such as mortality, would facilitate the comparability of physiological indicators.

Behavior

Behavioral changes (both voluntary and involuntary responses to stimuli) in fish are often assessed in conjunction with other metrics, particularly as a component of injury or mortality assessments where behaviors such as the loss of equilibrium or lethargy are considered indicators of injury or predictive of mortality (e.g., Neitzel et al. 2000, Guensch et al. 2002, Davis and Ottmar 2006, Diamond and Campbell 2009). Such combinations of metrics can be a successful way to maximize behavioral change information and can be expanded to assess the effects of passage through hydro-facilities.

Diamond and Campbell (2009), for example, created a condition index to predict delayed mortality of red snapper (*Lutjanus campechanus*) that were captured in recreational fisheries. When captured at depth and brought to the surface, these fish are exposed to decompression and can sustain barotraumas similar to those observed in fishes that are exposed to rapid decompression during hydro-facility passage. The condition index is based on the observation of a series of injuries (i.e., bleeding, protruding intestines,

everted stomach, exophthalmia) and behaviors (i.e., problems with submergence within a cage, ability to flap normally, and ability to gill normally). This index is partially similar to those derived solely from the observations of injuries (i.e., mortal injury [McKinstry et al. 2007]; FIT model [Halverson et al. 2012]) in that the variables included in the condition index must be validated for each species and life stage. Once established, such an index removes the need to hold fish for prolonged periods of time to assess delayed mortality.

A recent tool for behavioral assessment is the reflex action mortality predictor (RAMP) curve, which relies on a brief (< 30 seconds) analysis of fish reflexes (e.g., orientation, startle response, gag response, vestibular-ocular response, and body flex upon restraint [Davis 2010]) after exposure to a stressor.

Reflexes are an innate response to an environmental change and have a direct ecological relevance for fitness. Davis (2010) demonstrated that assessment of the reflexes in fish exposed to a specific stressor can be used to predict mortality. Using this method, the reflexes known to be present in an unstressed fish are assessed immediately following exposure to a stressor. These results correspond to a RAMP score (number of reflexes absent divided by the number of reflexes examined) between 0 and 1, with 0 indicating no reflex impairment and 1 indicating complete reflex impairment. This score can be compared to a predetermined RAMP curve to predict the likelihood of mortality for an individual.

The speed, low cost, and whole-organism evaluation of reflex assessment makes it an appealing tool, but like many of the metrics mentioned above, a RAMP curve (including key reflexes to evaluate and the relationship between impairment and mortality) would need to be established for each species and age group of interest and potentially each stressor (Davis 2010).

Behavioral metrics are used less frequently on their own to assess the effects of hydro-facility passage and can include observations of swimming ability, disorientation, lethargy, predator evasion, or other simple observations of change (Mesa et al. 1994; Haro et al. 1998; Tierney and Farrell 2004). Observing behavioral changes can be difficult in field studies, but telemetry tools can be used to track fish and

observe changes in swimming speed or location after stressor exposure (Sprankle 2005; Gravel and Cooke 2008; McMichael et al. 2010).

Discussion

The selection of a metric to evaluate stressor effects involves a number of different factors including logistical constraints (e.g., setting, permitting allowances), desired endpoint (i.e., end use of data, comparability needed), and available resources (e.g., funds, personnel, equipment). All factors that may be involved in the decision making process cannot be covered for all possible situations in this review. Instead, this discussion provides an overview of some of the important concepts and considerations that should be taken into account during the decision process. Although the focus of this discussion is on studies that involve evaluating the stressors associated with hydro-facility passage, these factors are important in the design of experiments with a variety of focus areas.

Study Setting

The setting of a study can dictate or influence which metric is most appropriate to evaluate actual or simulated hydro-facility passage. Laboratory studies are ideal to investigate individual stressors, allowing for precise control of the intensity, duration, and type of stressors to which fish are exposed, and to utilize metrics that require post-exposure evaluation, such as injury assessment, physiological markers, and some behaviors (e.g., reflexes, swimming performance, predator evasion; Table 1). Laboratory apparatuses that replicate specific stressors encountered during hydro-facility passage have successfully been used to test the response of fish in a controlled setting. For example, Stephenson et al. (2010) described an advanced system designed to test the effects of decompression on fish. The Mobile Aquatic Barotrauma Laboratory (MABL) includes sophisticated chambers that expose fish to complex pressure scenarios under a range of water quality parameters (e.g., total dissolved gas, temperature; Stephenson et al. 2010). Sensors in the chambers allow the pressures experienced by the fish to be recorded at a sampling rate of 1,000 Hz, making the experiments repeatable. Furthermore, the MABL has been used to examine the effects of

rapid decompression associated with hydro-turbine passage on a number of different species (e.g., juvenile Chinook salmon [*Oncorhynchus tshawytscha*; Brown et al. 2012]; juvenile Pacific and brook lamprey [*Entosphenus tridentatus* and *Lampetra richardsonii*, respectively; Colotelo et al., 2012]). Similarly, EPRI (2008) designed and constructed a blade strike apparatus that allowed control of the blade design and strike velocity. The system also recorded the fish orientation to the blade (i.e., dorsal, ventral, side) and location of the blade strike along the body (i.e., head, mid-section, and tail region). These types of experiments allow for a better understanding of the individual mechanisms of injury for fish exposed to hydro-facility related stressors. However, the design and construction of apparatuses that generate these exposures can require substantial capital investments and typically only expose fish to one stressor at a time, which may not be representative of what fish experience during downstream movement.

In comparison to laboratory studies, field studies allow researchers to investigate the effects of multiple stressors in a realistic hydro-facility environment, but can be logistically challenging. Additionally, field studies are bound by the operating configuration of the hydro-facility, which likely cannot be altered due to environmental conditions (e.g., discharge) or regulatory needs (e.g., power needs, environmental flow appropriations). Mortality can be easily assessed in these settings, and technological tools, such as telemetry, allow researchers to monitor survival rates post-passage, eliminating the need to recapture fish (Bell and Kynard 1985; Hasler et al. 2009; McMichael et al. 2010). However, other metrics that require post-passage evaluation of the fish (i.e., injury assessment, physiological markers, and behavior) may necessitate the complicated task of recapturing individuals using downstream collection facilities or tools such as the Hi-Z Turb'N Tag. Recapturing individuals likely subjects the fish to more stressors, which may confound results (Taylor and Kynard 1985; Heisey et al. 1992). For example, neutrally buoyant, externally attached transmitters can alter a fish's maximum sustainable swimming speed, which can affect movement through hydro-facilities (Janak et al. 2012). Internal tags have also been shown to increase barotrauma rates thus negatively biasing survival rates (Carlson et al. 2012). Additionally, the surgery process (including anesthesia and implantation/attachment of the tag) may elicit a stress response for the

fish, potentially altering behavior and survival (Jepsen et al. 2002). Thus, field studies may not be appropriate for all metrics. Logistical challenges and the need for resources to track fish survival (e.g., telemetry equipment, personnel) or recapture individuals (e.g., nets, personnel) can often make field studies more expensive than laboratory studies. Additionally, the magnitude and types of stressors that a given fish is exposed to during passage are unknown, limiting the ability to isolate the specific reasons for the response (e.g., mortality, injury, physiological markers, behavioral change). Further complicating field studies, working with protected species involves extensive permitting and monitoring take limits.

