

# Evaluation of Factors Affecting Juvenile and Larval Fish Survival in Fish Return Systems at Cooling Water Intakes





# **Evaluation of Factors Affecting Juvenile and Larval Fish Survival in Fish Return Systems at Cooling Water Intakes**

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# PRODUCT DESCRIPTION

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The Electric Power Research Institute (EPRI) has funded laboratory studies on biological efficacy of fine-mesh screens for safely collecting larval and juvenile fish. However, little information exists on effects of fish return systems on larval or early juvenile survival. This report presents results of two years of laboratory evaluations on factors affecting larval fish survival in fish return systems at cooling water intake structures (CWISs). This project is generating additional data necessary to determine overall biological efficacy of larval fish collection and return systems.

## Results & Findings

A summary of the results is as follows:

- Fish length: Survival for all species dropped during the transition from yolk-sac to post-yolk-sac larvae with exact length varying among species. Survival increased rapidly with increasing fish length with a peak when larvae attained a size of approximately 12 mm.
- Velocity: Velocity had no effect on survival within length groups.
- Drop height: With exception of common carp during initial testing, drop height ( $\leq 1.8$  m, or 6 ft) had no effect on survival within length groups.
- Length, drops, and bends: Increasing length (from 21.6 m [71 ft] to 131 m [430 ft]) of the fish return line and adding drops and bends did not affect survival within length groups.

For all species tested, survival ranged from 70-100% after the fish were approximately 11.0 mm in length. These results are similar to those observed for species tested in the fine-mesh screen studies; fish 12.0 mm or greater consistently showed high post-collection survival regardless of species, screen type, or approach velocity. This increase in survival appears to be correlated to scale development and general increase in body musculature. These results are consistent with and expand on results previously reported for larger juvenile fish during EPRI-sponsored impingement survival monitoring of coarse-mesh Ristroph screens in a laboratory flume where survival exceeded 90% for all species tested regardless of approach velocities (1-3 ft/s).

## Challenges & Objective(s)

Section 316(b) of the Clean Water Act establishes statutory requirements for fish protection at CWIS. In 2004, the U.S. Environmental Protection Agency (EPA) established a rule for implementing Section 316(b) for existing CWIS utilizing  $> 50$  MGD. The rule was eventually withdrawn by EPA following a legal challenge and subsequent court ruling. EPA is currently working on revising the rule for existing facilities, and a draft for public review and comment is expected in early 2011 with a final rule due in 2012. The specifics of any future of 316(b) rule

are uncertain, but it is expected that reductions in entrainment may be required at many facilities. Facilities needing to reduce entrainment will have limited options. Technologies such as fine-mesh traveling screens that can be deployed in existing intake bays with minimal structural modifications have the greatest potential for wide-scale application because they are relatively easy to retrofit. The fundamental fish protection component of fine-mesh traveling screens is the safe collection of all life stages—egg, larval, and juvenile—of fish and shellfish. Organisms collected off the traveling screen must be returned to the source water body via a fish return system. Such systems potentially subject organisms to additional stresses that can cause and contribute to increased mortality beyond any induced by the fine-mesh screen system. There is limited information on effectiveness of fish return systems and their optimum design features. EPRI undertook the literature review and laboratory investigations described in the report to fill information gaps.

### **Applications, Values & Use**

Information in the report will support Clean Water Act §316(b) policy development and future rule compliance efforts by the power industry, resource and regulatory agencies, and the public.

### **EPRI Perspective**

Data in this report provide additional information necessary to determine whether fine-mesh traveling screens are a viable option for reducing entrainment losses at CWISs, given the species and hydraulic conditions present. Results from other EPRI studies indicate that fine-mesh screen effectiveness is strongly influenced by species and life stages to be protected as well as plant design and operating characteristics (such as approach velocity and screen rotation speeds). Results of this study indicate that those species that survive the impingement and transfer process are not adversely affected by transport back to the source waterbody.

### **Approach**

The project team designed this study to evaluate effects of velocity, drop height, length, drops, and bends on larval fish survival through a fish return system. Testing was limited to freshwater species being tested in companion EPRI-sponsored studies evaluating performance of several types of fine-mesh screens.

### **Keywords**

Fine-mesh traveling screens  
Impingement  
Entrainment  
Clean water act §316(b)  
Fish protection technologies  
Fish return system

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# 1

## INTRODUCTION

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Section 316(b) of the Clean Water Act establishes the statutory requirements for fish protection at cooling water intake structures (CWIS). In 2004, the U.S. Environmental Protection Agency (EPA) established a rule for implementing Section 316(b) (the Rule; EPA 2004) for steam electric plants with existing CWIS utilizing > 50 MGD. The Rule was eventually withdrawn by EPA following a legal challenge and subsequent Circuit Court ruling that remanded several sections to EPA. EPA is currently working on revising the rule for existing facilities, with a proposed rule expected by early 2011 and a final rule in 2012.

The specifics of a future 316(b) Rule are uncertain, but it is expected that reductions in entrainment may be required at many facilities. Facilities needing to reduce entrainment will have limited options. Technologies such as fine-mesh traveling screens that can be deployed in existing intake bays with minimal structural modifications have the greatest potential for wide-scale application because they are relatively easy to retrofit. The fundamental fish protection component of fine-mesh traveling screens is the safe collection of all life stages – egg, larval and juvenile – of fish and shellfish. Organisms collected off the traveling screen must be returned to the source water body via a fish return system. Such systems potentially subject the organisms to additional stresses that can cause or contribute to increased mortality beyond any induced by the fine-mesh screen system. There is limited information on the effectiveness of fish return systems and their optimum design features. The only existing resource was that published by the American Society of Civil Engineers (ASCE 1982). The guidelines addressed design criteria to reduce abrasion, turbulence, shear, and velocity for transported fish. However, the criteria were evidently developed for juvenile and adult fish. For example, it is stated that the ...” system size must be based on the number and size of fish; use a minimum water depth of 6 in. (15.2 cm); have a minimum width 18 in. (45.7 cm); appropriate free board must be provided based on the jumping capability of the strongest fish to be transported; and, transport velocities must be greater than the sustained cruising speed of the fish, often 2 to 4 fps (0.61 to 1.22 m/sec).”... This last condition indicates a velocity that is commensurate with juvenile and adult fish swim speeds. Regardless, any new/retrofit fish return will have to be designed to not only assure juvenile and adult survival but egg and larval lifestages as well.

Information relative to return of early life stages is virtually non-existent. EPRI, therefore, initiated laboratory studies to investigate importance of various fish return designs and operations on larval fish survival. The results reported herein provide companion information to EPRI studies on the performance of fine-mesh traveling screens (EPRI 2010a) and the engineering, cost and operation and maintenance (O&M) issues that would be faced with retrofit of fine-mesh traveling screens at existing CWIS (EPRI 2010b).

The inherent variability in fish populations, natural conditions (e.g., temperature, storms, debris), and/or anthropogenic factors (e.g., maintenance, unscheduled outages) severely limit the ability to isolate the effects of any component of the fine-mesh screen/fish return system in the field. Laboratory studies allow controlled tests to isolate individual treatment parameters that can affect survival. To complement fine-mesh traveling screen survival studies (EPRI 2008, 2009, 2010a) this study was developed to determine if design features developed for adult fish apply to larval and early juvenile lifestages. The objectives were to determine the effects of velocity and discharge height and the length and configuration (i.e., bends, turns, drops) of the fish return on the ultimate survival of different species and lifestages of transported organisms.

The first component of this study was to conduct a literature review for data on fish return and passage systems to determine stressors affecting survival. The results are summarized in Section 2 and a detailed discussion is provided in Appendix A.

Based on the literature review, the initial study focused on the effects of velocity and discharge height, because it was anticipated that these would be key design variables in any retrofit consideration. The scope of the initial study was expanded to evaluate the effect of increased length of the system and the inclusion of drops, turns, and bends. The highest velocity initially tested and the underwater discharge conditions were selected to represent what EPRI envisioned as an optimum design for a retrofitted fish return system. The materials and methods for the studies are provided in Section 3. Results are presented in Section 4 and a discussion and summary is provided in Section 5.

# 2

## FACTORS AFFECTING PERFORMANCE OF FISH RETURN SYSTEMS

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A review of literature was conducted to identify specific stressors to fish that may be present within a return system at CWIS and to quantify the effect of each potential stressor on fish survival. Whenever possible, quantifiable units for reporting stressor levels (such as turbulence, shear and abrasion) were identified and acceptable ranges for safe fish movement or upper/lower thresholds were developed (Appendix A).

Key potential factors affecting survival of fish in water transport systems include water velocity, turbulence and shear, abrasion and impact, and to a limited extent pressure. A summary review of existing knowledge and their relative importance to CWIS and fish return systems for each of these factors are subsequently discussed. Details on each parameter are found in Appendix A.

In addition, during the summer of 2009 an EPRI team visited four power stations (Big Bend, Tampa Bay, FL; Brunswick, Cape Fear River, NC; Prairie Island, Mississippi River, MN; and AES Somerset, Lake Ontario, NY) that currently use fine-mesh traveling water screens. The objective of these visits was to gather practical engineering and operational information of fine-mesh screening systems including fish return lines as part of a different but related EPRI (2010b) project. These visits afforded the opportunity to also collect data on fish return systems implemented explicitly for returning larval life stages to source water bodies. For Big Bend (Brueggemeyer et al. 1988) and Brunswick (Thompson 2000), survival data collected at the end of the fish return are available. Those data represent total system effects (screen + fish return system) and although the specific stressors contributing to return system mortality cannot be determined from those data, they are indicative of survival reflecting affects of debris, temperature, biofouling, and survival of both fragile and hearty species. A summary of available survival data from these visits is provided in Appendix A.

### Velocity

Velocity is not a direct stressor to fish, but is one characteristic of the flow field that determines the relative intensity of other stressors (PSEG 2002). Fish can travel at uniform, high velocities within a body of water without deleterious effects. Other stressors such as turbulence and shear result from uneven or unsteady velocity conditions.

## **Turbulence and Shear**

Turbulence is a measurement of the fluctuation in velocity magnitude about a mean value. Turbulence creates shear which is defined as a stress which is applied parallel or tangential to a face of a material, as opposed to a normal stress which is applied perpendicularly.

The study of most relevance to examining shear effects was conducted at the Pacific Northwest National Laboratory (PNNL). The primary objective of the study was to “specify an index describing the hydraulic force that fish experience when subjected to a shear environment” (Nietzel et al. 2000). In this study, fish were exposed to a shear environment produced by a submerged jet with velocities ranging from 0 to 70 ft/sec. Test fish included juvenile rainbow trout, spring and fall Chinook salmon, and American shad.

In 2001, PSEG conducted a series of laboratory and field studies to assess mortality associated with the fish collection and return system at the Salem Nuclear Generating Station (Salem). Computational Fluid Dynamics (CFD) models of the existing Salem fish return system were developed and included calculations of turbulence and shear and the results compared to literature values. A test facility was built to evaluate the effect of changing the fish return system point-of-discharge from a subsurface discharge to one with a 1.3 ft drop and 6.0 ft drop. The facility simulated the end-of-pipe discharge and the return troughs to quantify stressors within these system components.

Alewife, a relatively fragile species, was selected for testing. The alewife that were evaluated ranged from 48-142 mm FL (mean 79.4 mm). The results indicate that survival was nearly 100% under all conditions tested (PSEG 2002).

## **Abrasion and Impact**

Abrasion in fish return systems can occur on rough surfaces. Conversely, other characteristics of the fish return system (such as the lack of sharp corners and physical impediments) act to minimize the potential for abrasion (ASCE 1982).

The survival rate for fish exposed to impact is determined not only by the relative velocity between the fish and the object struck, but is also affected by the physical characteristics (e.g., hardness, sharpness, roughness, etc.) of the object struck. For example, at the same velocity, impact against solid objects caused higher mortality than entry into water (ASCE 1982).

The majority of research in this area has been related to the safe passage of fish over high-head hydroelectric dams and conducted with salmonids. Early studies indicate that velocity at the time of impact was a greater predictor of injury and mortality than the height of the fall (Smith 1938; Holmes 1939; Richey 1956; and Regenthal 1957 as cited in Ruggles and Murry 1983).

Further study (data presented in Bell and DeLacy 1972) indicated fish experiencing impact greater than 16 m/s incurred damage to gills, eyes or internal organs. Survival of fish dropped from a helicopter into a hatchery pond was dependent upon the size of the fish dropped and the height of the fall. At any given height, smaller fish experienced greater survival at lower impact velocities than larger fish.

## **Pressure**

Pressure effects are most injurious to fish when they are exposed to changes substantially above or below the pressure to which they are acclimated. For example, a fish entrained in the cooling water flow from a depth of 30 feet and carried to an operating deck elevation 20 feet above water level will experience a change of one atmosphere of pressure. Pressure changes occurring within CWIS and fish return systems are dependent on the intake flow, depth of the intake, and the path that fish take as they are returned to the water body. The effect of pressure change on fish varies by species and lifestage.

Fish eggs and newly hatched larvae do not have swim bladders making them less susceptible to damage caused by brief drops in pressure (Cada 1990). In addition, some fish, including striped bass, that are physoclists (swim bladder not connected to digestive tract) as adults have a physostomous (swim bladder connected to the digestive tract by a tube) larval stage that may be less impacted by pressure than adult lifestages (Hadley et al. 1987).

## **Summary**

The review of literature showed that there are limited data on the survival of early lifestages of fish with stressors encountered at CWIS fish return systems. Based on the available data and existing CWIS design, pressure should not be a factor in larval or juvenile fish survival in fish return systems. Site-specific characteristics such as tidal or storm events that result in severe fluctuations in water level at CWIS could result in abrasion or impact (height) effects. Velocity is a factor that could have synergistic effects.

The initial variables selected for laboratory assessment were velocity and end-of-pipe discharge height. Velocity was selected because it will vary with each installation and can affect other stressors such as severity of abrasion, turbulence and shear. Drop height has always been perceived to be important and has been the focus of many studies with adult and juvenile fish. A system was constructed to allow testing of three drop heights and two velocity conditions. The drop heights included an underwater discharge (no drop), 0.61 m (2 ft) drop, and 1.22 m (4 ft) drop. Drop heights to evaluate the extremes (e.g., 50 ft) that may be realized in the field were not attempted because a fish return for a facility with this condition could be designed, in most cases, to preclude such a drop. Based on practical experience with existing systems, velocities of 0.6 m/sec (2 ft/sec) and 1.8 m/sec (6 ft/sec) were selected to represent the range of potential return line velocities for a new/retrofit return line. The study was then expanded to assess the effect of length and configuration (i.e. bends, turns, drops) of the fish return on the ultimate survival of different species and lifestages of transported organisms.



# 3

## LABORATORY METHODS

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A primary consideration in the design of the initial test apparatus was to keep, to the extent practicable, the system components similar while varying both velocity and drop height. A parallel system was designed which resulted in the larvae traveling the same distance and allowed use of the same collection system. The pitch of the lines was different which resulted in the velocity in one line being 0.6 m/sec (2 ft/sec) and the velocity in the other being 1.8 m/sec (6 ft/sec). The fish return system was constructed to allow assessment of various stressors during larval fish transport (e.g., turbulence, impact (height of fall), and velocity). The system was constructed to allow testing of three drop heights and the two velocities. The 0.6 m/sec (2 ft/sec) velocity was selected to represent the low end of potential return line velocities; the 1.8 m/sec (6 ft/sec) was selected to represent a logical design point (based on the ASCE guidelines) for a new/retrofit return line. After the initial velocity and drop height tests the system was modified to lengthen the return line and add drops, turns, and bends. The 1.8 m/sec (6 ft/sec) velocity and underwater discharge were selected as test components. One of the initial lines was retained to act as a control and supplement the initial database with additional species and length effects data.

### Test Facility

All testing was conducted in a closed-loop system. Water from a reservoir was supplied to the loop via a 5-hp pump. Photographs of the test system are provided in Figure 3-1 through Figure 3-4. Water was pumped from the reservoir to a head tank using the single pump and an independent valve setting was used to regulate flow into one of two return pipes; one per velocity condition. A section of the test device is provided in Figure 3-5, while Figure 3-6 shows each of the three collection box configurations.

The pipes were each 15.2 cm (6 in.) diameter but had a different slope to achieve transport velocities of 0.6 m/sec (2 ft/sec) in one and 1.8 m/sec (6 ft/sec) in the other. Due to the facility's available space, each pipe's slope was adjusted to maintain the proper water depth and velocity. This resulted in a pipe length of approximately 21.6 m (71 ft). Each pipe contained an injection point on the upstream end and a 23 degree elbow with a 0.91 m (3 ft) section of clear pipe at the discharge end.

An adjustable collection box was used to obtain three different conditions at the outfall; underwater, 0.61 m (2 ft) drop, and 1.22 m (4 ft) drop heights. The box was 0.91m (3 ft) wide x 2.13 m (7 ft) long x 1.22 m (4 ft) deep, with a bottom which was slightly pitched, creating an "upstream" and "downstream" end. A 25.4 cm (10-in.) diameter stand pipe with a valve and a 5.08 cm (2 in.) drain pipe controlled by a gate valve were located on the upstream side of the

collection tank behind an angled barrier screen (with 350- $\mu$ m mesh) to maintain water level (Figure 3-1). This design allowed the box to be drained when making a collection without removing organisms. When draining the collection box, a gentle spray was used to prevent organisms from adhering to the sides of the box or on the barrier screen. When water became concentrated in the downstream end, a collection pan with screened (200- $\mu$ m mesh) overflow windows was placed at the outfall of the downstream 5.08 cm (2-in.) collection pipe. The collection gate valve was then opened and the remaining water and organisms in the box sluiced into the collection pan (Figure 3-3). Water flowing from the stand pipe, 5.08 cm (2-in.) drain pipe and the collection pipe/tray was returned to the reservoir.