One advantage of field studies is the ability to characterize the hydro-facility environment and evaluate passage simultaneously. Environment characterization can be used to better understand the stressors that fish were exposed to during downstream passage and may allow for correlation with the effects observed. Tools, such as the Sensor Fish, have been frequently used to characterize hydro-turbines and spillways and can be used for such comparisons; however, the relationships between the forces captured using the Sensor Fish, the conditions that fish are experiencing, and injury and mortality rates has not been studied extensively (Deng et al. 2010).

Comparability

Although logistical concerns may appear to be the most important factor when designing a study, the comparability of the results is imperative to allow for scientific discussion, to inform hydro-facility decision-making, and to be of use in a modeling context. Studies that expose fish to multiple stressors simultaneously (e.g., field studies, reduced scale turbine laboratory studies) complicate comparability because the magnitude and types of stressors to which the individual fish were exposed are unknown (Table 1). These studies may be most helpful to evaluate passage through hydro-facilities post-construction, whereas, studies that focus on single stressors may be more useful in identifying the mechanisms and thresholds of injury information that is useful in the turbine design process (Richmond et al. 2014).

Comparability of results relies on three elements: the difference in response based on species/life stage, the differences in methodology and endpoint within a given metric, and the ability to understand each metric in a common context. The variation in response to a stressor for different species and life stages requires that metrics be validated as comparable before use. The validation process would need to be done so that the “minimal” and “significant” responses of the metric would be equivalent across different life stages and species despite changes in assessment or categorization based on the unique needs to the subset of fish. For example, a [age, species] fish with a plasma cortisol level of [level] versus a [different age, same species] fish with a plasma cortisol level of [same level] may be in very different states of stress response, but as long as that has been categorized in such a way that the two different plasma cortisol levels correspond to different stress responses or amounts, then the metric can be used across the different life stages and yield information that can be understood in a single context.

Comparability within categories of metrics is derived from standardization within each metric system. The assessment of response must be standardized within a metric so that it can be repeated in other studies knowing that the results are being determined in the same way and can thus be compared to each other. Intra-metric comparability also relies on a way of relating a variety of stress effects (e.g., types of injuries) from different stressors (e.g., pressure change) to each other. Using a categorization system, indices, or pre-determined endpoints help to facilitate comparisons. Mortality is the simplest example because there is a single, clear, non-subjective endpoint regardless of the source or extent of the response. Injury assessment, physiological markers, and behavior all have the ability to be linked to a single endpoint, but in order to utilize a single endpoint, the methodology of the endpoints chosen would need to be consistent and able to be applied in multiple contexts. The McKinstry et al. (2007) mortal injury metric and the FIT model (Halvorsen et al. 2012) are two examples of ways to create consistency within injury assessment metrics. Both indices create systems of assessing injuries independent of the stressor,

which allows the results within the system to be compared to each other. Expanding such indices to a variety of species and life stages can allow for additional comparability.

The final element of comparability is cross-metric comparability so that the evaluation of a fish's response to a stressor can be understood independent of the metric. Cross-metric comparability is predicated on the consistency needed for intra-metric comparability but also relies on validation across metrics. Multiple metrics are needed to evaluate the array of fish responses to stressors due to the inherent nature of different stressors eliciting different responses, which is why cross-metric comparability is also inherently complex and cannot be accounted for in its entirety (e.g., trying to compare the injuries caused by blade strike to a plasma cortisol level after exposure to turbulence). Cross-metric comparability can be assisted by using multiple metrics when possible in order to relate indices or endpoints. If this type of validation is not possible, intensity models (e.g. "minimal" to "significant" effects) can also bridge the understanding between metrics. Similar to intensity models, using a fixed point of reference can also help relate different metric results. A fixed point of reference could be as simple as mortality (e.g., this particular set of behaviors is generally observed before mortality) to give context even if mortality does not occur in a study.

Resources

The resources associated with a study to evaluate the effects of hydro-facility passage on fish are highly variable and depend on many factors. The study cost variability is primarily due to the setting (field or laboratory), evaluation metric chosen, and whether the study relies on prior work that validates the metric for the species and life stage of interest. When evaluating the resources needed for a study design, it is therefore necessary to consider not only the needed resources for each individual component of the study, but also the additive costs created from the specific configuration of choices (e.g., choosing to use physiological indicators in a field study necessitating recapture).

Overall, field studies generally require more resources than laboratory studies due to the additional logistic considerations and the costs of telemetry systems, transmitters, and/or recapturing individuals (Table 1). Telemetry studies also require processing and interpretation of the data collected to identify survival and behaviors of fish, requiring additional resources (McMichael et al., 2010; Figure 1). In comparison, laboratory studies that utilize existing fish housing and testing facilities may require fewer resources (Figure 2).

The metrics evaluated in a given study can require a variety of analytical equipment, extended periods of monitoring, or, for immediate mortality, no additional resources beyond observation (Table 1). The range of metric-derived resources needed, therefore, typically spans from immediate mortality when no post-treatment monitoring is required (less resources) to physiological markers that require tissue collection supplies (e.g., blood and organ tissue), assay supplies, laboratory equipment, and additional labor to process and analyze collected samples (more resources). The costs for the physiological resources can quickly increase with large sample sizes, the need for pre-processing samples, and the need to further develop assays if not commercially available. Delayed mortality monitoring, behavior observations, and injury assessment all require post-treatment observations and fall in the middle of the range. However, reliance on additional laboratory equipment (e.g., imaging technology, respirometers) can increase the resources needed for injury assessment and behavior observations.

Invasiveness

It may not be prudent to lethally sacrifice individuals in a given study, particularly when working with species of conservation concern or limited availability (Cooke 2008). This is an important consideration in field studies where individuals that do not die as a result of the treatment may be released back into the natural environment. For example, injury metrics often require necropsies, whereas many physiological and behavior metrics can be sub-lethally assessed, allowing wild fish to be released. Furthermore, if using telemetry, mortality and behavior can be assessed without resampling individuals, but telemetry

requires surgery before fish are released. For laboratory studies, the importance of invasiveness may relate more to the resources available for a given study (e.g., personnel, equipment, funding, time) as laboratory animals are generally not permitted to be released into a natural environment. Behavior, injury, and physiological markers can all require extensive post-treatment assessments of fish and assessment of collected tissues (e.g., histology, assays).

Diagnostic Properties

Diagnostic examinations of fish post-exposure can be vital to understand the mechanisms of injury/mortality. Injury assessments and physiological indicators allow researchers to isolate specific organs or systems of the body that are affected by exposure (Table 1). Physiological indicators measure specific physiological responses to a stressor (e.g., gene expression, blood chemistry). Similarly, necropsies allow identification of specific types of injury (e.g., abrasions, lacerations, bruising, descaling) and can isolate those to specific organs. For example, Brown et al. (2012b) identified through necropsy and x-rays that the expansion and rupture of the swim bladder was the primary source of injuries for juvenile Chinook salmon exposed to rapid decompression associated with hydro-turbine passage. This information is important in understanding the mechanisms of injury for juvenile Chinook salmon, and it can also be useful for predicting the susceptibility of other species to injury/mortality from rapid decompression and may help with prioritization of other species to examine. Based on the collected information, Brown et al. (2012b) suggested that fish that lack undissolved gases within their body cavity (i.e., swim bladders), such as lamprey, would not be susceptible to rapid decompression. This theory was supported when juvenile brook lamprey were exposed to a rapid decompression that would have resulted in 97% mortality for juvenile Chinook salmon and did not sustain any injuries (Colotelo et al. 2012). Metrics that provide detail on the mechanisms of the response are particularly useful for studies that focus on single stressors; however, they may provide more information than required.

For studies not requiring extensive diagnostic detail, metrics involving whole body responses, such as mortality and behavior, may be ideal. One advantage of measuring whole body responses is that fish can be exposed to multiple stressors and the cumulative effect can be measured. For example, fish that pass through hydro-turbines may be exposed to multiple stressors (e.g., blade strike, rapid decompression, shear forces). Exposure to the individual stressors may not have negatively affected the fish; however, the cumulative effects may be enough to alter the behavior or survival of an individual. Evaluations of the performance of hydro-facilities often focus on examining the whole body responses, namely survival (Skalski et al. 2010). In these studies, fish are released upstream of a hydro-facility and survival is monitored through the downstream reaches of the river. In these scenarios, it may be too difficult to link the specific types of effects (e.g., injuries, physiological responses) with the types and magnitude of the stressors, since there are infinite possibilities.

Evaluation of Metrics for Use with the Biological Performance Assessment Tool

The process to design and manufacture a new hydro-turbine can be costly and require several years to complete. Therefore, it is important during the design process to understand the relationship between design characteristics, the physical forces that fish may experience during passage, and the likelihood of negative effects for those fish (Brown et al. 2012). To assist in this role, tools, such as the BioPA tool, can be used to compare the biological performance of multiple turbine designs prior to construction (Richmond et al. 2014). The BioPA tool uses simulated data from computational fluid dynamics (CFD) models to compute a suite of biological performance indicators (based on the probability of exposure to stressors from streamtrace sampling) for each turbine design. This allows engineers and biologists to identify designs and operations that will minimize the extent and magnitude of stressors that fish may be exposed to during passage (Richmond et al. 2014). The BioPA tool relies on information collected from a variety of sources, including the relationships between the magnitude of the stressors (dose) that occur within hydro-turbines and the responses of the fish that encounter these stressors as determined in laboratory experiments. While the BioPA tool currently focuses on hydro-turbine designs, the general

approach may also be applicable to the design of other structures within a hydro-facility, including spillways, sluiceways, and other routes that water and fish may pass through.

The dose-response relationship for juvenile Chinook salmon exposed to rapid decompression and evaluated using the mortal injury metric, as described by Brown et al. (2012a), has been successfully integrated into the BioPA tool allowing for the successful comparison of new turbine designs for Priest Rapids Dam on the Columbia River (Richmond et al. 2013). However, there is a need to integrate more dose-response relationships that include additional species and stressors to expand the applicability of the BioPA tool. One of the most prominent reasons for the lack of integration of other studies is the lack of comparability among the biological endpoints used in different studies. Many of the studies that have evaluated the effects of hydro-facility passage (or examined specific stressors) have used various metrics. Additionally, the physical conditions that fish are exposed to can vary substantially (e.g., rate of pressure change, speed of blade strike) or may not be representative of the conditions that occur during hydro-facility passage. Therefore, there is a need to identify priority metrics and provide guidance for future studies with results that may be integrated into the BioPA tool.

The BioPA tool evaluates the probability of exposure to a certain dose of a stressor; if the dose-response relationship is known for the species and stressor, the likelihood of negative effects can be calculated (Richmond et al. 2014). Since these analyses are linked to specific stressors, studies that focus on individual stressors would be easily transferred. Thus laboratory based studies would be the ideal study setting to determine dose-response relationships. In addition, the dose-response relationship is critical for evaluation tools such as HBET. With the known dose-response relationship, HBET can estimate likelihood of injury to fish that might experience the exposure observed by measurement technologies such as Sensor Fish, leading to reduction of the use of live fish and cost during the evaluation stage.

The evaluations of hydro-facilities by regulatory agencies commonly focus on survival rates of fish that pass through. Typically, the Federal Energy Regulatory Commission requests studies that evaluate entrainment rates of fish through the facility during the licensing process for hydro-facilities. These studies are generally coupled with survival studies to estimate the impacts that entrainment has on fish populations (FERC 1995). Similarly, specific dam passage survival rates are required in the lower Snake and Columbia rivers of the Pacific Northwest of the United States at hydro-facilities operated by the USACE (NOAA 2008). For this reason, dose-response relationships included in the BioPA tool should use mortality as a metric or should use an index that can tie to mortality (e.g., mortal injury metric [McKinstry et al. 2007], condition index [Diamond and Campbell 2009], RAMP [Davis 2010]). Indices have the added benefit of incorporating diagnostic information about the mechanisms and thresholds of damage which is useful in identifying biological design criteria for new hydro-turbines (Trumbo et al. 2014).

Indices have additional features that make them good candidates for studies that can be incorporated into the BioPA tool. Once the critical injuries or behaviors are identified and validated as predictors of mortality, long term holding of individuals is no longer required, reducing the resources needed (i.e., post-treatment holding). Furthermore, critical injuries or behavior provide insight on important variables to look for when establishing dose-response relationships for other species. This presents an established methodology to identify critical variables and increases the comparability among studies. Based on this framework, future studies that establish dose-response relationships for fish exposed to hydro-turbine related stressors should focus on indices that relate to mortality but also possess diagnostic properties to help identify mechanisms and thresholds of damage.