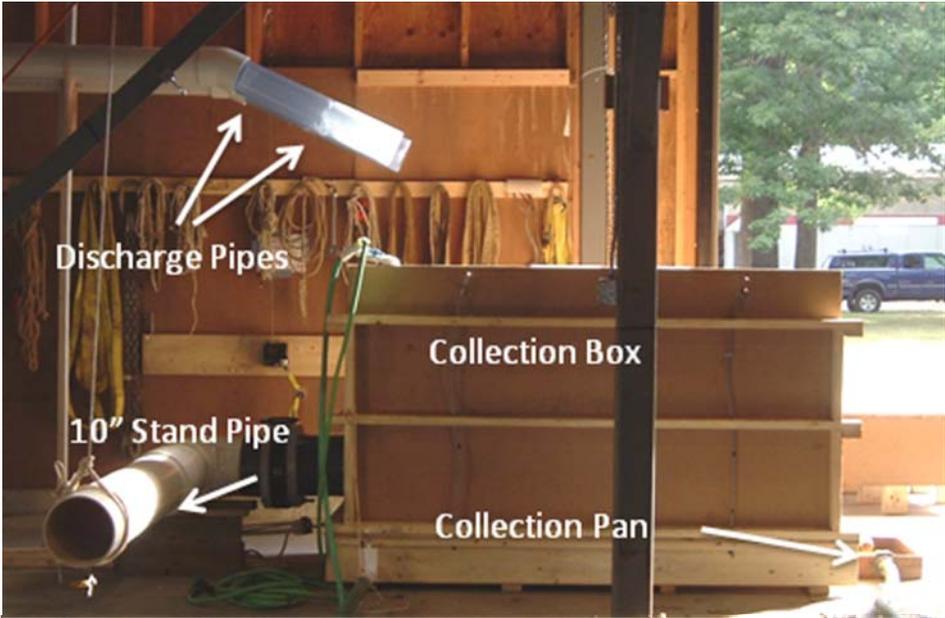
Water quality within the reservoir was maintained with a 15-hp pump that recirculated reservoir water through a separate filtration plumbing circuit. The plumbing circuit included a bag filter to remove particulate matter, an ultraviolet sterilizer to reduce potential pathogens, and a 100-ton chiller to maintain water temperature.

To modify the original system to include added length, drops, bends, and turns, one return line was retained from the original testing and an additional line with more complexity was added that allowed the use of the same collection box. Both lines were constructed to maintain a velocity of 1.8 m/sec (6 ft/sec) and an underwater discharge. Photographs of the test system are provided on Figure 3-7 through Figure 3-10.

The system was constructed to allow testing from three release locations, one on the original line (i.e. “head of short line”) and two on the extended line (Figure 3-10). The “head of long line” release point was selected to expose the organisms to the full length of the extended test line. The “upstream “S” line” release point was selected to allow assessment of the affect of the tight radius turns section without the effect of a longer distance of travel. Finally, the release point for the control fish was directly into the collection tank. The 1.8 m/sec (6 ft/sec) velocity was selected to represent a potential design velocity for a new/retrofit return line. The underwater discharge was selected to represent the ideal design condition and to remove drop height as a stressor from the test system. The original line was tested in conjunction with the extended line to provide a comparison to the initial data and expand on the length-species survival data set compiled during the initial testing.

The return pipes were each 15.24 cm (6 in.) diameter; however, each had a different transport distance (i.e., approximately 21.6 m (71 ft) (original line) and approximately 131 m (430 ft) for the new line). Due to limited interior building space, the new line was run outside and along the full length of the building prior to re-entering the facility. This line included a 0.61m (2 ft) drop, a large radius turn, and several tight turns which included four 180 degree and two 45 degree turns. Each line was constructed with an organism release point at the upstream end just beyond its head tank. The new line had an additional injection point installed just upstream of the tight radius turn segment. Each line contained a 23 degree elbow with a 0.91 m (3 ft) section of clear pipe at the discharge end for the visual observation of water turbulence. Both lines were discharged into the collection box used during the initial testing (Figure 3-1).

A.



B.

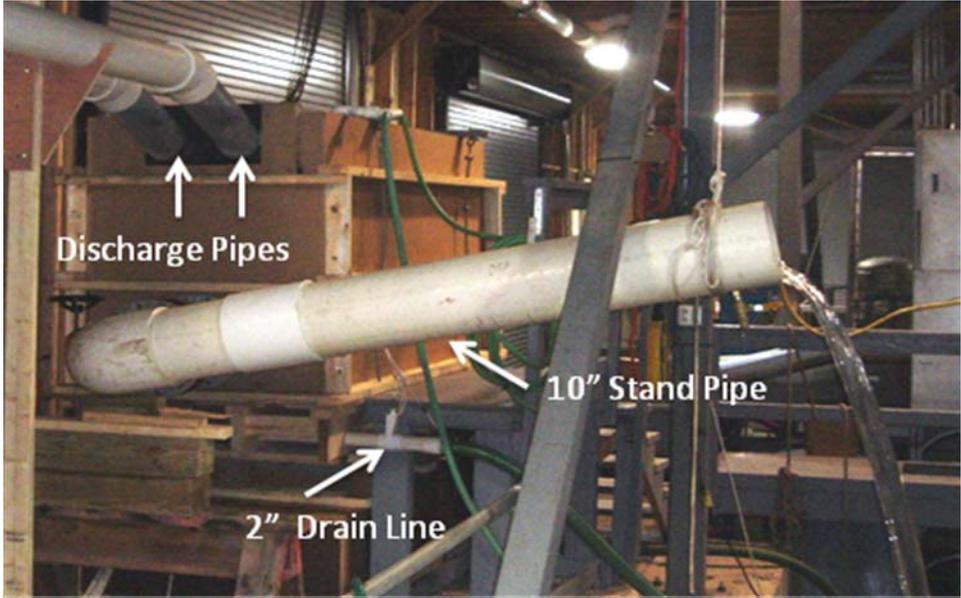


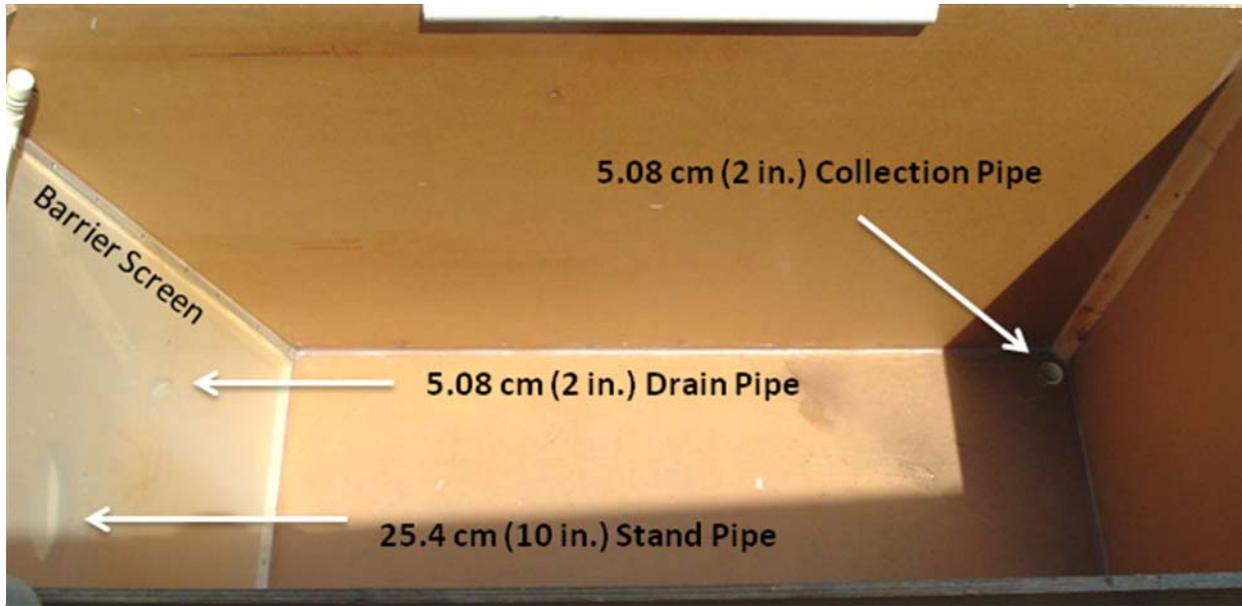
Figure 3-1  
Test facility collection tank at 4 ft (A) and underwater drop heights (B)



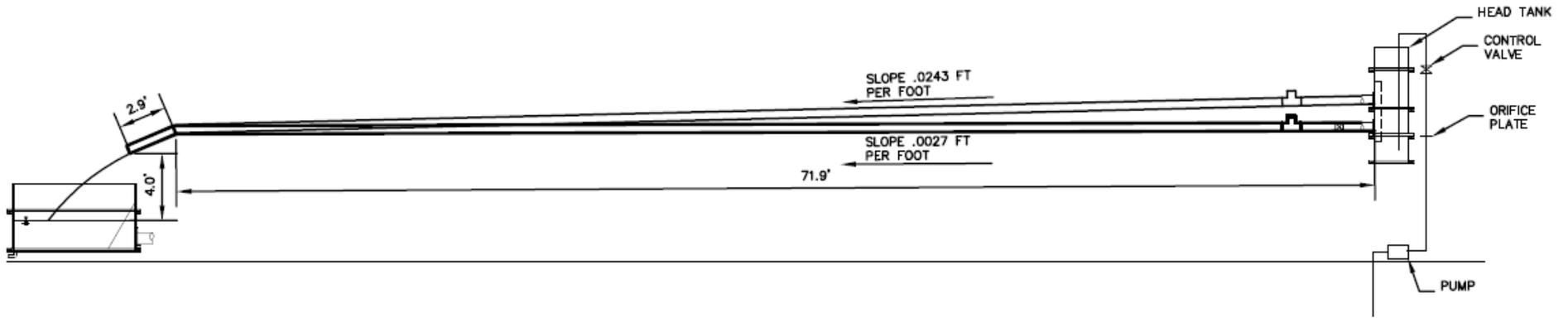
**Figure 3-2**  
Head tank and 5 hp pump



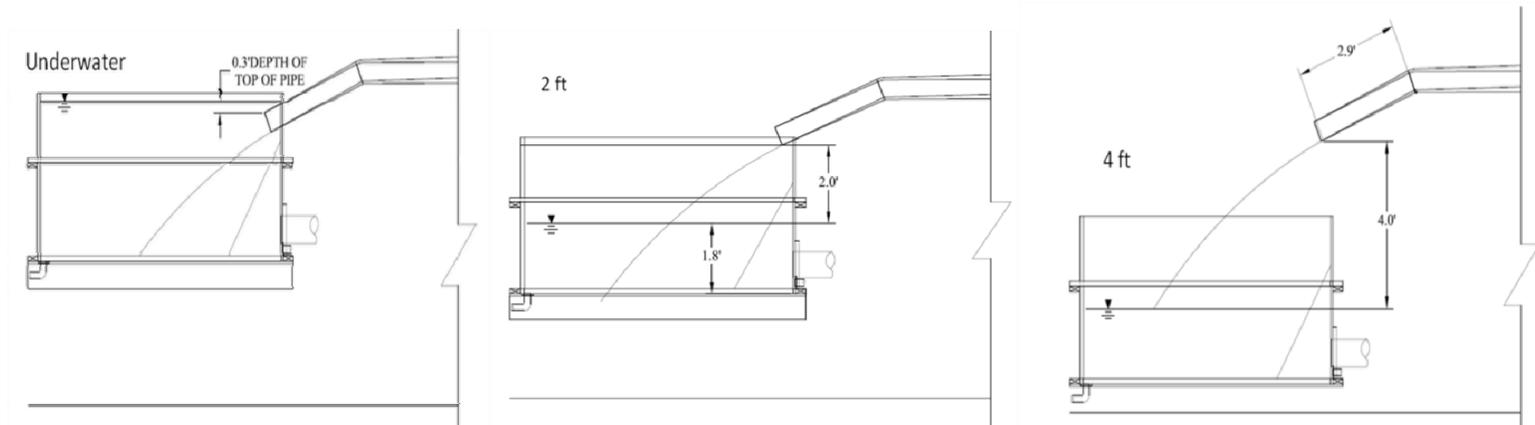
**Figure 3-3**  
Collection tray with collection pan



**Figure 3-4**  
Interior of collection box with various pipe locations



**Figure 3-5**  
Section view of fish return system testing loop with collection box at the 4 ft drop height



**Figure 3-6**  
Configuration of collection box at each drop height; underwater, 2 ft, and 4 ft



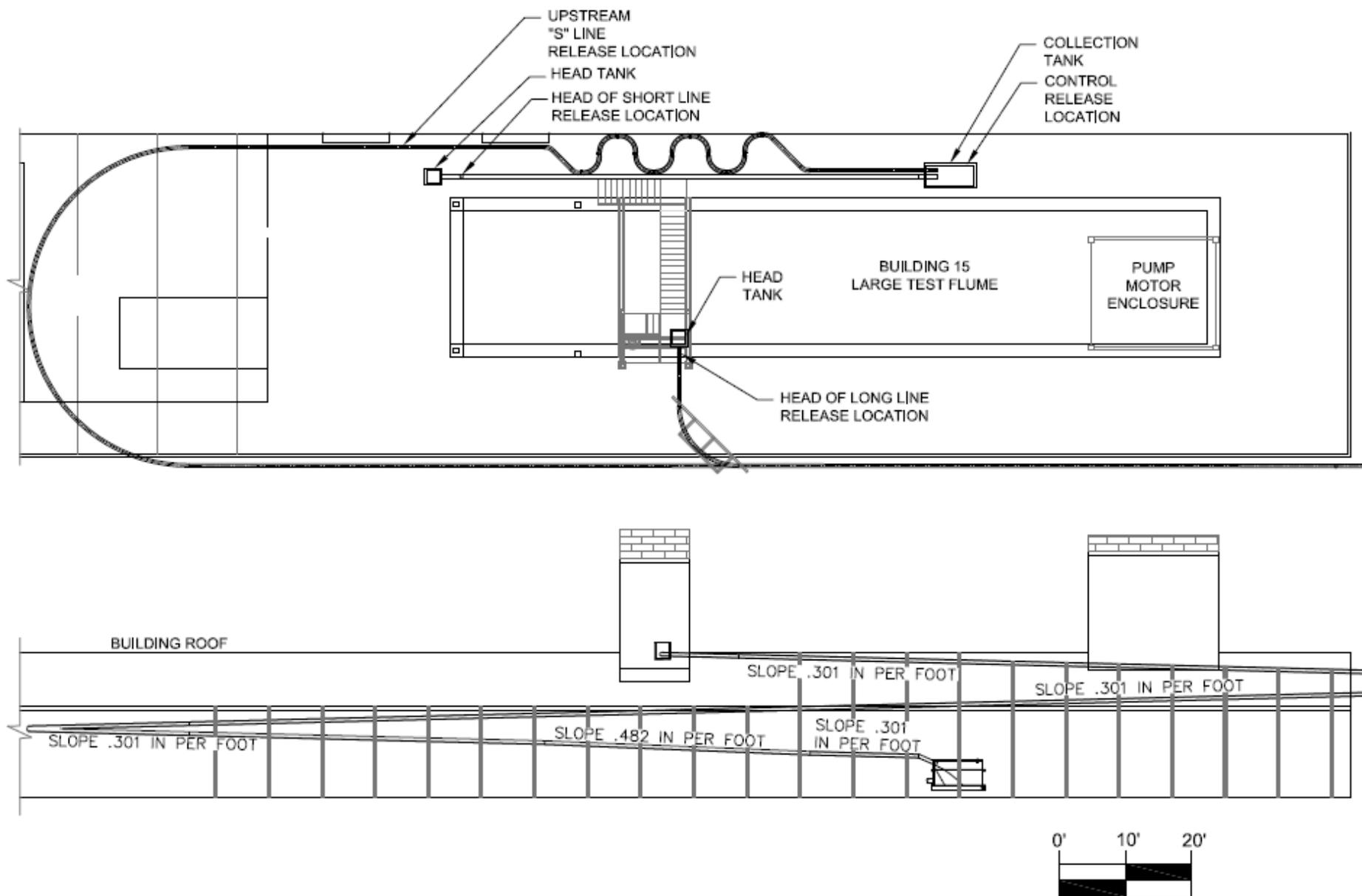
**Figure 3-7**  
Modified return line exiting facility running along the length of the testing building and re-entering the building (water flows from top left center to bottom left)



**Figure 3-8**  
Modified return line tight radius turns looking downstream



**Figure 3-9**  
Modified testing return lines discharging into collection box; original line on right and modified line on left



**Figure 3-10**  
**Plan and section view of the modified fish return line testing facility**

### **Larval and Juvenile Fish Holding Facilities**

Fish larvae were held in a multi-tank closed-loop system that drained into a shared reservoir (Figure 3-11). The larger juvenile fish were held in modified 250 gallon tanks within an adult recirculating system (Figure 3-12). Water was pumped from the reservoir through filters and then back to the fish holding tanks completing the loop. Bag filters and an activated charcoal filter were used to remove solid waste materials and other impurities. An ultraviolet sterilizer and a recirculating biological filter were used to control potential pathogens and nitrogenous waste products. Water quality parameters (dissolved oxygen [DO], temperature, hardness, alkalinity, ammonia, pH, and salinity) were monitored daily and salinity levels were maintained at 1-3 ppt. Water temperatures were maintained between 16 and 24 °C through the use of a chiller or heater to ensure survival and desired growth rate. Water changes (5–20%) were performed as needed. The larvae were fed rotifers (*Brachionus plicatilis*) and a crushed commercial pellet feed as they developed and their dietary needs changed. Fish in the holding facility were routinely examined externally for disease, fungus, or infection by parasites.



**Figure 3-11**  
**Larval fish holding facility**



**Figure 3-12**  
**Modified 250 gallon tanks holding juvenile test fish**

### ***Post-testing Holding System***

To assure comparability of results with on-going EPRI fine-mesh screening studies (EPRI 2008, 2009, 2010b), the same latent mortality (LM) holding system was used (Figure 3-13). The LM system featured a rack plumbed into the larval holding facility. This ensured that the water quality in the LM tanks matched those in the holding facility. The tanks in the LM rack were supplied with a steady drip of filtered water, overflowing through a 100- $\mu$ m, nylon screen into a water bath. LM tank water temperatures were regulated by a high-flow water bath; resulting in identical water qualities between the tanks in the bath rack and holding system. Outflow from the water bath drained back into the reservoir of the larval holding system.

The extended testing provided an additional challenge with regards to LM holding since both small and larger fish would be tested. In an effort to minimize stress to the test fish, it was important to move to larger LM tanks when larger-sized organisms were tested. As a result both

the initial larval LM rack (described above) and a modified juvenile LM system were used (described below, Figure 3-14).