Conclusion

With the diversity of hydro-facility passage-related effects on fish and the many factors that influence study design, it is unsurprising that such a wide variety of metrics has been created or adapted to

585 hydropower by the scientific community. The lack of comparability among metrics, however, hinders
586 applications of results and deters incorporation into design tools such as the BioPA and evaluation tools
587 such as HBET. All metrics have advantages and limitations that should be thoroughly evaluated before a
588 study is performed, however, as much as possible, a high degree of comparable metrics between studies is
589 critical for future research. Increased comparability of metrics will allow the results of future studies to
590 be more accessible for scientific discussion, informative for hydro-facility decision-making, and critical in
591 identifying mechanisms and thresholds of damage.

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References

Abernethy, C.S., Amidan, B.G. and Čada, G. 2001. Laboratory studies of the effects of pressure and dissolved gas supersaturation on turbine-passed fish. Available from Pacific Northwest National Laboratory, Richland, WA. PNNL-13470.

Andrews, C., Payne, J. and Rise, M. 2014. Identification of a gene set to evaluate the potential effects of loud sounds from seismic surveys on the ears of fishes: a study with *Salmo salar*. *J. Fish Biol.*, 84(6): 1793-1819. doi:10.1111/jfb.12398.

Bell, C.E. and Kynard, B. 1985. Mortality of adult American shad passing through a 17-megawatt Kaplan turbine at a low-head hydroelectric dam. *N. Am. J. Fish Manage.*, 5(1): 33-38. doi:10.1577/1548-8659(1985)5<33:MOAASP>2.0.CO;2.

Blackwell, B.F. and Juanes, F. 1998. Predation on Atlantic salmon smolts by striped bass after dam passage. *N. Am. J. Fish Manage.*, 18(4): 936-939. doi:10.1577/1548-8675(1998)018<0936:POASSB>2.0.CO;2.

Brown, R.S., Ahmann, M.L., Trumbo, B.A. and Foust, J. 2012b. Fish protection: cooperative research advances fish-friendly turbine design. *Hydro Rev.*, 31(8): 48-53.

Brown, R.S., Carlson, T.J., Gingerich, A.J., Stephenson, J.R., Pflugrath, B.D., Welch, A.E., Langeslay, M.J., Ahmann, M.L., Johnson, R.L. and Skalski, J.R. 2012a. Quantifying mortal injury of juvenile Chinook salmon exposed to simulated hydro-turbine passage. *T. Am. Fish Soc.*, 141(1): 147-157. doi:10.1080/00028487.2011.650274.

- Brown, R.S., Carlson, T.J., Welch, A.E., Stephenson, J.R., Abernethy, C.S., Ebberts, B.D., Langeslay, M.J., Ahmann, M.L., Feil, D.H. and Skalski, J.R. 2009. Assessment of barotrauma from rapid decompression of depth-acclimated juvenile Chinook salmon bearing radiotelemetry transmitters. *T. Am. Fish Soc.*, 138(6): 1285-1301. doi:10.1577/T08-122.1.
- Brown, R.S., Colotelo, A.H., Pflugrath, B.D., Boys, C.A., Baumgartner, L.J., Deng, Z.D., Silva, L.G., Brauner, C.J., Mallen-Cooper, M. and Phonekhampeng, O. 2014. Understanding barotrauma in fish passing hydro structures: a global strategy for sustainable development of water resources. *Fisheries*, 39(3): 108-122. doi:10.1080/03632415.2014.883570.
- Brown, R.S., Cook, K.V., Pflugrath, B.D., Rozeboom, L.L., Johnson, R.C., McLellan, J.G., Linley, T.J., Gao, Y., Baumgartner, L.J. and Dowell, F.E. 2013. Vulnerability of larval and juvenile white sturgeon to barotrauma: can they handle the pressure? *Conserv. Physiol.*, 1(1). doi:10.1093/conphys/cot019.
- Brown, R.S., Deng, Z.D., Cook, K.V., Pflugrath, B.D., Li, X., Fu, T., Martinez, J.J., Li, H., Trumbo, B.A. and Ahmann, M.L. 2013. A field evaluation of an external and neutrally buoyant acoustic transmitter for juvenile salmon: implications for estimating hydroturbine passage survival. *PLOS One* 8(10): e77744. doi:10.1371/journal.pone.0077744.
- Brown, R.S., Pflugrath, B.D., Colotelo, A.H., Brauner, C.J., Carlson, T.J., Deng, Z.D. and Seaburg, A.G. 2012. Pathways of barotrauma in juvenile salmonids exposed to simulated hydroturbine passage: Boyle's law vs. Henry's law. *Fish. Res.*, 121: 43-50. doi:10.1016/j.fishres.2012.01.006.
- Čada, G.F. 1991. Effects of hydroelectric turbine passage on fish early life stages. Available from Oak Ridge National Laboratory, Oak Ridge, TN. CONF-910778-2.

- 647 Čada, G.F. 2001. The development of advanced hydroelectric turbines to improve fish passage survival.
648 Fisheries, 26(9): 14-23. doi:10.1577/1548-8446(2001)026<0014:TDOAHT>2.0.CO;2.
649
- 650 Čada, G.F., Garrison, L.A. and Fisher, R.K. 2007. Determining the effect of shear stress on fish mortality
651 during turbine passage. Hydro Review, 26(7): 52.
652
- 653 Carlson, T. J., Brown, R.S., Stephenson, J.R., Pflugrath, B.D., Colotelo, A.H., Gingerich, A.J., Benjamin,
654 P.L., Langeslay, M.J., Ahmann, M.L., Johnson, R.L., Skalski, J.R., Seaburg, A.G. and Townsend, R.L.
655 2012. The influence of tag presence on the mortality of juvenile Chinook salmon exposed to simulated
656 hydroturbine passage: Implications for survival estimates and management of hydroelectric facilities. N.
657 Am. J. Fish. Manage. 32(2): 249-261. doi: 10.1080/02755947.2012.661384
658
- 659 Casper, B.M., Popper, A.N., Matthews, F., Carlson, T.J. and Halvorsen, M.B. 2012. Recovery of
660 barotrauma injuries in Chinook salmon, *Oncorhynchus tshawytscha* from exposure to pile driving sound.
661 Plos One, 7(6): e39593. doi:10.1371/journal.pone.0039593.
662
- 663 Colotelo, A.H., Pflugrath, B.D., Brown, R.S., Brauner, C.J., Mueller, R.P., Carlson, T.J., Deng, Z.D.,
664 Ahmann, M.L. and Trumbo, B.A. 2012. The effect of rapid and sustained decompression on barotrauma
665 in juvenile brook lamprey and Pacific lamprey: Implications for passage at hydroelectric facilities. Fish.
666 Res., 129: 17-20. doi:10.1016/j.fishres.2012.06.001.
667
- 668 Cook, T.C., Hecker, G.E., Amaral, S., Stacy, P., Lin, F. and Taft, E. 2003. Pilot scale tests
669 Alden/Concepts NREC Turbine. Available from Alden Research Laboratory (US), Holden, MA.
670 DOE/ID/13733.
671

- 672 Cooke, S.J. 2008. Biotelemetry and biologging in endangered species research and animal conservation:
673 relevance to regional, national, and IUCN Red List threat assessments. *Endang. Species Res.*, 4(1-2): 165-
674 185. doi:10.3354/esr00063.
- 675
- 676 Cooke, S.J. and O'Connor, C.M. 2010. Making conservation physiology relevant to policy makers and
677 conservation practitioners. *Conserv Lett* **3**:159-166. doi: 10.1111/j.1755-263X.2010.00109.x
- 678
- 679 Coutant, C.C., and Whitney R.R. 2000. Fish behavior in relation to passage through hydropower turbines:
680 a review. *Trans Am Fish Soc* **129**(2): 351-380. doi:10.1577/1548-
681 8659(2000)129<0351:FBIRTP>2.0.CO;2
- 682
- 683 Davis, M. and Ottmar, M. 2006. Wounding and reflex impairment may be predictors for mortality in
684 discarded or escaped fish. *Fish. Res.*, 82(1): 1-6. doi:10.1016/j.fishres.2006.09.004.
- 685
- 686 Davis, M.W., 2010. Fish stress and mortality can be predicted using reflex impairment. *Fish Fish.*, 11(1):
687 1-11. doi:10.1111/j.1467-2979.2009.00331.x.
- 688
- 689 Deng, Z., Guensch, G.R., McKinstry, C.A., Mueller, R.P., Dauble, D.D. and Richmond, M.C., 2005.
690 Evaluation of fish-injury mechanisms during exposure to turbulent shear flow. *Can. J. Fish. Aquat. Sci.*,
691 62(7): 1513-1522. doi:10.1139/f05-091
- 692 Deng, Z., Carlson, T.J., Ploskey, G.R., Richmond, M.C. and Dauble, D.D., 2007. Evaluation of blade-
693 strike models for estimating the biological performance of Kaplan turbines. *Ecol. Model.*, 208(2): 165-
694 176. doi:10.1016/j.ecolmodel.2007.05.019.
- 695

Deng, Z., Carlson, T.J., Duncan, J.P., Richmond, M.C. and Dauble, D.D., 2010. Use of an autonomous sensor to evaluate the biological performance of the advanced turbine at Wanapum Dam. *J. Renew. Sustain. Energy*, 2(5): 053104. doi:10.1063/1.3501336.

Deng, Z., Carlson, T.J., Dauble, D.D. and Ploskey, G.R., 2011. Fish passage assessment of an advanced hydropower turbine and conventional turbine using blade-strike modeling. *Energies*, 4(1): 57-67. doi:10.3390/en4010057.

Deng, Z. D., J. J. Martinez, A. H. Colotelo, T. K. Abel, A. P. LeBarge, R. S. Brown, B. D. Pflugrath, R. P. Mueller, T. J. Carlson, A. G. Seaburg, R. L. Johnson, and M. L. Ahmann. 2012. Development of external and neutrally buoyant acoustic transmitters for juvenile salmon turbine passage evaluation. *Fish. Res.* 113(1):94-105. doi:10.1016/j.fishres.2011.08.018.

Deng, Z., Lu, J., Myjak, M.J., Martinez, J.J., Tian, C., Morris, S.J., Carlson, T.J., Zhou, D. and Hou, H., 2014. Design and implementation of a new autonomous sensor fish to support advanced hydropower development. *Rev. Sci. Instrum.*, 85(11): 115001. doi:10.1063/1.4900543.

Diamond, S.L. and Campbell, M.D., 2009. Linking “sink or swim” indicators to delayed mortality in red snapper by using a condition index. *Mar. Coast. Fish. Dynam. Manage. Ecosys. Sci.*, 1(1): 107-120. doi: 10.1577/C08-043.1.

Elston, R., Colt, J., Abernethy, S. and Maslen, W., 1997. Gas bubble reabsorption in Chinook salmon: pressurization effects. *J. Aquat. Anim. Health*, 9(4): 317-321. doi:10.1577/1548-8667(1997)009<0317:GBRICS>2.3.CO;2.

721 EPRI (Electric Power Research Institute). 2007. Investigation of hydro-turbine leading edge shapes
722 favorable to fish survival. Prepared by Alden Research Laboratory, Inc., Holden, MA, EPRI Report No.
723 1012561.
724
725 EPRI. 2008. Evaluation of the effects of turbine blade leading edge design on fish survival. Prepared by
726 Alden Research Laboratory, Inc., Holden, MA, EPRI Report No. 1014937.
727
728 EPRI. 2011. 2010 Tests examining survival of fish struck by turbine blades. Prepared by Alden Research
729 Laboratory, Inc., Holden, MA, EPRI Report No. 1024684.FERC. 1995. Preliminary Assessment of Fish
730 Entrainment at Hydropower Projects: A Report on Studies and Protective Measures, Volume 1.
731 Washington, DC. Paper No. DPR-10.
732
733 FERC. 1995. Preliminary Assessment of Fish Entrainment at Hydropower Projects: A Report on Studies
734 and Protective Measures, Volume 1. Washington, DC. FERC Paper No. DPR-10.
735
736 Gerwick, L., Corley-Smith, G. and Bayne, C.J., 2007. Gene transcript changes in individual rainbow trout
737 livers following an inflammatory stimulus. *Fish Shellfish Immun.*, 22(3): 157-171.
738 doi:10.1016/j.fsi.2006.04.003.
739
740 Gordon, N. D., T. A. McMahon, and B. L. Finlayson. 1992. Stream hydrology: An introduction for
741 ecologist. John Wiley & Sons, New York, NY. pp.526.
742
743 Gonzalez, S.F., Huising, M.O., Stakauskas, R., Forlenza, M., Verburg-van Kemenade, B.L., Buchmann,
744 K., Nielsen, M.E. and Wiegertjes, G.F., 2007. Real-time gene expression analysis in carp (*Cyprinus*
745 *carpio* L.) skin: inflammatory responses to injury mimicking infection with ectoparasites.
746 *Develop.Compar. Immunol.*, 31(3): 244-254. doi:10.1016/j.dci.2006.06.010.