The juvenile LM system featured a series of 250 gallon tanks which each could hold ten modified 2 gallon bucket tanks (Figure 3-15). The tanks in the LM system were supplied and maintained the same as the larval system.



**Figure 3-13**  
**Larval latent mortality rack holding system**



**Figure 3-14**  
**Juvenile latent mortality system**



**Figure 3-15**  
**Juvenile latent mortality tanks within LM system**

## Test Species

Four species were evaluated during initial testing and five species during the extended testing (Table 3-1). Channel catfish were dropped after initial testing due to high survival (> 94%) and replaced with white sucker and bluegill. Because of the costs associated with the disposal of saltwater, potential test species were limited to freshwater species. Selection of species was based primarily on their availability and secondarily on their occurrence and abundance at CWISs. These were the same species and lifestages tested for fine-mesh screen evaluations (EPRI 2009, 2010a).

**Table 3-1**  
**Species used in initial and extended testing**

Family	Common Name	Scientific Name
Ictaluridae	Channel catfish <sup>1</sup>	<i>Ictalurus punctatus</i>
Catostomidae	bigmouth buffalo	<i>Ictiobus cyprinellus</i>
	white sucker <sup>2</sup>	<i>Catostomus commersonii</i>
Cyprinidae	common carp	<i>Cyprinus carpio</i>
	golden shiner	<i>Notemigonus crysoleucas</i>
Centrarchidae	Bluegill <sup>2</sup>	<i>Lepomis macrochirus</i>

1. Dropped after initial testing
2. Added during extended testing

## Experimental Design

The initial tests were designed to determine the survival of larval fish exposed to several velocities at various drop heights at the outfall of a fish return line.

Survival testing included three replicates with each combination of species, lifestage, velocity, and drop height. For each species at a given size, 21 replicates were targeted (2 velocities) × (3 drop heights) × (3 replicates per condition) + (3 controls [1 per height]). To ensure that results for each fish length were comparable all 21 replicates for a given species were completed within one test week. Tests were conducted 3 days/week, each day consisting of seven replicates at a single drop height; this included three replicates at each velocity plus one control per day. The control was used to separate mortality associated with handling (removal from holding facility, counting into groups, collection from fish return, and post-processing of samples) from mortality associated with passage through the return line system. In addition, sets of replicates were repeated as the larvae grew to determine the effect of larval length on survival. Each replicate was held for 48 hours to assess latent effects.

The extended study was designed to determine the survival of fish exposed to increased return line length, drops, and wide and tight bends. Additionally, larger length classes were evaluated to supplement the initial database.

In the extended return line experiments, testing also included three replicates with each combination of species, lifestage, and release locations. For each species at a given size, 12 replicates were targeted (3 release locations)  $\times$  (3 replicates per condition) + (3 controls [1 per day/location]). As in the initial experiments to ensure that results for each fish length were comparable all 12 replicates for a given species were completed within one test week. Tests were conducted 3 days/week, each day consisting of four replicates at a single release location; this included three replicates at that location plus one control per day. The control was used to separate mortality associated with handling (removal from holding facility, counting into groups, collection process, and post-processing of samples) from mortality associated with passage through a fish return line. In the interest of time, multiple species were tested simultaneously; these test groups were separated by species prior to being held for latent mortality. In addition, sets of replicates were repeated as the larvae grew to determine the effect of larval length on survival. Each replicate and control was held for 48 hours to assess latent effects.

## ***Test Procedures***

### **Treatment Replicates**

Survival replicates were conducted as follows:

1. Larvae were removed from the holding system, placed into a beaker of holding water, and transported to the larval workbench. Using a pipette, groups of 50 larvae were counted into LM containers and topped off with water from the holding system.
2. Each container was marked with the test condition, species, and count, and then placed into the LM rack until testing.
3. The collection box was positioned for the proper drop height associated with that day's testing condition.
4. The standpipe valve was adjusted to the appropriate position to maintain the water level within the collection box for the current drop height and velocity being tested. Both the drain valve and collection valve were closed.
5. The pump was activated and the supply valve opened to initiate flow to the test system.
6. The water level within the collection box was verified to ensure the appropriate drop height.
7. A container of fish from the LM rack was removed and introduced into the test system via the injection point at the upstream end of the return pipe for the velocity being tested.
8. Once the test organisms were introduced the pump was shut off and the supply valve closed to minimize the turbulence within the collection box.
9. The collection box was drained using the 2 in. drain valve upstream of the barrier screen.
10. The barrier screen and the exposed portions of the collection box were gently rinsed to prevent adhesion of larvae.
11. After the water had drained to approximately 1 inch, the collection drain gate valve was opened and a collection pan was used to collect larvae.

12. During collection, the box was rinsed toward the collection drain, preventing any adhesion of larvae to the box as the water drained. Finally, the collection drain and gate valve were rinsed.
13. The screens of the collection pan were gently rinsed and the collected larvae were then transported to the fish holding/sample analysis room in the holding facility.

The same basic procedures were followed for the extended tests only modified to reflect the different holding facilities and handling procedures (e.g., lack of use of pipette).

### Control Replicates

Control replicates were conducted as follows:

1. The collection box was filled and maintained with the appropriate water level for the drop height for the test day.
2. With the pump off and both the drain valve and collection valve closed, organisms were introduced into the collection box. For the extended studies, the organisms were held in the box for 3 minutes.
3. The larvae were then collected as described above.

### Sample Processing

Collected fish were processed as follows:

1. The volume of water in the collection pan was reduced and the collected fish were then poured into a plastic sorting tray. Using squeeze bottles (with fish holding water), any remaining larvae in the collection pan were rinsed to the sorting tray.
2. Using a pipette or dip net, live fish were removed, enumerated, and placed into a LM tank (labeled with the appropriate test code). Dead larvae were counted and placed into labeled beakers for proper disposal.
3. All counts and additional observations were recorded on testing datasheets.
4. After sorting was completed, the LM tanks were transferred to the LM holding system.

### Latent Mortality Assessment

LM assessments were made at 24 and 48-hours after testing as follows:

1. At 24 hours, dead fish were removed and the number recorded on the test datasheet.
2. The LM tanks containing the remaining live larvae were returned to the LM rack.
3. At 48 hours, the contents of the LM tanks were poured into sorting tray. Dead fish were removed, counted, and recorded as the number dead at 48 hrs. Remaining fish were then enumerated and recorded as live at the end of the 48 hours.

## Fish Length Determination

For the initial tests all test species arrived as eggs and were tested as soon after hatching as possible. Testing with any given species continued each week with the same cohort until insufficient numbers or fish were available to complete a full set of replicates during the test day. Morphometric measurements were recorded during each test day for each of the species tested, as follows:

1. Twenty (20) larvae of each species were randomly selected from the holding system as a representative subsample.
2. During the initial testing, each larva was measured to the nearest 0.1 mm for either notochord length (in larvae that had not yet developed fin rays) or total length (in larvae that had developed fin rays) with a microscope and ocular micrometer. For the extended studies, a digital image was taken of the larval sub-sample using a digital camera and the photo was uploaded to a laboratory computer.
3. The digital image processing program ImageJ (NIH 2007) was used to make morphometric measurements of each larva's body length (standard or notochord). This program calculates measurements by comparing the unknown distance in pixels of a line designated by the user to the known distance in pixels of a reference scale [a metric ruler was used in this case; (Figure 3-16)]. Measurements were taken to the nearest 0.01 mm and saved for statistical analyses.



**Figure 3-16**  
**Photograph of bluegill arranged on Petri dish and numbered**

## **Statistical Methods**

Survival was calculated in three ways prior to statistical analysis; initial survival, latent mortality, and total survival. Initial survival was calculated by enumerating the number alive at the end of the test and dividing by the total number of live and dead organisms collected. Latent mortality survival was calculated using the total number of live organisms at the end of 48 hours and dividing it by the total number held for latent mortality observation. Total survival was calculated using the total number alive after the 48 hour observation and dividing it by the total number collected during that replicate.

In addition to the descriptive statistics, data were analyzed using logistic regression in the generalized linear model (GLM) (McCullagh and Nelder 1989). In this form the logit link function was employed and an over-dispersed binomial distribution was chosen to model random error to account for extra-binomial variation from date to date.

The initial studies were designed to test velocity and drop height effects on fish survival; for the extended studies the primary design variable was release location. A GLM was applied with a model composed of a factor with seven levels ( $2 \times 3$  factorial + control) and mean fish length was modeled as a covariate. The  $2 \times 3$  structure represents two levels of velocity crossed with three levels of release height. The factorial information was recovered using linear contrasts of the seven treatments. However, graphical analysis of the extended studies data disclosed that the survival-to-fish-length relation differed for two size groups of fish. To model this differing response, the fish were blocked by size and a different survival-to-length slope parameter was estimated for each size group.

The analyses were computed using the GLM functions as implemented in the “r” statistical programming language (Wood 2006; Chambers and Hastie 1992; R Development Core Team 2009).



# 4

## LABORATORY RESULTS

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For the initial testing, over 260 replicates were conducted between May 27 and July 16, 2008. Testing focused on four species: channel catfish, common carp, golden shiner and bigmouth buffalo. These species were being tested at the same time in another EPRI-funded, fine-mesh screen study (EPRI 2010a). This study evaluated the effects of drop heights and velocities in a fish return system on larval fish mortality. Velocity and drop heights examined were selected based on the expected range of what might be designed for use at CWIS. In most cases, analysis failed to detect significant differences between treatment results and controls, indicating that drop height and velocity had no impact on total survival of the test species.

For the extended studies, over 220 replicates were conducted between May 26 and July 22, 2009. White sucker and bluegill were added and channel catfish dropped during these studies (in addition to the initial species tested). Again, these species were being tested at the same time in the companion EPRI sponsored fine-mesh screen study (EPRI 2010a). These tests were designed to evaluate the effects of travel distance, elevation drops, and bends as additional stressor elements.

The statistical analysis of total survival by species is provided below.

### Initial Testing

The length data for the species tested over the duration of the study is provided in Table 4-1.

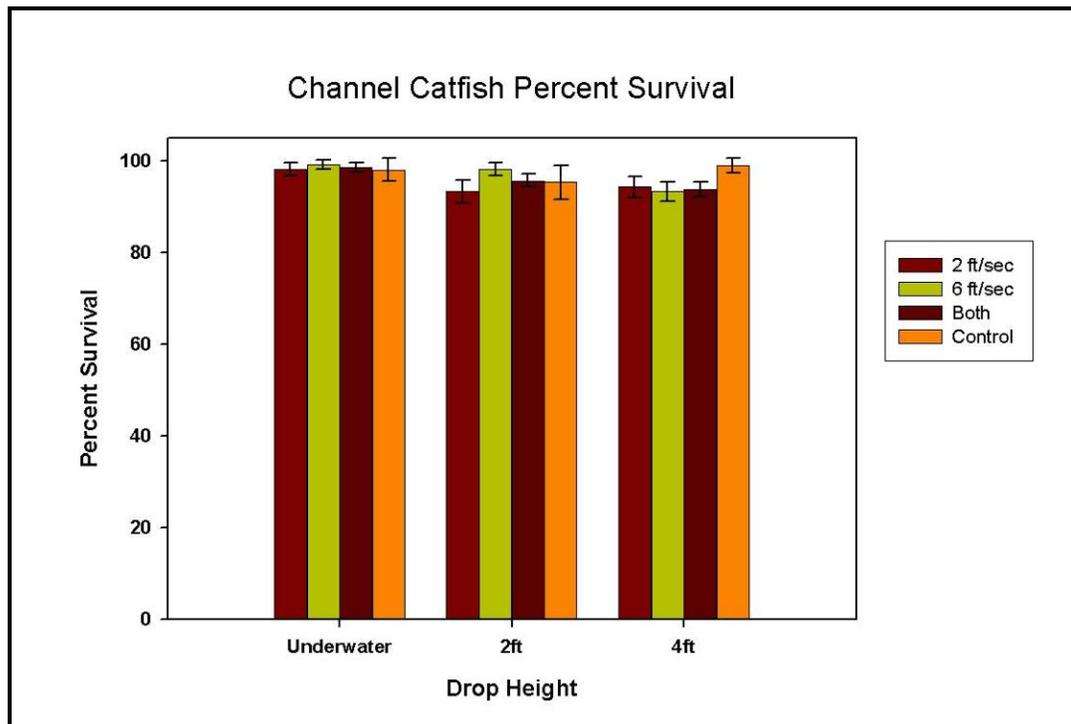
**Table 4-1**  
**Mean, minimum, and maximum of length of larvae tested, including the standard deviation around the mean, and the number of organisms measured to estimate length**

Species	Total Length				
	Mean (mm)	Standard Deviation	Minimum (mm)	Maximum (mm)	Number Measured
Bigmouth buffalo	8.2	2.1	6.2	11.7	260
Channel catfish	14.3	3.3	10.4	17.2	200
Golden shiner	6.4	2.2	4.2	11.3	180
Common Carp	9.4	2.8	5.6	12.8	500

### Channel Catfish

Over 67 replicates with channel catfish were conducted during three weeks of testing. Of the four species tested, channel catfish exhibited the highest survival for both velocities and all drop heights (Figure 4-1). The initial survival observed for all conditions was high (> 94%) and in 84% of the treatment replicates initial survival was 100% regardless of condition. Statistical analysis of the initial survival data showed no significant difference between treatments and controls ( $P=0.1975$ ). However, analysis of total survival showed a statistically significant difference between the treatment and the control ( $P=0.0084$ ; Table 4-4); specifically for the 4 ft drop at 6 ft/sec ( $P=0.0417$ , Table 4-5). Additionally, the total survival for the underwater discharge condition was statistically higher (99%,  $P=0.0045$ , Table 4-6) than those treatments run at the 4 ft drop (94%) and there was no significant difference ( $P=0.1702$ ) between the velocities. While there was a statistical difference between the underwater and 4 ft drop height, this may not be biologically significant given the high total survival at both drop heights ( $\geq 94\%$ ).

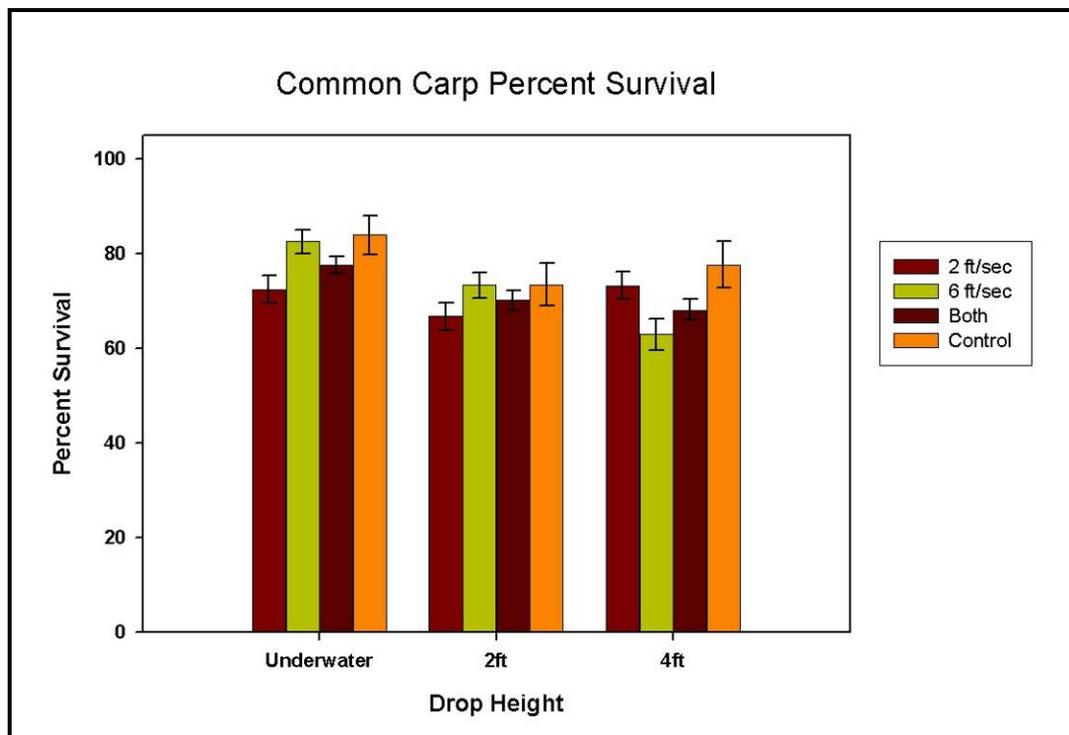
In comparing fish lengths and drop heights, survival is high among all length groups for both velocities and control conditions for the underwater discharge. Channel catfish results showed no trend between total survival and length, given that total survival was high for all length classes. This trend is supported by the statistical analysis which showed no significant length effect on total survival ( $P=0.0616$ , Table 4-4).



**Figure 4-1**  
Channel catfish total survival averaged over all conditions and replicates conducted during the study. Error bars represent 95% confidence intervals.

### Common Carp

High numbers of common carp were available and tested because of their excellent survival in the holding system; 141 replicates were conducted over the course of seven weeks; the full length of the study period. Overall, total survival was high but did not match that observed with channel catfish (Figure 4-2). The statistical analysis revealed that there was a significant difference in total survival among treatments ( $P=0.0002$ , Table 4-4), more specifically among the 2 ft-2 ft/sec ( $P=0.0039$ ) and 4 ft-6 ft/sec ( $P=0.0034$ ) treatment conditions (Table 4-5). Additionally, when the survival among each variable was statistically compared, the results indicate that there is no significant difference between the velocities ( $P=0.1590$ ); however, both the 2 ft and 4 ft drop heights have significantly lower survival than the underwater discharge ( $P=0.0458$  and  $0.0471$ , respectively; Table 4-6). This indicates that, like channel catfish, common carp show increased mortality when exposed to greater drop heights independent of the velocity. Total survival for the controls for smaller length groups ( $< 8$  mm) were noticeably lower than larger length groups as seen in Table 4-2 (8-11 mm and  $>11$  mm). This trend of increasing survival may coincide with a lifestage transition from yolk-sac to post-yolk-sac. Yolk-sac absorption is complete for most carp larvae at lengths from 6.5 to 7.0 mm (Wang and Kernehan 1979). This trend in control survival confirms that these smaller larvae are less hearty than larger larvae and are more vulnerable to handling and other stressors. Other research has demonstrated that larval lifestage transition is a period when larvae are more vulnerable to stress (e.g., EPRI 2008, ESEERCO 1981). The statistical analysis of length effect on survival showed a significant increase in survival as organisms increased in length ( $P=<0.0001$ , Table 4-4).



**Figure 4-2**  
Common carp total survival averaged for all conditions and replicates conducted during the study. Error bars represent 95% confidence intervals.

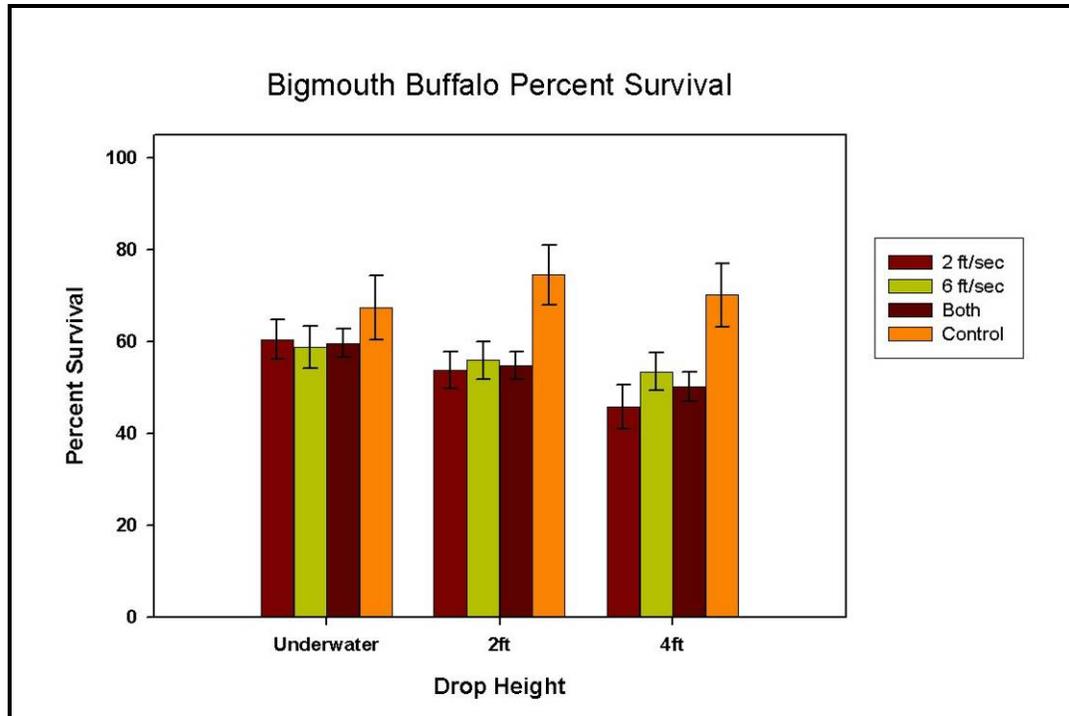
**Table 4-2**  
Common carp average control survival at various length groups

Length Group	Percent Survival
< 8 mm	64
8-11mm	85
>11mm	85

*Bigmouth Buffalo*

Over 80 replicates were conducted during four weeks with bigmouth buffalo. The average total survival at each drop height and velocity condition was below 60% and the control total survival was approximately 70% (Figure 4-3). However, there were no statistically significant differences in total survival between the control and treatment replicates ( $P=0.4441$ , Table 4-4).

When a comparison of survival is made with respect to larval length, a more defined decrease in survival was observed for all conditions at the 7 mm length (Table 4-3). This trend was observed with common carp and was likely a result of lifestage transition from the yolk-sac to post-yolk-sac. Survival of carp increased with increasing length and hardiness. Bigmouth buffalo also showed high control survival as lengths increased over 8 mm. Statistical analysis showed a significant increase in survival as fish increased in length ( $P<0.0001$ , Table 4-4).



**Figure 4-3**  
Bigmouth buffalo total survival averaged for all conditions over the duration of the study. Error bars represent 95% confidence intervals.

**Table 4-3**  
**Bigmouth buffalo average control total survival at various length groups**

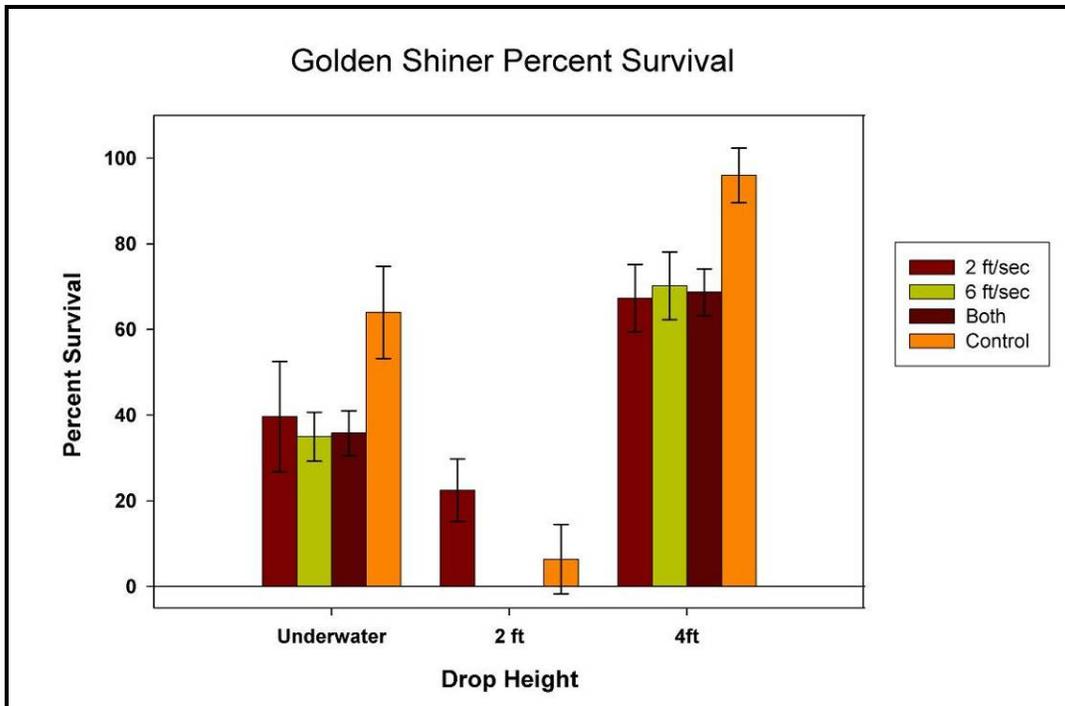
Length Group	Percent Survival
6 mm	82
7 mm	54
> 8mm	80

### *Golden Shiner*

Data for golden shiner were limited due to poor survival in the holding facility and during early lifestage testing. In the interests of conserving larval numbers, testing with shiners was conducted iteratively; that is, if the observed initial mortality was high during a treatment at a gentle condition (i.e., underwater height at 2 ft/sec) the remaining testing was not completed for the week. This assumes that mortality would only increase under harsher conditions. During the tests, the drop height increased as the week progressed and testing was terminated after two replicates of poor initial survival.

For the first two days of testing, shiner survival numbers (including controls) were considerably lower than other species at the yolk-sac lifestage, which resulted in a small number of conditions tested during the first week (Figure 4-3). In addition, the golden shiner holding system experienced nitrogen super-saturation when a seal on the pump inlet entrained air. These fish exhibited large gas bubbles in the gastrointestinal tract and hyper-inflated swim bladders, which resulted in high mortality. The remaining larvae were allowed to acclimate and grow for an additional week before testing. Poor survival (61% average) at the underwater, 6 ft/sec velocity condition delayed additional testing another week. The remaining fish were tested at the 4 ft drop height under both velocity conditions. The control survival during this test was considerably higher (96%) than previous weeks.

The statistical analysis of total survival indicated that there was a significant difference between treatments and controls ( $P=0.0123$ , Table 4-4), specifically at the 4 ft discharge height ( $P=0.0285$ , Table 4-5). A significantly higher survival was observed for the 6 ft/sec velocity when compared to 2 ft/sec ( $P=0.0425$ , Table 4-6) and the 4 ft discharge when compared to the underwater ( $P=0.0100$ , Table 4-6). These findings are most likely the result of the limited numbers tested and the poor survival observed with this species over the course of the testing.



**Figure 4-4**  
**Golden shiner total survival averaged for all conditions over the duration of the study. Replicates were not conducted at 2 ft/sec at the UW drop condition. Due to low survival at 2 ft/sec, no replicates were conducted at the 6ft/sec, 2 ft drop condition.**

**Table 4-4**  
**P-values for the length and total survival regression models for all species tested during the initial testing <sup>a</sup>**

Species	Length	Treatment Effects
Channel Catfish	0.0616	0.0084
Common Carp	<0.0001	0.0002
Bigmouth Buffalo	<0.0001	0.4441
Golden Shiner	<0.0001	0.0123

<sup>a</sup> Significant  $\leq 0.05$

**Table 4-5**  
**Pertinent *P*-values for those treatments in which total survivals were lower than controls for all species tested <sup>a</sup>**

Species	UW-2 ft/sec	UW-6 ft/sec	2 ft-2 ft/sec	2 ft-6 ft/sec	4 ft-2 ft/sec	4 ft-6 ft/sec
Channel Catfish	ns	ns	Ns	ns	ns	0.0417
Common Carp	ns	ns	0.0039	ns	ns	0.0034
Bigmouth Buffalo	ns	ns	Ns	ns	ns	ns
Golden Shiner	ns	ns	Ns	ns	0.0285	0.0410

<sup>a</sup> Significant  $\leq 0.05$ ; ns = Not Significant; UW = Underwater

**Table 4-6**  
**Summary of pertinent *P*-values from the drop height and velocity comparisons for the total survival regression models for all species tested <sup>a</sup>**

Species	UW v 2 ft	UW v 4 ft	2 ft v 4 ft	2 ft/sec v 6 ft/sec
Channel Catfish	ns	0.0045	ns	ns
Common Carp	0.0458	0.0471	ns	ns
Bigmouth Buffalo	ns	Ns	ns	ns
Golden Shiner	ns	0.0100*	ns	0.0425

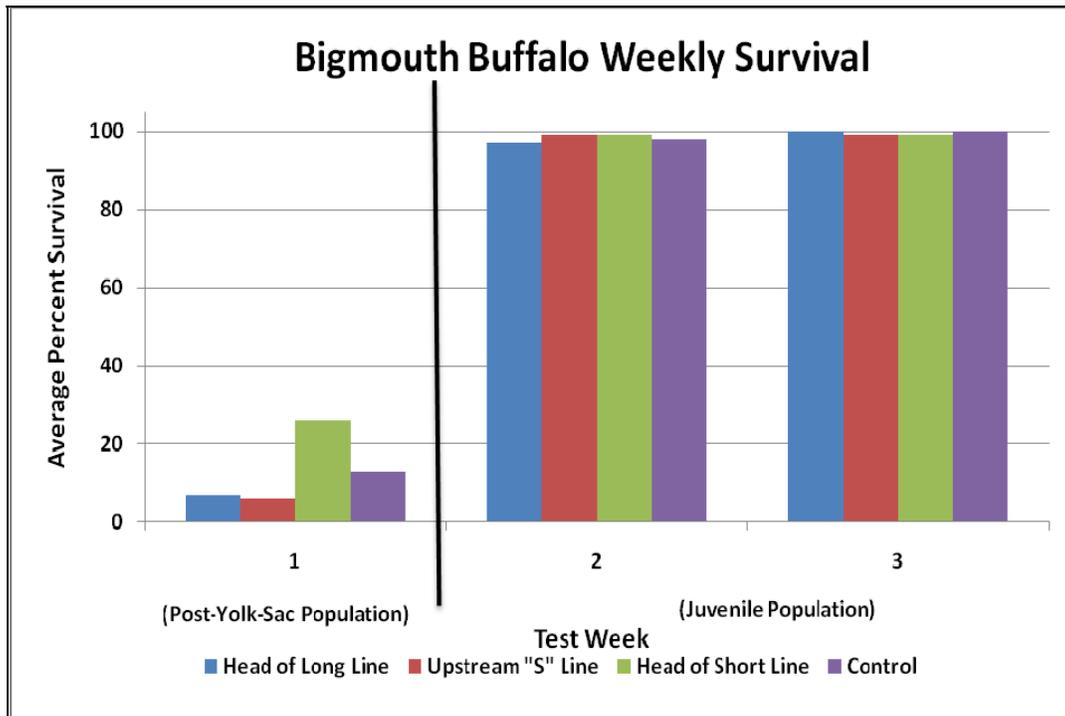
<sup>a</sup> Significant  $\leq 0.05$ ; \*4 ft survival was significantly higher than UW; ns = Not Significant

## Extended Testing

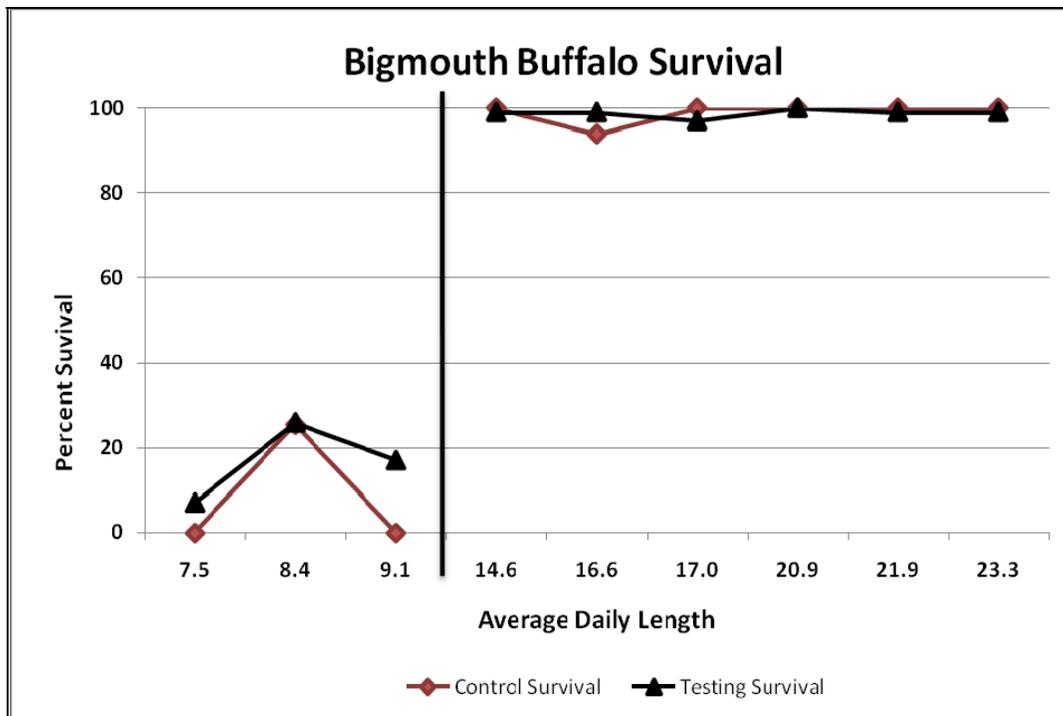
### *Bigmouth Buffalo*

Fifty-four bigmouth buffalo replicates were tested over three weeks in 2009. This species experienced high mortality early in the testing period (Figure 4-5). This mortality was observed and documented simultaneously in both the treatment and control groups, as well as in the holding system. The holding system mortality event reduced the number of test fish available in the lower length range ( $< 10$  mm) thereby limiting the test period to one week for this length class. An additional shipment of juvenile bigmouth buffalo ( $> 14$  mm) were received and available for testing during the additional two weeks of testing. This juvenile group showed consecutive weeks of high survival at approximately 100% for all release locations and controls (Figure 4-6). This high survival resulted in the termination of testing after a total test period of three weeks.

There was a strong relationship ( $P < 0.0001$ , Table 4-8) between fish length and total survival, with larger bigmouth buffalo exhibiting higher survival (Figure 4-6). The high mortality experienced in the holding system resulted in fewer numbers of bigmouth buffalo available for testing. Consequently, the effect of transition from yolk-sac to post-yolk-sac could not be demonstrated.



**Figure 4-5**  
Bigmouth buffalo average total survival for controls and all release locations for each test week



**Figure 4-6**  
Bigmouth buffalo control and testing total survival by average daily length in mm

**Table 4-7**  
*P*-values for total survival regression models for all species tested during extended testing<sup>a</sup>

Species	<i>P</i> -value
Bigmouth buffalo	0.9169
Bluegill	0.2634
Golden shiner	0.4828
Common carp	0.8715
White sucker	0.7391

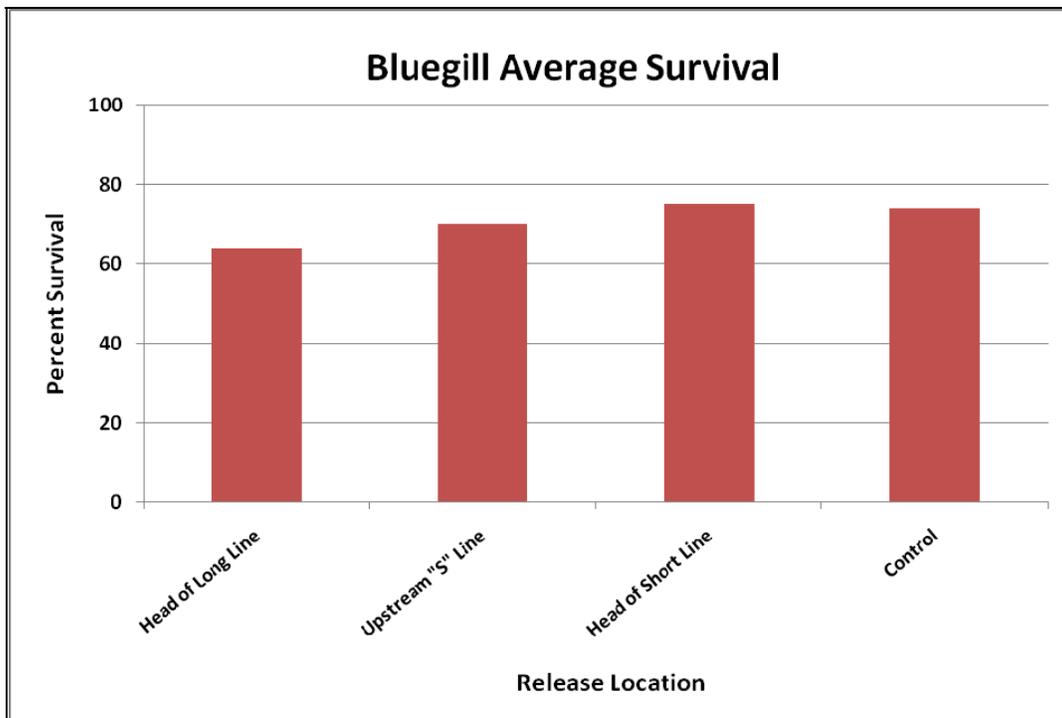
<sup>a</sup> Significant  $\leq 0.05$

### Bluegill

Twenty-four replicates were completed over a two week testing period with bluegill. The average total survival for all release locations were similar (64-75%) across the study period (Figure 4-7) and the statistical analysis revealed no significant differences in total survival by release location ( $P=0.2634$ ). Total survival was moderate to low during the first week of testing and coincided with the mortality seen with the controls and in the holding system. The difficulty in testing bluegill at the earlier life stages stems from their vulnerability to stress and handling,

which made collection and shipment to the test site difficult. A second shipment of hardier juvenile bluegill was received and tested during the second week of testing. Total survival for all test conditions increased to an average greater than 98% (Figure 4-8). Bluegill testing was not continued since this juvenile size class showed consecutive high total survival values regardless of the test condition.