- 747
748 Gravel, M.-A. and Cooke, S.J., 2008. Severity of barotrauma influences the physiological status,
749 postrelease behavior, and fate of tournament-caught smallmouth bass. *N. Am. J. Fish Manage.*, 28(2):
750 607-617. doi:10.1577/M07-013.1.
751
- 752 Guensch, G.R., Mueller, R.P., McKinstry, C.A. and Dauble, D.D., 2002. Evaluation of fish-injury
753 mechanisms during exposure to a high-velocity jet. Available from Pacific Northwest National
754 Laboratory, Richland, WA. PNNL-14173.
755
- 756 Halvorsen, M.B., Casper, B.M., Woodley, C.M., Carlson, T.J. and Popper, A.N., 2012. Threshold for
757 onset of injury in Chinook salmon from exposure to impulsive pile driving sounds. *Plos One*, 7(6):
758 e38968. doi:10.1371/journal.pone.0038968.
- 759 Hasler, C.T., Pon, L.B., Roscoe, D.W., Mossop, B., Patterson, D.A., Hinch, S.G. and Cooke, S.J., 2009.
760 Expanding the “toolbox” for studying the biological responses of individual fish to hydropower
761 infrastructure and operating strategies. *Environ. Rev.*, 17(NA): 179-197. doi:10.1139/A09-008.
762
- 763 Haro, A., M. Odeh, J. Noreika, and T. Castro-Santos. 1998. Effect of water acceleration on downstream
764 migratory behavior and passage of Atlantic salmon smolts and juvenile American shad at surface
765 bypasses. *T. Am. Fish. Soc.*, 127(1):118-127. doi:10.1577/1548-
766 8659(1998)127<0118:EOWAOD>2.0.CO;2.
767
- 768 Heisey, P.G., Mathur, D. and Rineer, T., 1992. A reliable tag-recapture technique for estimating turbine
769 passage survival: application to young-of-the-year American shad (*Alosa sapidissima*). *Can. J. Fish.*
770 *Aquat. Sci.*, 49(9): 1826-1834. doi:10.1139/f92-202.
771

- Hou H, Z Deng, JJ Martinez, T Fu, GE Johnson, TJ Carlson, and JP Duncan. 2015. Turbine Biological Evaluation Tools and Their Applications using Sensor Fish Data. HydroVision International 2015, Portland, OR, USA.
- Ingerslev, H.-C., Lunder, T. and Nielsen, M.E., 2010. Inflammatory and regenerative responses in salmonids following mechanical tissue damage and natural infection. *Fish Shellfish Immun.*, 29(3): 440-450. doi:10.1016/j.fsi.2010.05.002.
- International Energy Agency, 2015. 2015 Key World Energy Statistics. Paris, France. Available from <http://www.iea.org/publications/freepublications/publication/key-world-energy-statistics-2015.html> [accessed 30 April 2016].
- Janak, J.M., Brown, R.S., Colotelo, A.H., Pflugrath, B.D., Stephenson, J.R., Deng, Z.D., Carlson, T.J. and Seaburg, A.G., 2012. The effects of neutrally buoyant, externally attached transmitters on swimming performance and predator avoidance of juvenile Chinook salmon. *T. Am. Fish. Soc.*, 141(5): 1424-1432. doi:10.1080/00028487.2012.688915.
- Jepsen, N., Koed, A., Thorstad, E. and Baras, E., 2002. Surgical implantation of telemetry transmitters in fish: how much have we learned? In: *Developments in Hydrobiology: Aquatic Telemetry*. Edited by E. Thorstad, I. Fleming and T. Næsje, Springer Netherlands, pp. 239-248.
- Kao, S. C., R. A. McManamay, K. M. Stewart, N. M. Samu, B. Hadjerioua, S. T. DeNeale, D. Yeasmin, M. Fayzul, K. Pasha, A. A. Oubeidillah, and B. T. Smith. 2014. New Stream-Reach Development: A Comprehensive Assessment of Hydropower Energy Potential in the United States. Prepared by Oakridge National Laboratory for the US Department of Energy, Washington, DC. GPO DOE/EE-1063.

- 798 Mathur, D., Heisey, P.G., Euston, E.T., Skalski, J.R. and Hays, S., 1996. Turbine passage survival
799 estimation for chinook salmon smolts (*Oncorhynchus tshawytscha*) at a large dam on the Columbia River.
800 Can. J. Fish. Aquat. Sci., 53(3): 542-549. doi: 10.1139/f95-206.
801
- 802 Matsche, M. A., and J. Gibbons. 2012. Annual variation of hematology and plasma chemistry in
803 shortnose sturgeon, *Acipenser brevirostrum*, during a dam-imposed spawning run. Fish Physiol.
804 Biochem., 38(6):1679-1696. doi:10.1007/s10695-012-9664-7.
805
- 806 McKinsty, C., Carlson, T. and Brown, R., 2007. Derivation of a mortal injury metric for studies of rapid
807 decompression of depth-acclimated physostomous fish. Available from Pacific Northwest National
808 Laboratory, Richland, WA. PNNL-17080.
809
- 810 McMichael, G.A., Eppard, M.B., Carlson, T.J., Carter, J.A., Ebberts, B.D., Brown, R.S., Weiland, M.,
811 Ploskey, G.R., Harnish, R.A. and Deng, Z.D., 2010. The juvenile salmon acoustic telemetry system: a
812 new tool. Fisheries, 35(1): 9-22. doi:10.1577/1548-8446-35.1.9.
813
- 814 Mesa, M.G., 1994. Effects of multiple acute stressors on the predator avoidance ability and physiology of
815 juvenile Chinook salmon. T. Am. Fish. Soc., 123(5): 786-793. doi:10.1577/1548-
816 8659(1994)123<0786:EOMASO>2.3.CO;2.
817
- 818 Mesa, M.G., Gee, L.P., Weiland, L.K. and Christiansen, H.E., 2013. Physiological responses of adult
819 rainbow trout experimentally released through a unique fish conveyance device. N. Am. J. Fish Manage.,
820 33(6): 1179-1183. doi:10.1080/02755947.2013.833560.
821