In a comparison of fish length and control total survival there is a noticeable shift in survival from 12.5 to 17.5 mm in length (Figure 4-9). This difference in survival by length was significant ( $P < 0.0001$ , Table 4-8). Due to the limited number of test organisms of the earlier post-yolk-sac bluegill, the critical length at which this survival change happens was not captured in the data. The relationship between bluegill control and test survival is similar to that observed with bigmouth buffalo indicating that transport within the fish return line is not a factor causing mortality among bluegill.



**Figure 4-7**  
**Bluegill average total survival for controls and all release locations**

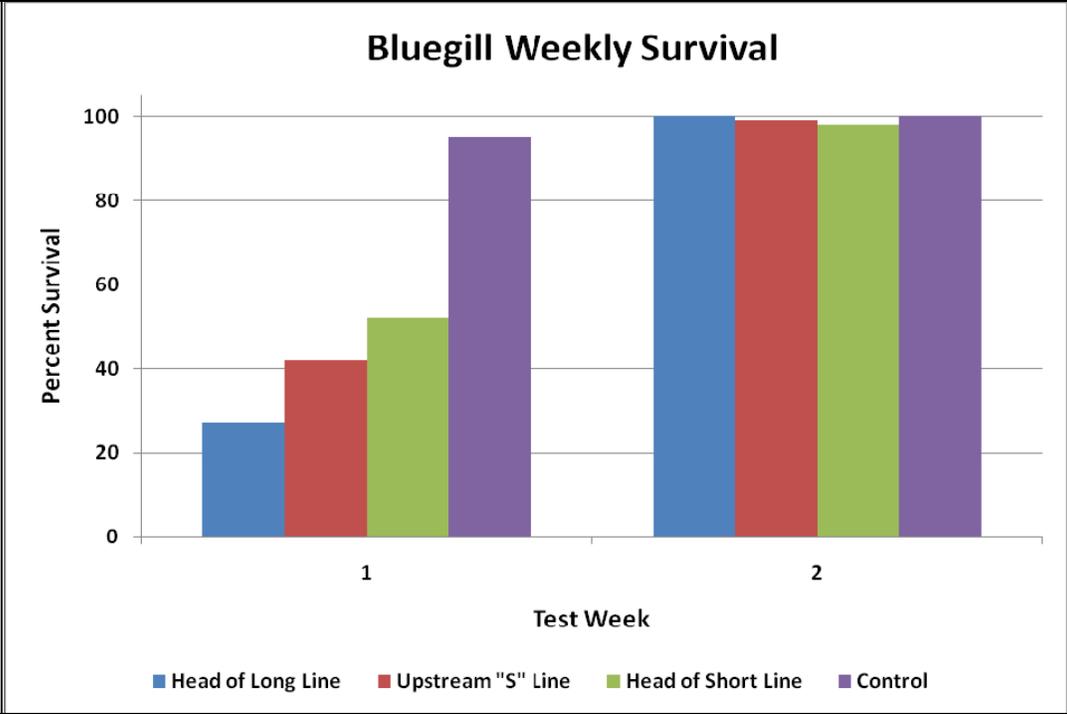


Figure 4-8  
Bluegill average total survival for all release locations for both testing weeks

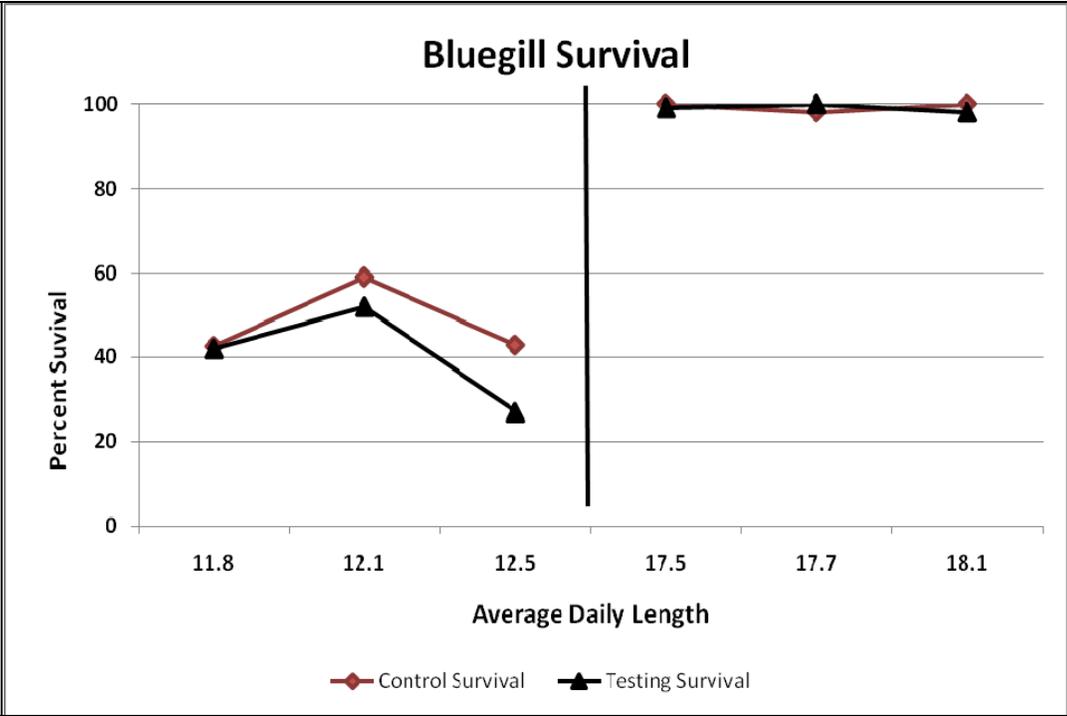


Figure 4-9  
Bluegill control and testing total survival by average daily length in mm

**Table 4-8**  
**P-values for the survival by length regression models for all species tested during extended testing <sup>a</sup>**

Species	p-value
Bigmouth buffalo	<0.0001
Bluegill	<0.0001
Golden shiner	<0.0001
Common carp	<0.0001
White sucker <sup>b</sup>	0.6761

<sup>a</sup> Significant  $\leq 0.05$

<sup>b</sup> Limited length range tested – precluded correlation

### Golden Shiner

Seventy-two replicates were completed for golden shiner during the study period. The average total survival for treatment fish was moderate (67-81%) for all release locations (Figure 4-10). The upstream “s” line release location had the lowest total survival (67%); however, this lower total survival was not statistically significant ( $P=0.4828$ ). When the data are broken out by test week it is apparent that this moderate to low average total survival for each release location was a result of testing during the first week (Figure 4-11). Plotting control survival against the daily length data confirms that survival was lower during the earlier weeks of testing when shiners were smaller and potentially more fragile (Figure 4-12). When total survival is plotted along with control survival at each fish length tested, results observed for the other species were also observed for golden shiner (Figure 4-12). This trend shows that transport within a fish return line is not the major contributor to the mortality observed during this testing. It appears that golden shiners are vulnerable to stressors until they reach a length of 7.0-9.0 mm, at which point they begin to experience increased survival (Figure 4-12). The statistical analysis confirms that difference in total survival by length was significant ( $P<0.0001$ , Table 4-8) and that as the larvae increase in length so does survival.

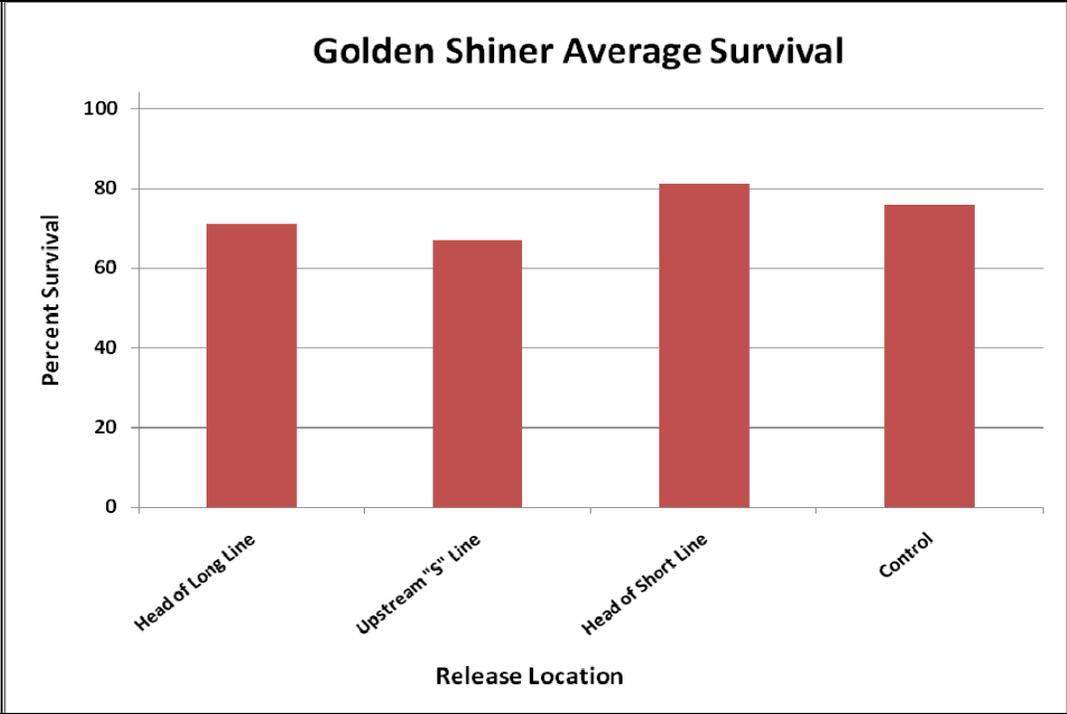


Figure 4-10 Golden shiner average total survival for controls and all release locations

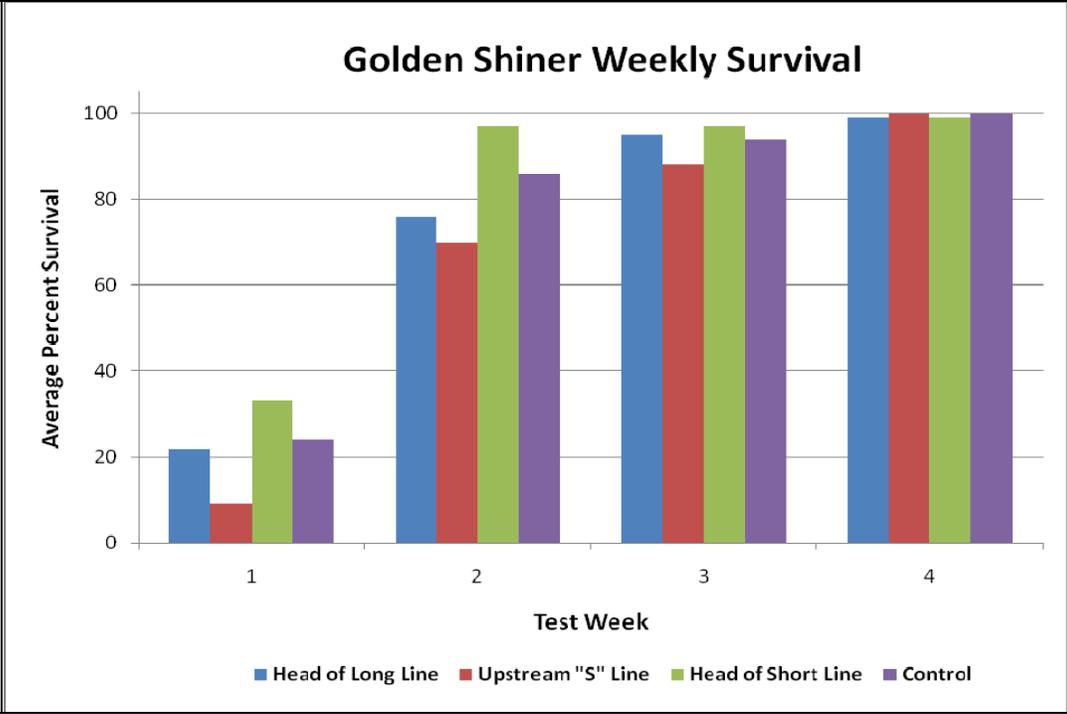
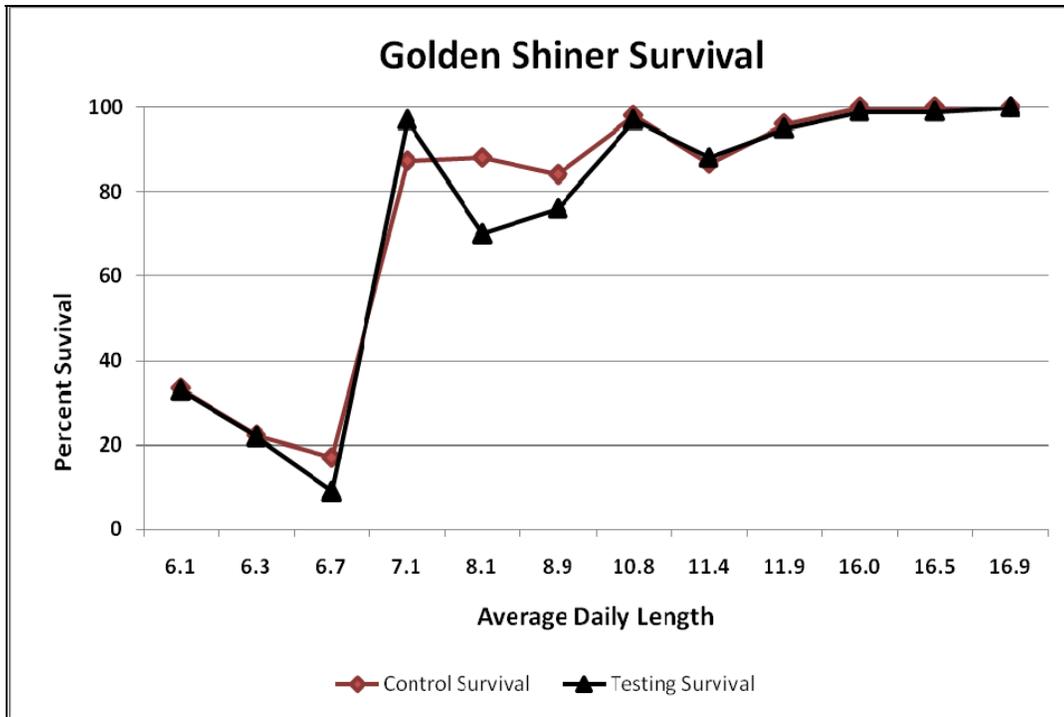


Figure 4-11 Golden shiner average total survival for each drop location during each test week



**Figure 4-12**  
Golden shiner control and testing total survival by average daily length in mm

*Common Carp*

Sixty common carp replicates were tested over the full length of the study period. The average total survival was moderately high for all conditions (Figure 4-13). The survival for the head of the long line location was noticeably lower at 72%, however, when the data are broken out over each week of testing it appears that this lower average is only a result of the first week and not significant throughout the testing period (Figure 4-14). In addition, the total survival by release location was not statistically significant ( $P=0.8715$ ). As with bluegill and golden shiner, a majority of the mortality appeared early in testing and appears associated with handling and the collection box, since this trend is also apparent within the control groups. Control total survival was also very low during the first week of testing (17%), but showed a substantial increase during the remainder of the testing period (Figure 4-15).

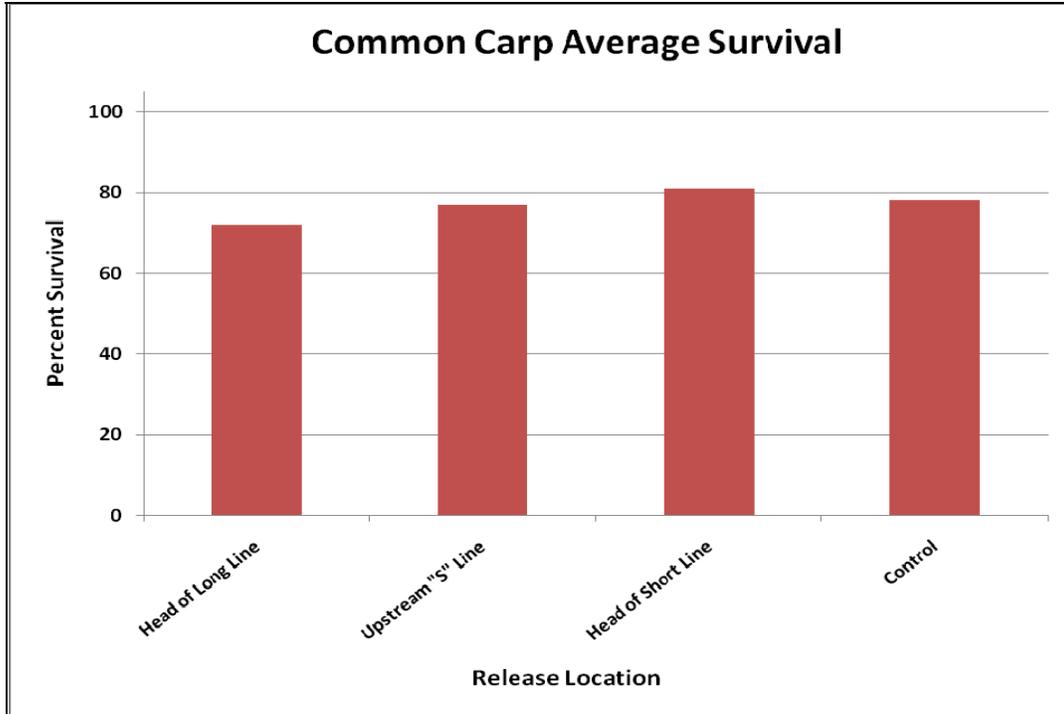


Figure 4-13  
Common carp average total survival for controls and all release locations

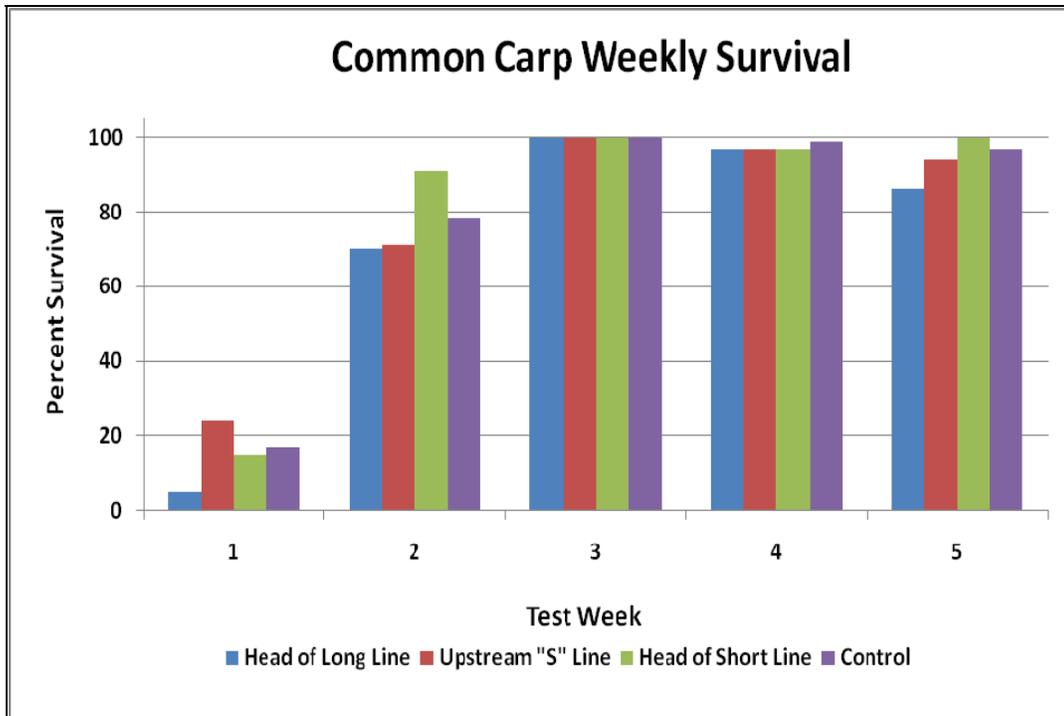
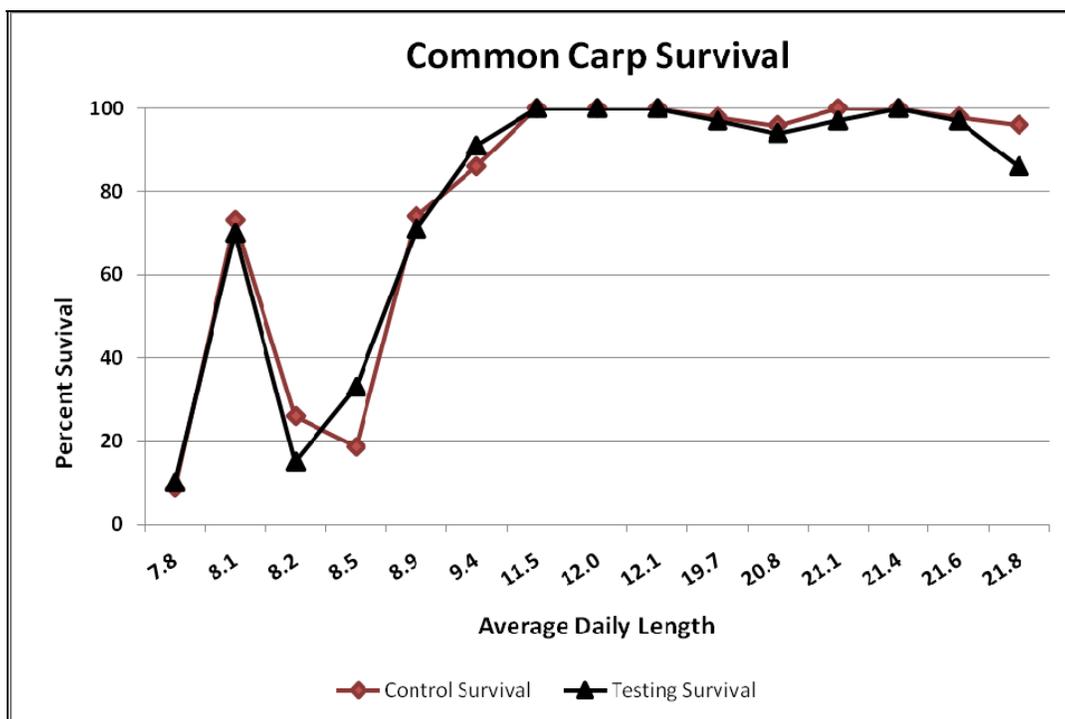


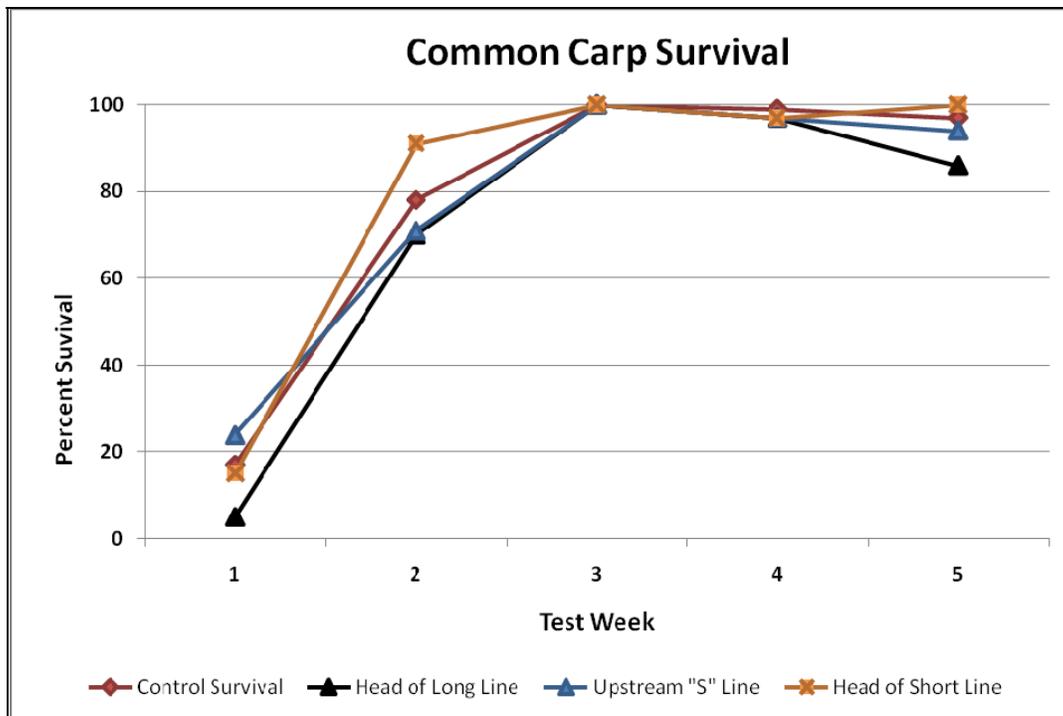
Figure 4-14  
Common carp average total survival for each drop location during each test week, which have been adjusted for control mortality

In a comparison of fish lengths and both daily test and control total survivals it is apparent that survival is highly variable below 9 mm; however, once the carp reach a length range of 9.0-12.0 mm survival improves dramatically and remained nearly 100% through 20 mm in length (Figure 4-15). Additionally, when graphing average daily testing total survival along with the daily control total survival, each line shows a similar trend (Figure 4-15). Length was a significant predictor of survival with larger fish experiencing greater total survival ( $P < 0.0001$ , Table 4-8). As described in the methods section, each test day consisted of a different release location; therefore, each line on Figure 4-16 represents a daily average total survival at one release location. The similar testing and control survival trends indicate that release location and exposure to a return line does not appear to be a substantial contributor to mortality for the common carp lengths tested during 2009.

When weekly total survival is plotted for each drop location and for all controls, again a similar survival trend is observed (Figure 4-16). The average total survival among each drop location and control for the first two weeks were within 20-25 percent of each other, however, the trend from week one to week two was similar for each condition. Additionally, the following testing weeks were not only similar in their survival trends but also amongst each other within a given week. This confirms the trend seen in the comparison between control and test groups within a similar length group and supports the finding that transport within a fish return line does not result in mortality to carp; rather it is the vulnerability of the species at earlier life stages or shorter lengths that influences mortality.



**Figure 4-15**  
Common carp control and testing total survival at the average daily lengths in mm

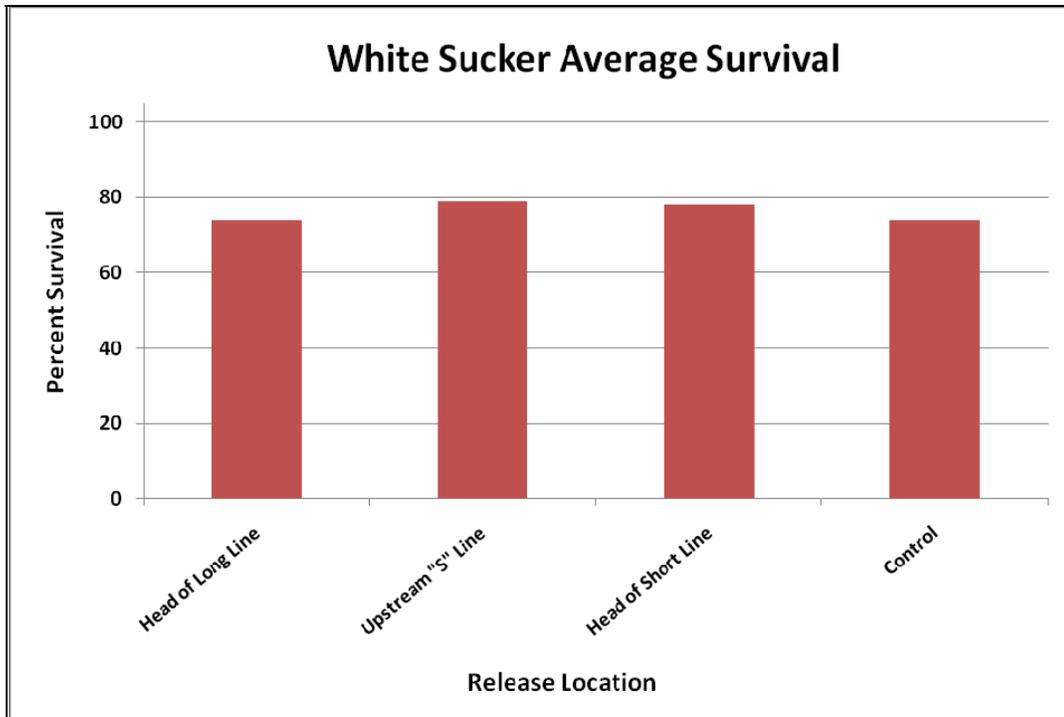


**Figure 4-16**  
Common carp weekly average total survival for controls and test release location

#### White Sucker

White sucker were only available for a limited period of time and only used for shakedown testing of the modified fish return system before the primary test species (i.e. bigmouth buffalo, bluegill, golden shiner, and common carp) became available and the full series of test experiments were conducted. Twenty-seven replicates were conducted with white sucker during the shakedown testing. These results provided valuable additional data to compliment the data collected for the primary test species during the extended testing. There was very little variability in the average total survival regardless of release location (74-79%; Figure 4-7). In fact, there were no statistical differences between the treatments and the controls ( $P=0.7391$ ). These results indicate that the majority of the mortality observed was natural or associated with handling.

With a limited length range tested over the study period (13.0-14.5 mm) it was not possible to determine a correlation between fish length and survival ( $P=0.6761$ , Table 4-8). The consistent total survival observed for all lengths and at all release locations indicates that the test system did not impart differential mortality among the white sucker tested.



**Figure 4-17**  
**White sucker average total survival for controls and all release locations**

In summary, these studies included a variety of conditions which were believed to potentially affect larval fish survival through a fish return system. As was the case with tests of fine-mesh screens, species selection was limited by commercial availability. With the exception of high mortality during the period of transition from yolk-sac larvae to post-yolk-sac larvae, for the species and conditions tested the fish return system does not appear to add additional mortality. Ultimately, site-specific factors such as screen mesh size and the lifestage(s) of species exposed to the screens will determine the total survival of the organisms handled.

# 5

## SUMMARY AND DISCUSSION

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### Laboratory Evaluation of Fish Return Systems

This study was designed to evaluate the effects of velocity, drop height, length, drops and bends on larval fish survival through a fish return system. Testing was limited to the freshwater species being tested in companion EPRI-sponsored studies evaluating the performance of several types of fine mesh screens (EPRI 2010a). These species were selected because they were readily available as larvae or could be easily cultured in the laboratory. The fine-mesh screen study was conducted initially with 0.5 mm mesh screens and expanded to evaluate 2.0 mm mesh screens. This resulted in smaller fish being tested initially and larger fish being tested during the extended tests. A summary of the results for each condition is as follows:

- Fish length: Survival for all species dropped during the transition from yolk-sac to post-yolk-sac larvae with the exact length varying among species. Survival increased rapidly with increasing fish length with a peak when larvae attained a size of approximately 12 mm.
- Velocity: Velocity had no effect on survival within length groups.
- Drop height: With the exception of common carp during the initial testing, drop height ( $\leq 1.8$  m or 6 ft) had no effect on survival within length groups.
- Length, drops and bends: Increasing the length (from 21.6 m (71 ft) to 131 m (430 ft)) of the fish return line and adding drops and bends did not affect survival within length groups.

Survival varied among species and lifestages within species. Total survival varied from less than 20% for bigmouth buffalo, golden shiner, and common carp to approximately 40% for bluegill and to greater than 90% for channel catfish during the first week of testing and mirrored the mortality seen with the controls and the holding system. The mortality observed during the first week of testing for buffalo, shiners and carp coincides with the transition from yolk-sac to post-yolk-sac; other research has demonstrated that larval lifestage transition is a period during which larvae are more vulnerable to stresses (EPRI 2009, ESEERCO 1981). For all species tested, survival ranged from 70-100% after the fish were approximately 11.0 mm in length. These results are similar to those observed for species tested in the fine-mesh screen studies; fish 12 mm or greater consistently showed high post-collection survival regardless of species, screen type, or approach velocity (EPRI 2009; 2010a). This increase in survival appears to be correlated to scale development and general increase in body musculature. These results are consistent with and expand on results previously reported for larger juvenile fish during EPRI-sponsored impingement survival monitoring of coarse-mesh Ristroph screens in a laboratory flume (EPRI 2006; Black 2007). In those tests using juvenile and adult fish  $\geq 50$  mm, post-impingement survival exceeded 90% regardless of species or approach velocities (i.e. 1-3 ft/s).

These results are subject to several qualifying points as follows:

- Testing was limited to a few species that are readily available as larvae or can be easily cultured in the laboratory. The tested organisms, by their nature, are hardier than pelagic species, such as clupeids and bay anchovy which dominate actual CWIS entrainment. For example, an ongoing analysis of entrainment at power plants indicates that the top five entrained species at coastal/estuarine locations are clupeids (American Shad, blueback herring, bay anchovy, Atlantic menhaden, and Gulf menhaden) and, similarly, unidentified clupeids and gizzard shad dominate at plants using freshwater systems for cooling<sup>1</sup>.
- Results may, however, be representative of the hardier recreational species that encounter CWIS.
- Testing was limited to freshwater species. The results are believed to be indicative of screen performance with estuarine and marine species of comparable hardiness as there is no information that indicates or suggests that either group is more or less sensitive than the other.
- Results are from ideal laboratory testing conditions. Field conditions that could lower post-collection survival include the presence of debris, suspended sediments, elevated temperatures, variable water quality and the lack of control organisms.

The retrofit or installation of traveling water screens with fish protection features at existing CWIS will require a fish return system. The ASCE published guidelines for fish conveyance structures were developed through an understanding of the hydraulic conditions likely to produce fish injury and how to avoid them, common sense, and the best professional judgment of fish passage experts (ASCE 1982). The application of these guidelines should not have an adverse effect on early lifestages of fish that are greater than approximately 11-12 mm in length.

Site-specific factors such as height of screens above low water elevation, quantity and type of debris, and potential for biofouling will dictate the ultimate design and complexity of the fish return. However, use of smooth, non-abrasive material (e.g., fiberglass) and assuring a sub-surface discharge of the return line should not adversely affect fish survival. Where biofouling is an issue (e.g., at marine facilities), a redundant fish return line allows one line to be periodically cleaned while the other line is in use. Such systems are installed at Progress Energy's Brunswick Station at the mouth of the Cape Fear River, NC and Tampa Electric Company's (TECO) Big Bend Station in Tampa Bay, FL.

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<sup>1</sup> Preliminary results from EPRI's ongoing national survey of impingement and entrainment data collected by power plants between 2004-2008 in response to the 2004 EPA §316(b) Phase II Rule which has since been remanded.