- 822 Metcalfe, J. D. and G. P. Arnold. 1997. Tracking fish with electronic tags. *Nature*
823 387(6634):665-666.
824
- 825 Miracle, A., Denslow, N.D., Kroll, K.J., Liu, M.C. and Wang, K., 2009. Spillway-induced
826 salmon head injury triggers the generation of brain alphaII-spectrin breakdown product
827 biomarkers similar to mammalian traumatic brain injury. *PloS one*, 4(2).
828 doi:10.1371/journal.pone.0004491.
829
- 830 Morrissey, M.B., Suski, C.D., Esseltine, K.R. and Tufts, B.L., 2005. Incidence and physiological
831 consequences of decompression in smallmouth bass after live-release angling tournaments. *T.*
832 *Am. Fish. Soc.*, 134(4): 1038-1047. doi:10.1577/T05-010.1.
833
- 834 Muir, J., 1959. Passage of young fish through turbines. *Proc. Amer. Soc. Civ. Eng. (Power*
835 *Division)*, 85(1): 23-46.
836
- 837 Navarro, J.E., McCauley, D.J. and Blystra, A.R., 1996. Turbine passage at four low-head
838 hydroelectric facilities in northeast Michigan. *N. Am. J. Fish Manage.*, 16(1): 182-191.
839 doi:10.1577/1548-8675(1996)016<0182:TPAFLH>2.3.CO;2.
840
- 841 Neitzel, D.A., Dauble, D.D., Čada, G., Richmond, M.C., Guensch, G.R., Mueller, R.P., Abernethy, C.S.
842 and Amidan, B., 2004. Survival estimates for juvenile fish subjected to a laboratory-generated shear
843 environment. *T. Am. Fish. Soc.*, 133(2): 447-454. doi:10.1577/02-021.
844

- 845 Neitzel, D.A., Richmond, M.C., Dauble, D.D., Mueller, R.P., Moursund, R.A., Abernethy, C.S., Guensch,
846 G.R. and Čada, G., 2000. Laboratory studies on the effects of shear on fish. Available from Pacific
847 Northwest National Laboratory, Richland, WA. PNNL-13323.
- 848
- 849 NOAA (National Oceanic and Atmospheric Administration). 2008. Consultation on Remand for
850 Operation of the Federal Columbia River Power System, 11 Bureau of Reclamation Projects in the
851 Columbia Basin and ESA Section 10(a)(1)(A) Permit for Juvenile Fish Transportation Program [Revised
852 and reissued pursuant to court order, *NWF v. NMFS*, Civ. No. CV 01-640-RE (D. Oregon)], NOAA
853 National Marine Fisheries Service, Northwest Region, Seattle, Washington.
- 854
- 855 Noga, E. and Udomkusonsri, P., 2002. Fluorescein: a rapid, sensitive, nonlethal method for detecting skin
856 ulceration in fish. *Vet. Pathol. Online*, 39(6): 726-731. doi:10.1354/vp.39-6-726.
- 857
- 858 Pracheil, B.M., C. R. DeRolph, C.R., Schramm, M.P., and Bevelhimer, M.S., In Press. A fish-eye view of
859 riverine hydropower systems: The current understanding of the biological response to turbine passage.
860 *Rev. Fish Biol. and Fish.*, **000**:000-000.
- 861
- 862 Pribyl, A., Schreck, C., Parker, S. and Weis, V., 2012. Identification of biomarkers indicative of
863 barotrauma and recovery in black rockfish *Sebastes melanops*. *J. Fish Biol.*, 81(1): 181-196. doi:
864 10.1111/j.1095-8649.2012.03322.x.
- 865
- 866 Qi, Z.-H., Liu, Y.-F., Wang, W.-N., Wu, X., Xin, Y., Lu, Y.-F. and Wang, A.-L., 2011. Molecular
867 characterization and functional analysis of a complement C3 molecule in the orange-spotted grouper
868 (*Epinephelus coioides*). *Fish Shellfish Immun.*, 31(6): 1284-1290. doi:10.1016/j.fsi.2011.09.018.
- 869

- 870 Richmond, M. C., J. A. Serkowski, L. L. Ebner, M. Sick, R. S. Brown, and T. J. Carlson. 2014.
871 Quantifying barotrauma risk to juvenile fish during hydro-turbine passage. *Fish. Res.* 154:152-164.
872 doi:10.1016/j.fishres.2014.01.007.
873
- 874 Skalski, J.R., Townsend, R.L., Steig, T.W. and Hemstrom, S., 2010. Comparison of two alternative
875 approaches for estimating dam passage survival of salmon smolts. *N. Am. J. Fish Manage.*, 30(3): 831-
876 839. doi:10.1577/M09-103.1.
877
- 878 Sprankle, K., 2005. Interdam movements and passage attraction of American shad in the lower
879 Merrimack River main stem. *N. Am. J. Fish Manage.*, 25(4): 1456-1466. doi:10.1577/M04-049.1.
880
- 881 Stephenson, J.R., Gingerich, A.J., Brown, R.S., Pflugrath, B.D., Deng, Z., Carlson, T.J., Langeslay, M.J.,
882 Ahmann, M.L., Johnson, R.L. and Seaburg, A.G., 2010. Assessing barotrauma in neutrally and negatively
883 buoyant juvenile salmonids exposed to simulated hydro-turbine passage using a mobile aquatic
884 barotrauma laboratory. *Fish. Res.*, 106(3): 271-278. doi:10.1016/j.fishres.2010.08.006.
885
- 886 Stier, D.J. and Kynard, B., 1986. Use of radio telemetry to determine the mortality of Atlantic salmon
887 smolts passed through a 17-MW Kaplan turbine at a low-head hydroelectric dam. *T. Am. Fish. Soc.*,
888 115(5): 771-775. doi:10.1577/1548-8659(1986)115<771:UORTTD>2.0.CO;2.
889
- 890 Stokesbury, K.D. and Dadswell, M.J., 1991. Mortality of juvenile clupeids during passage through a tidal,
891 low-head hydroelectric turbine at Annapolis Royal, Nova Scotia. *N. Am. J. Fish Manage.*, 11(2): 149-
892 154. doi:10.1577/1548-8675(1991)011<0149:MOJCDP>2.3.CO;2.
893