# 6

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# A

## APPENDIX – FACTORS AFFECTING FISH SURVIVAL

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### Background

A review of historic, recent, and ongoing research indicated the following as key stressors associated with fish mortality in fish return systems: pressure; impact and abrasion; turbulence and shear; and velocity. As such, these were used as key terms in an electronic literature search using several reference databases including: Applied Science and Technology Index; Aquatic Sciences and Fisheries Abstracts; Biological Abstracts; Digital Dissertations; IDEAL; JSTOR; Science Citation Index; and Web of Science. In addition, resources available through the World Wide Web were searched via the Google search engine. Government and university reference libraries, as well as the Alden's in-house library, were searched for documents and publications detailing fish stressors at both CWISs and other facilities. To the extent possible, relevant "gray literature" was obtained.

Much of the available literature on stressors is based on observations of fish that have interacted with man-made structures (e.g., fish return system, hydroelectric project spillway, hydroelectric turbine). Fish may be exposed to multiple stressors during their exposure to such structures. When injury occurs under complex flow situations, as is common in fish returns, it can be difficult to determine which stressor caused the injury. The situation is further complicated by the relationship between stressors and/or other physical forces. For example, velocity, which is not a direct stressor, plays a role in the magnitude of shear forces and turbulence. Likewise, the potential for impact injury is related to the velocity of the fish at the time of impact. Similar types of injury can be caused by different stressors (Table A-1).

**Table A-1**  
**Primary injuries observed during laboratory studies of pressure, shear, and strike (from Turnpenny et al. 1992)**

Observed Injury	Source of Injury		
	Pressure	Shear	Strike/Impact
Ruptured swim bladder	Yes	No	No
Eye hemorrhaging	Yes	Yes	No
Scale loss	No	Yes	Yes
Mucous loss	No	Yes	Yes
Internal hemorrhaging	No	Yes	Yes
Egg loss	Yes	No	Yes
Gill/Operculum damage	No	Yes	No

However, the bulk of data available on fish return systems and stressors is for juvenile and adult fish.

### **Fish Return Survival of Early Life Stages**

Most facilities that have evaluated fine-mesh screens for protecting early lifestages of fish have sampled the wash water directly downstream of the screens and did not sample at the outfall of the return pipe or other components of the fish return system. The exceptions are Tampa Electric Company's (TECO) Big Bend Station and Progress Energy's Brunswick Nuclear Generating Station which provide the evaluation of egg and larval survival at both the screens and the outfall of the return pipe.

Fine-mesh screens were incorporated into the intake structures of both Units 3 and 4 at Big Bend Power Station operated by TECO. Prior to installation, full-scale prototype studies were conducted in 1979; the results concluded that the 0.5 mm fine-mesh screen technology was a viable effective alternative for removal of organisms from the circulating cooling water. Six continuously operated dual-flow traveling screens with 0.5 mm mesh were installed at the station, and studies of their biological effectiveness were conducted in 1985 (Brueggemeyer et al. 1988). The fish return system required the incorporation of three Hidrostral pumps to provide the energy needed to transport collected organisms to a remote discharge location. The pumps are located in a sump that collects the combined screen wash discharge from all six screens. To account for possible pump effects on organism survival, samples were collected both from the sump and at the remote organism return discharge. Control organisms were collected from the intake canal upstream of the screens. Sampling and holding methods were similar to those used in the prototype study. This included sampling March through September from three locations: 1) screenwash station; 2) return discharge; 3) control station at the intake canal. The number of observations made and the number of organisms held for the survival study were not reported. According to Brueggemeyer et al. (1988), sampling methods were similar to those used to assess

the prototype screen. From this, one can assume that sampling was conducted 5 days per week (March – May 16) or 5 days per month (June – August), sampling during the day (0900–1500) and during the night (1900–2400, May 16 – August); control samples taken once per week (March) and twice per week (April – August).

Invertebrate survival was high at the screens and at the outfall of the fish return. Survival of Sciaenidae (drums) larvae was similar at the screens and at the outfall (Table A-2). Survival of bay anchovy at the screens was lower than survival of those collected at the end of the fish return. Survival of eggs for both species (Sciaenidae and bay anchovy) was lower at the end of the return than off the screen wash. No statistical analyses were conducted, so it is unknown if the observed differences in survival between the screen wash and return discharge were significant.

**Table A-1**  
**Comparison of Initial Survival (%) During the Fine-Mesh Screen Survivability Studies at Big Bend Station (Brueggemeyer et al. 1988)**

Taxa	Initial Survival (%)		
	Screen wash	Return Discharge	Control
Fish Eggs:			
bay anchovy	48	29	72
Sciaenidae	63	40	72
Fish Larvae:			
bay anchovy	16	58	16
Sciaenidae	61	56	85
Invertebrates:			
Caridea	72	70	65
Xanthidae	93	90	88
Pinnotheridae	99	83	77

Brunswick is a nuclear-fueled station located on the estuarine portion of the Cape Fear River about 22 miles south of Wilmington, NC. Three of the four 9.4-mm (0.37-in.) mesh intake traveling screens on each of the station's units are overlaid with 1-mm (0.04-in.) fine-mesh polyester screens. The fourth screen for each unit has overlays on every other basket. The fine-mesh screens were operated only when the intake water temperature was less than 18°C (65°F). Survival studies were conducted between 1984 and 1987 to document the survival of impinged

fish (Carolina Power and Light 1985; Thompson 2000). Larval, juvenile, and adult fish and key invertebrates were collected and held for 96 h). Organisms were collected at two screen rotation speeds – slow 0.76 m/min (2.5 ft/min) and fast 1.98 m/min (6.5 ft/min). Velocity approaching the screens was approximately 0.61 m/sec (2.0 ft/sec).

Survival rates were high for all invertebrate species, including shrimp and blue crab (Table A-3). Larval survival was highly variable by species. Menhaden spp., bay anchovy, weakfish, and goby all had poor survival (< 20%). Other species showed moderate to high survival, especially at faster screen rotation speeds (mullet, 70%; flounder *Paralichthys* spp., 93%).

**Table A-2**  
**Brunswick Station Impingement Survival Study Results: Screen Mortality — 1984 and 1985**  
**(Carolina Power and Light 1985; Thompson 2000)**

Taxon Collected	Screen Speed	Number of Trials	Number Collected	Number Stocked	Initial Mortality a (%)	Latent Mortality b (%)	Total Survival c (%)
croaker — group 1	F	15	2,903	1,285	39.6	52.2	28.9
croaker — group 2	F	5	584	338	36.0	43.8	36.0
spot — group 1	F	8	1,349	620	19.0	61.8	31.0
pink and white shrimp	F	6	264	219	1.4	5.5	92.7
brown shrimp	F	2	87	81	7.9	25.9	69.0
Penaeid post-larvae	F	2	188	120	4.3	5.8	90.2
blue crab	F	4	170	79	2.4	5.1	92.7
blue crab megalops	F	2	159	71	1.9	11.3	88.9
weakfish	F	4	282	191	19.4	82.2	12.6
searobin	F	4	132	124	2.3	8.1	89.8
blackcheek tonguefish	F	3	110	95	5.5	15.8	79.6
bay anchovy	F	2	249	114	54.2	100.0	0.0
striped mullet — group 1	F	1	62	52	16.1	19.2	67.7
striped mullet — group 2	F	1	37	37	0.0	8.1	91.9
flounder	F	1	91	78	8.9	1.3	90.0
menhaden	F	1	32	30	6.3	83.3	15.6
croaker — group 1	S	12	2,105	772	60.1	77.3	9.6
croaker — group 2	S	6	597	420	15.4	57.9	35.6
spot — group 1	S	9	1,806	767	39.1	87.6	7.6
spot — group 2	S	3	333	219	27.9	61.2	28.0
pink and white shrimp	S	1	48	44	8.3	11.4	81.2
brown shrimp	S	3	249	241	3.2	7.9	89.2
Penaeid post-larvae	S	2	131	119	9.2	15.1	77.1
blue crab	S	1	26	20	7.7	0.0	92.3
blue crab megalops	S	2	203	135	3.0	11.1	86.3
bay anchovy	S	1	596	59	90.1	100.0	0.0
hardback shrimp	S	1	123	41	66.7	34.1	22.0

F = Fast-screen operation.

S = Slow-screen operation.

a=Number of organisms that were found dead in collection gear ÷ number collected.

b=Number of organisms that died after being stocked in tanks ÷ number stocked.

c=100 - [(a) (Number collected) + (b) (Number stocked) + (b) (Other live organisms collected but not stocked)] ÷ number collected.

The total number of organisms collected in this study was not reported (Carolina Power and Light 1985; Thompson 2000), making it difficult to determine the extent of these data. In particular, comparisons between screen rotation speeds could be biased due to unequal sample sizes. Mean survival data were presented, but initial and latent survival measurements were not included. Also, durations of sampling, gear types, holding facilities, and holding durations were not reported.

Neither of these studies attempted to isolate factors that could affect survival.

### **Stressors**

Fish response to stressors is species and lifestage-specific. For example, studies by Grasser et al. (1979) indicated that fish larvae were injured passing over a relatively low dam (3.1-m high). Several other studies indicate that later lifestages (juvenile and adult) show no adverse effects after passage over dams of similar height. In addition, species-specific differences in mortality were observed, with filiform shad larvae experiencing greater damage than the more robust Catostomidae (suckers) larvae. Much of the available data are for salmonids which are not typically impinged or entrained at CWIS, but these results are still useful in understanding the response of fish to stressors.

### **Pressure**

Fish responses to both pressure increases (multiple atmospheres) and exposure to partial vacuum (i.e., pressure below 1 atmosphere) have been observed. Research on pressure has been conducted under both laboratory and field conditions. Because observations of fish injury under field conditions are complicated by the presence of multiple stressors and the difficulty in determining the source of injury, laboratory studies are better suited to the study the effects of an isolated stressor. Pressure changes occurring within CWIS and fish return systems are dependent on the intake flow, depth of the intake, and the path that fish take as they are returned to the water body.

The body of research concerning pressure effects indicates that fish are generally more sensitive to pressure drops than to increases (Cada 1990). Studies by Cada (1997), Cook et al. (1997), and Abernethy et al. (2001) suggest that sub-atmospheric pressures can be harmful to fish, particularly if they are either physoclistous (having no direct airway between the swim bladder and the gut) and/or acclimated to higher pressures (i.e., deep water habitats). The authors differ in their suggested maximum percent drop in pressure before swim bladder rupture (60%, 30%, and 10% of acclimated pressure levels, respectively). However, they agree that there are species- and family-specific factors that should be taken into account when determining the effect of pressure decreases on entrained fish and that limiting those decreases will help prevent injury.

The effect of pressure change on fish varies by species and lifestage. In general, the physiology of the swim bladder and associated venting mechanism will determine the relative susceptibility of each species to pressure changes. Physostomous fish (e.g., most soft-rayed fishes like salmon,

trout, catfish, minnows, shad, and gar) have a pneumatic duct that connects the swim bladder directly to the gut. These fish are able to shunt air via the pneumatic duct to and from their air bladders. Because of this adaptation, physostomous fish can quickly expel excess gas and are thus more resilient to pressure decreases. Physoclists (e.g., most spiny-rayed fishes such as perch, bass, and bluegill sunfish) have no mechanism for rapid release of air from the swim bladder and so are more susceptible to swim bladder damage when exposed to sub-atmospheric pressures. Under normal conditions, physoclists move oxygen into their swim bladder via a rete mirabile system (highly vascularized countercurrent mechanism whereby oxygen is diffused from the blood across a membrane into the swim bladder). Equalizing internal and external pressures via the rete mirabile system may take hours depending on the pressures and volumes to be equalized.

The effect of pressure changes on fish can vary by lifestage. Fish eggs and newly hatched larvae do not have swim bladders which makes them less susceptible to damage caused by brief drops in pressure (Cada 1990). In addition, some fish that are physoclists as adults have a physostomous larval stage that may be less impacted by pressure than adult lifestages, including striped bass (Hadley et al. 1987).

Several studies have been conducted to assess the effects of pressure on the early lifestages of fish. A summary of these studies is provided in Table A-4.

**Table A-3**  
**Survival of the early lifestages of several species of fish exposed to pressure change**

Species	Lifestage – Average Size	Pressure Exposure	Survival	Reference
striped bass ( <i>Morone saxatilis</i> )	Eggs and larvae	Exposure to 0.4 atm Exposure to 0.14 atm – back to atmospheric – pressurized to 3.3 atm	No significant mortalities for either scenario.	Beck et al. (1975) in Keevin et al. (2000)
Atlantic herring ( <i>Clupea harengus</i> )	Larvae & Early Juveniles 11-39 mm	Rapid increase from 1 atm to 5 atm and quickly back to 1 atm	11-20 mm fish had no increased mortality.  25-29 mm exhibited increased mortality over controls.  30-39 mm fish had no increased mortality	Hoss and Blaxter (1979) in Keevin et al. (2000)
Atlantic herring ( <i>Clupea harengus</i> )	Juvenile 120-150 mm	Rapid increase in pressure from 1 atm to 3 atm	Resulted in rupture of prootic membrane. Smaller juveniles and larvae were less at risk.	Hoss and Blaxter (1979) in Keevin et al. (2000)
herring ( <i>Alosa</i> spp.)	6-8 mm larvae without bulla or swim bladder	Exposure to 5 atm followed by decompression	Not harmed	Bishai (1961) in Keevin et al. (2000)
bluegill ( <i>Lepomis macrochirus</i> )	Larvae	Exposed to turbulence, shear and pressures from ~2 atm to 0.5 atm in simulated power plant condenser tube	Little or no mortality was observed.	Kedl and Coutant (1976) in Keevin et al. (2000)
common carp ( <i>Cyprinus carpio</i> )	Larvae			
white bass ( <i>Morone chrysops</i> )	Larvae			
striped bass ( <i>Morone saxatilis</i> )	Larvae			
common carp ( <i>Cyprinus carpio</i> )	Larvae	Exposed to pressures as low as 0.5 atm in simulated power plant condenser tube	No harmful effects	Ginn et al. (1978) in Keevin et al. (2000)

The effects of pressure on the early lifestages of bigmouth buffalo (*Ictiobus cyprinellus*), blue catfish (*Ictalurus furcatus*), bluegill (*Lepomis macrochirus*), largemouth bass (*Micropterus salmoides*), and walleye (*Sander vitreus*) were evaluated to determine the potential impact to

eggs and larvae (Keevin et al. 2000). A pressure vessel was used to simulate pressure changes resulting from water column mixing. Fish were subjected to one of three experimental treatments:

- Pressure gradually raised to 4.4 atm over 1 h, held for 30 min, returned to 1 atm in 5 sec;
- Pressure raised to 4.4 atm within 5 sec, held for 10 min, and returned to 1 atm in 5 sec; and
- Pressure raised to 4.4 atm within 5 sec, held for 30 min, and returned to 1 atm in 5 sec.

For each group of experimental treatment fish, a control group was used to estimate mortality associated with handling and exposure to the pressure vessel. Very little mortality was observed for any of the treatments and no significant difference was detected between treatment and control groups.

Although not of value for eggs and larvae, the following discussion of stressors for juvenile and adult fish is provided for completeness. The effects of pressure on adult fish have been studied extensively. The species tested and the exact test protocols used to examine pressure effects on adult fish varied between studies, but generally one of the following three scenarios was used:

- Surface-acclimated fish were exposed to sub-atmospheric pressures;
- Surface-acclimated fish were pressurized for a length of time then exposed to sub-atmospheric pressures; or
- Deepwater-acclimated (i.e., higher pressure) fish were exposed to sub-atmospheric pressures.

Since many of these studies were conducted to mimic site specific conditions, the length of time that fish were pressurized or depressurized and the magnitude of the pressure change often were set to represent best and worst case scenarios at that site. A summary of several pressure-related investigations are presented in Table A-5 through Table A-8. Whenever possible the following information is given: species tested; pressures to which fish were exposed or the proportional increases and decreases in pressure; type of injury sustained; and survival.