- 894 Stoot, L.J., Cairns, N.A., Cull, F., Taylor, J.J., Jeffrey, J.D., Morin, F., Mandelman, J.W., Clark, T.D., and
895 Cooke, S.J., 2014. Use of portable blood physiology point-of-care devices for basic and applied research
896 on vertebrates – a review. *Conserv. Physiol.*, 2: cou011. doi: 10.1093/conphys/cou011.
897
- 898 Talbot, A.T., Pottinger, T.G., Smith, T.J. and Cairns, M.T., 2009. Acute phase gene expression in rainbow
899 trout (*Oncorhynchus mykiss*) after exposure to a confinement stressor: a comparison of pooled and
900 individual data. *Fish Shellfish Immun.*, 27(2): 309-317. doi:10.1016/j.fsi.2009.05.016.
901
- 902 Taylor, R.E. and Kynard, B., 1985. Mortality of juvenile American shad and blueback herring passed
903 through a low-head Kaplan hydroelectric turbine. *T. Am. Fish. Soc.*, 114(3): 430-435. doi:10.1577/1548-
904 8659(1985)114<430:MOJASA>2.0.CO;2.
905
- 906 Tierney, K. and Farrell, A., 2004. The relationships between fish health, metabolic rate, swimming
907 performance and recovery in return-run sockeye salmon, *Oncorhynchus nerka* (Walbaum). *J. Fish Dis.*,
908 27(11): 663-671. doi:10.1111/j.1365-2761.2004.00590.x.
909
- 910 Trumbo, B.A., Ahmann, M.L., Renholds, J.F., Brown, R.S., Colotelo, A.H. and Deng, Z., 2014.
911 Improving hydroturbine pressures to enhance salmon passage survival and recovery. *Rev. Fish Biol.*
912 *Fisher.*, 24(3): 955-965. doi: 10.1007/s11160-013-9340-8.
913
- 914 Turnpenny, A. 1998. Mechanisms of fish damage in low-head turbines: an experimental appraisal. In *Fish*
915 *migration and fish bypasses*. Edited by M. Jungwirth, S. Schmutz, and S. Weiss. Fishing News Books,
916 Blackwell Science Ltd., Oxford, UK. pp. 300–314.
917

- 918 US Energy Information Administration. 2014. International Energy Statistics. Available from
919 [http://www.eia.gov/cfapps/ipdbproject/iedindex3.cfm?tid=2&pid=33&aid=12&cid=regions&syid=2005&](http://www.eia.gov/cfapps/ipdbproject/iedindex3.cfm?tid=2&pid=33&aid=12&cid=regions&syid=2005&eyid=2011&unit=BKWH)
920 [eyid=2011&unit=BKWH](http://www.eia.gov/cfapps/ipdbproject/iedindex3.cfm?tid=2&pid=33&aid=12&cid=regions&syid=2005&eyid=2011&unit=BKWH) [accessed 15 October 2015].
921
- 922 Wagner, T. and Congleton, J.L., 2004. Blood chemistry correlates of nutritional condition, tissue damage,
923 and stress in migrating juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Can. J. Fish. Aquat. Sci.*,
924 61(7): 1066-1074. doi:10.1139/f04-050.
925
- 926 Weber, E.D., Borthwick, S.M. and Helfrich, L.A., 2002. Plasma cortisol stress response of juvenile
927 Chinook salmon to passage through Archimedes lifts and a hidrostral pump. *N. Am. J. Fish Manage.*,
928 22(2): 563-570. doi:10.1577/1548-8675(2002)022<0563:PCSROJ>2.0.CO;2.
929
- 930 Wendelaar Bonga, S.E., 1997. The stress response in fish. *Physiol. Rev.*, 77(3): 591-625. PMID:
931 9234959.
932
- 933 Wertheimer, R.H., 2007. Evaluation of a surface flow bypass system for steelhead kelt passage at
934 Bonneville Dam, Washington. *N. Am. J. Fish Manage.*, 27(1): 21-29. doi:10.1577/M05-216.1.
935
- 936 Winemiller, K.O., McIntyre, P.B., Castello, L., Fluet-Chouinard, E., Giarrizzo, T., Nam, S., Baird, I.G.,
937 Darwall, W., Lujan, N.K., Harrison, I., Stiassny, M.L.J., Silvano, R.A.M., Fitzgerald, D.B., Pelicice,
938 F.M., Agostinho, A.A., Gomes, L.C., Albert, J.S., Baran, E., Petrere, M., Zarfl, C., Mulligan, M.,
939 Sullivan, J.P., Arantes, C.C., Sousa, L.M., Koning, A.A., Hoeinghaus, D.J., Sabaj, M., Lundberg, J.G.,
940 Armbruster, J., Thieme, M.L., Petry, P., Zuanon, J., Vilara, G.T., Snoeks, J., Ou, C., Rainboth, W.,
941 Pavanelli, C.S., Akama, A., Soesbergen, A.V., and Saenz, L. 2016. Balancing Hydropower and
942 Biodiversity in the Amazon, Congo, and Mekong. *Science* 351(6269): 128-129. doi:
943 10.1126/science.aac7082.

944

945 Zarfl, C., Lumsdon, A.E., Berlekamp, J., Tydecks, L., and Tockner, K., 2015. A global boom in

946 hydropower dam construction. *Aquat Sci* 77:161-170. doi: 10.1007/s00027-014-0377-0.

947

948 Zydlewski, J., Zydlewski, G. and Danner, G.R., 2010. Descaling injury impairs the osmoregulatory ability

949 of Atlantic salmon smolts entering seawater. *T. Am. Fish. Soc.*, 139(1): 129-136. doi:10.1577/T09-054.1

Draft

1 *Tables*

- 2 Table 1. A summary of different available metrics (i.e., mortality, injury, physiological markers, behavior) and rankings with regard to
3 the resources needed, invasiveness, comparability among stressors and species, and diagnostic properties. Metrics are ranked on a
4 scale from 0 to 5, with 0 being the least favorable and 5 being the most favorable.

Draft

Study Setting	Metric	Resources Needed	Invasiveness	Comparability Among Stressors	Comparability Among Species	Diagnostic Properties	Ideal Studies
Lab	Mortality	2	2	5	5	3	Exposure to controlled stressors; need to understand whole body responses
	Injury	0	0	4	4	5	Exposure to controlled stressors; need to understand mechanisms of injury/mortality
	Physiological Markers	0	1	4	4	5	Exposure to controlled stressors; need to understand mechanisms of injury/mortality
	Behavior	1	2	4	4	4	Exposure to controlled stressors; need to understand whole body responses
Study Setting	Metric	Resources Needed	Invasiveness	Comparability Among Studies	Comparability Among Species	Diagnostic Properties	Ideal Studies
Field	Mortality	1	1	3	5	3	Exposure to multiple stressors in realistic environment; need to understand whole body responses
	Injury	0	0	3	4	3	Exposure to multiple stressors in realistic environment; need to understand types of injury/mortality
	Physiological Markers	0	1	3	4	3	Exposure to multiple stressors in realistic environment; need to understand types of injury/mortality
	Behavior	0	2	3	4	3	Exposure to multiple stressors in realistic environment; need to understand whole body responses

Figure 1. Factors to consider when planning a field study including the sampling method (i.e., telemetry vs. recapture) and the metric (i.e., mortality, injury, physiological markers, behavior). Shading in the corners corresponds to a high (black), medium (gray), or low (white) amount of resources required.

161x120mm (300 x 300 DPI)

Figure 2. Factors to consider when planning a laboratory study including the metric (i.e., mortality, injury, physiological markers, behavior). Shading in the corners corresponds to a high (black), medium (gray), or low (white) amount of resources required.

153x137mm (300 x 300 DPI)