**Table A-4**  
**Critical pressure drop percentages by species**

Species/Family	Critical Pressures	Effect	Reference
perch	exposed to 60% of acclimated pressure or lower	Swim bladder rupture	Jones (1951) in Cada (1997)
bluegill	exposed to 10% of acclimated pressure or lower	Swim bladder rupture	Abernethy et al. (2001)

**Table A-5**  
**Swim bladder rupture pressures and estimated volume expansion required for rupture**  
**(Turnpenny 1992)**

Species	Number Tested (n)	Acclimation Pressure (atm)	Rupture Pressure (atm)	Volume Expansion for Rupture (%)
pout ( <i>Trisopterus luscus</i> )	14	1.3	0.6 +/- 0.1	203
whiting ( <i>Merlangius merlangus</i> )	10	1.3	0.5 +/- 0.1	250
bass ( <i>Dicentrarchus labrax</i> )	12	1.3	0.6 +/- 0.1	215
sand smelt ( <i>Atherina boyeri</i> )	8	1.0	0.6 +/- 0.1	163
brown trout ( <i>Salmo trutta</i> )	18	1.0	No rupture*	n/a
herring ( <i>Clupea harengus</i> )	20	1.0	No rupture*	n/a

\*tested to 0.2 atm: gas venting prevented rupture  
n/a = not applicable

**Table A-6**  
**Swim bladder damage and survival of fish exposed to varying pressures (Turnpenny 1992)**

Species	Exposure	Results	Notes
Atlantic salmon ( <i>Salmo salar</i> )	Brought from 3.9 to 0.1 atm	10%-30% swim bladder rupture	7-day overall survival 90%
European eel ( <i>Anguilla anguilla</i> )	Tested down to 0.1 atm	No ruptured swim bladder evident	7-day survival 100%. Freshwater phase eels maintain a reduced volume of air in their swim bladders so damage due to pressure reductions is unlikely for this lifestage
sole ( <i>Solea solea</i> )	Tested down to 0.1 atm	No adverse reaction	7-day survival 100%. Sole are benthic and possess no swim bladder. No damage of any type was observed as a result of pressure changes
bass ( <i>Dicentrarchus labrax</i> )	Tested down to 0.1 atm	15% swim bladder rupture at 0.3 atm, 94% rupture at 0.1 atm	No mortalities occurred despite damage to swim bladders
dragonet ( <i>Callionymus lyra</i> )	Tested down to 0.1 atm	100% swim bladder rupture at 0.1 atm, 0% at 0.3 atm	7-day survival 86%
corkwing wrasse ( <i>Crenilabrus melops</i> )	Small group tested down to 0.1 atm	No immediate swim bladder damage evident	Only 5 fish were tested and they were immediately sacrificed to check for damage -No 7-day survival data were available
golden grey mullet ( <i>Liza aurata</i> )	Tested down to 0.1 atm	79% swim bladder rupture at 0.1 atm, 0% at 0.3 atm	Fish exposed to 0.1 atm had 60% 7-day survival

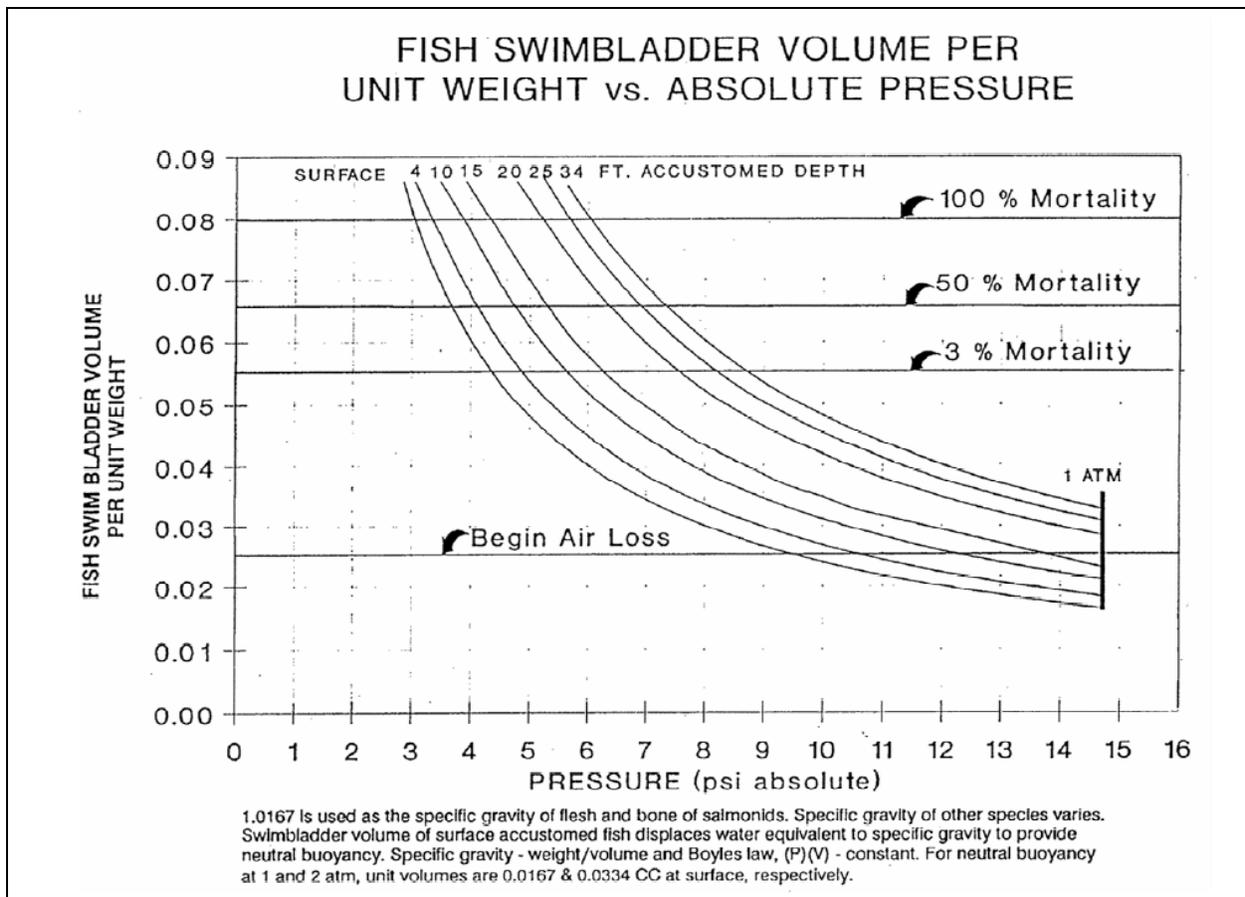
**Table A-7**  
**Mortality of fish exposed to rapid and brief pressure reductions in laboratory test chambers**  
**(modified from Cada et al. 1997; Turnpenny 1992)**

Species	Acclimation pressure, Pa (atm)	Exposure pressure, Pe (atm)	Pe / Pa	Mortality (%)	Source
sockeye salmon	1.0	0.7	0.7	0.0	Harvey (1963)
sockeye salmon	3.4	1.0	0.3	0.5	Harvey (1963)
sockeye salmon	1.0	0.7	0.7	2.0	Harvey (1963)
sockeye salmon	2.0	0.7	0.4	21.0	Harvey (1963)
perch	3.0	1.0	0.3	70.0	Tsvetkov et al. (1972)
largemouth bass	1.0	1.0	1.0	0.0	Feathers and Knable (1983)
largemouth bass	1.9	1.0	0.5	25.0	Feathers and Knable (1983)
largemouth bass	3.6	1.0	0.3	41.7	Feathers and Knable (1983)
largemouth bass	3.6	1.0	0.3	45.8	Feathers and Knable (1983)
bluegill	1.0	0.2	0.2	33.0	Hogan (1941)
bluegill	1.0	0.2	0.2	50.0	Hogan (1941)
crappie	1.0	0.4	0.4	100.0	Hogan (1941)
crappie	1.0	0.2	0.2	50.0	Hogan (1941)
largemouth bass	1.0	0.2	0.2	80.0	Hogan (1941)
largemouth bass	1.0	0.2	0.2	100.0	Hogan (1941)
largemouth bass	1.0	0.2	0.2	50.0	Hogan (1941)
Atlantic salmon, brown trout, rainbow trout	1.0	0.1	0.1	0.0	Turnpenny et al. (1992)
brown trout	3.4	0.3	0.1	10.0	Turnpenny et al. (1992)
rainbow trout	3.4	0.3	0.1	0.0	Turnpenny et al. (1992)
herring	3.4	0.3	0.1	4.0	Turnpenny et al. (1992)
Coho salmon	1.0	0.1	0.1	0.0	Muir (1959)
coho salmon	1.0	0.1	0.1	10.0	Muir (1959)

In addition to the potential for physiological damage as a result of exposure to changes in pressure, secondary effects, such as gas bubble trauma (GBT), may also occur. Survival studies conducted at the Pacific Northwest National Laboratory (PNNL) with bluegill, Chinook salmon,

and rainbow trout examined the occurrence of GBT and the role of pressure changes in the survival of fish acclimated to different water depths (Abernethy et al. 2001). GBT can occur when fish are exposed to high-pressure systems that allow water to become super-saturated with dissolved gasses (120% or greater); a condition unlikely to occur within a fish return system. Results indicated that there were species-specific differences in measured tolerances to both GBT and pressure changes. Bluegill were least affected by GBT followed by Chinook salmon and rainbow trout. However, bluegill were extremely susceptible to swim bladder rupture from pressure drops that had a nadir of 0.1 atm. Chinook salmon experienced burst swim bladders when acclimated to 1.9 atm prior to testing, but rainbow trout did not exhibit burst swim bladders regardless of total dissolved gasses or acclimation pressure (Abernethy et al. 2001).

Fish acclimated to deeper waters are more susceptible to injury and mortality caused by pressure reductions (Abernethy et al. 2001). Physostomes, while less likely to be injured by swim bladder rupture during a low-pressure event, are susceptible to embolism from gasses dissolved in their blood and tissues, particularly when they are acclimated to deeper-water habitats. Differential mortality rates experienced by salmonids acclimated to varying pressures is given in Figure A-1. Note that as pressure drops, mortality increases relative to initial acclimation depth (USACE1991).



**Figure A-1**  
**Differential survival of salmonids acclimated to different depths/pressures (USACE 1991)**

One approach to determine the potential effect of a stressor on the survival of a multi-species assemblage is to focus on the most susceptible member of that assemblage and thus bound the lower limit of survival. Prior to the work by Turnpenny (1992) on sand smelt and herring, studies were conducted at Alden Research Laboratory to observe the effect of pressure on another fragile species; alewife (SWEC 1975). Mean length of alewife tested was 10 cm (range 2.5 to 20.0 cm). During pressure chamber testing, fish were quickly brought from 1 to 2.4 atm and held for 15 min. Pressure was then returned to 1 atm over a period of 2 minutes. As chamber pressure was increased and their air bladders became compressed, the alewife experienced some signs of disorientation and had trouble maintaining position. However, by the end of the 15 minutes the fish were swimming normally again. Following the release of pressure, the alewife appeared in good condition and no burst swim bladders were evident. One week survival of treatment fish was close to control groups, though high mortality was observed at the end of the fifth test. These mortalities coincided with the occurrence of a bacterial infection in both groups of fish.

Pressure, as a potential factor in the survival of fish, at a facility can be assessed by comparing existing conditions within the debris and/or fish return system to those determined to be injurious at other sites. Existing literature indicates that pressure effects are most injurious to fish when:

- Pressures are substantially above or below atmospheric pressures;
- Fish are acclimated to high-pressure (deep water) prior to exposure to low-pressure;
- The magnitude of the pressure drop is large;
- Pressure drops occur over a short period of time; and/or
- The duration of exposure to low-pressure is long.

## **Velocity**

Velocity is not a direct stressor to fish, but is one characteristic of the flow field that determines the relative intensity of other stressors (PSEG 2002). Fish can travel at uniform, high velocities within a body of water without deleterious effects. Other stressors such as turbulence (the fluctuation in velocity magnitude over time in one location) and shear (the relative difference in velocity and direction between two moving bodies of water) result from uneven or unsteady velocity conditions. The magnitude of potential adverse impact to fish from abrasion, impact, shear, and turbulence are subsequently discussed.

## **Abrasion and Impact**

Abrasion or other injuries to the skin can result in one or more of the following conditions (Ruggles and Murray 1983):

- Flooding of internal tissues with excess water (through osmosis);
- Acute toxicity resulting from the liberation of toxins sequestered in the injured tissue and/or;
- Creation of pathways for the penetration of pathogenic organisms.

Often abrasion injuries do not result in immediate mortality, but difficulties in osmoregulation, exposure to histamine-like toxins or the onset of latent fungal infections can lead to delayed mortality.

Abrasion in fish return systems can occur on rough surfaces. Conversely, other characteristics of the fish return system (such as the lack of sharp corners and physical impediments) act to minimize the potential for abrasion.

Development of criteria for the safe passage of fish over high-head hydroelectric dams led researchers to examine the effects of fish exposed to freefall conditions. The majority of research in this area has been conducted with salmonids since most of the high-head dams in the U.S. are in the Pacific Northwest, and anadromous salmonids are abundant and important commercially and recreationally. Smith (1938 as cited in Ruggles and Murry 1983) and Holmes (1939 as cited in Ruggles and Murry 1983) showed juvenile salmon (5 to 10 cm) could survive freefalls of up to 56 m. At the Glins Dam on the Elwha River, survival of 92% was observed for yearling Coho salmon that freefell 55 m into a pool (Regenthal 1957 as cited in Ruggles and Murry 1983).

These early studies indicate that velocity at the time of impact was a greater predictor of injury and mortality than the height of the fall. Laboratory studies were conducted at the University of Washington to calculate terminal velocities of fish of varying sizes. While fish 10 to 13 cm length reached terminal velocities of 16 m/s in falls of 30.5 m, larger fish (~60 cm) reached terminal velocities in excess of 58 m/s and would continue to accelerate during falls as high as 213 m (Richey 1956 as cited in Ruggles and Murry 1983). Further, testing with live sockeye salmon (*Oncorhynchus nerka*) ~18 cm long reached terminal velocity of 16 m/s when falling from a 45-m high tower (Richey 1956 as cited in Ruggles and Murry 1983).

Further study (data presented in Bell and DeLacy 1972) indicated fish experiencing impact greater than 16 m/s incurred damage to gills, eyes or internal organs. Survival of fish dropped from a helicopter into a hatchery pond was dependent upon the size of the fish dropped and the height of the fall (Table A-9). At any given height, smaller fish experienced greater survival and lower impact velocities than larger fish.

**Table A-8**  
**Survival of salmon smolts dropped from various heights (from data presented in Bell and DeLacy 1972)**

Fish Size (cm)	30.5-m drop		61-m drop		91.5-m drop	
	% Survival	n	% Survival	n	% Survival	n
15-18	98.5	200	97.5	199	98.5	200
25-28	94.8	198	82.0	189	81.4	189
30-38	67.0	6	83.4	6	20.0	5

Sweeney and Rutherford (1981 as cited in Ruggles and Murry 1983) observed the mortality of Atlantic salmon smolts and kelts following a fall from either 10.6 or 18 m. No significant initial

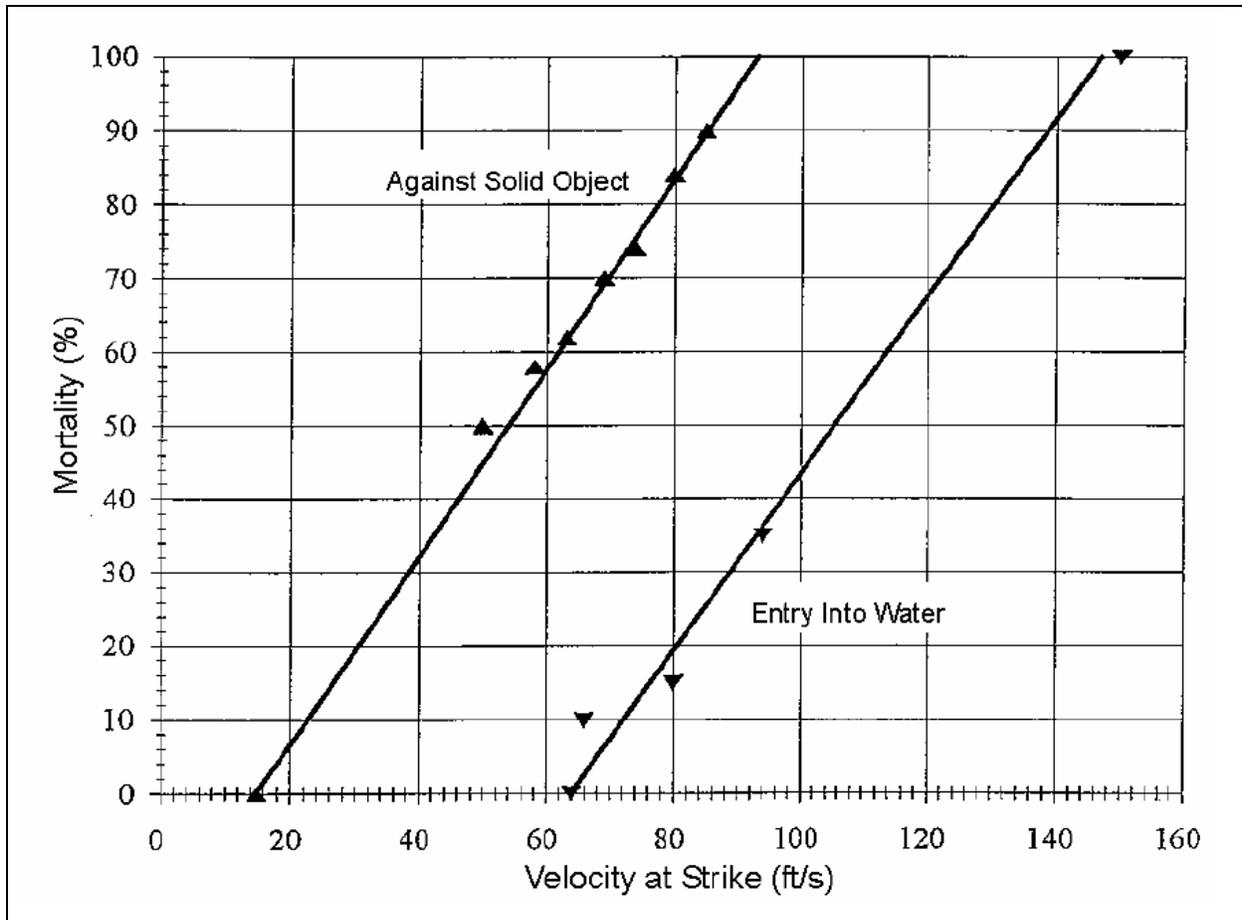
mortalities were observed for fish experiencing falls from either height. During the 8-day delayed mortality observation period, the kelts dropped 18 m suffered 12.5% mortality. By contrast, kelts dropped 10.6 m experienced no delayed mortality.

Based on the observation of Richey (1956) and Regenthal (1957) it appears that the terminal velocity of fish 18 mm and smaller is less than the lethal impact velocity for salmonids tested. Bell and DeLacy (1972) point out that fish falling within a column of water may experience injuries as a result of shear forces resulting from the rapid deceleration of the water as it enters the receiving pool and that those injuries are similar to those resulting from impact. Bell and DeLacy (1972) acknowledge that additional mortalities may have been caused by repeated exposure to a stressor (e.g., fish getting caught in turbulent flows). Shear and other stressors likely added to the observed mortalities, but no measurement of shear or other stressors were collected. A compilation of survivals observed under different hydraulic conditions are summarized in Table A-10 (Bell and DeLacy 1972).

**Table A-9**  
**Summary of expected survival of salmonids exposed to different hydraulic conditions (from Bell and DeLacy 1972)**

Hydraulic Condition	Survival and Conclusions
Fish striking a fixed object at velocities <20 ft/s.	No data, but expected survival to be low based on data collected at higher velocities.
Falling in constricted areas where deceleration was controlled by baffles and walls.	Survival dropped quickly for velocities over 40 ft/s and was likely as low as 70% in the 20-30 ft/s range.  Some mortality may have been from shear.
50 ft/s entering a pool from freefall.	98-100% survival
60 ft/s entering a pool from freefall.	80% survival
80 ft/s and greater entering a pool from freefall.	Approaching 0% survival.
Entering a pool within a column of water and decelerating with the jet without mechanical deflection.	Survival may equal best freefall conditions (98-100%).
Entering a pool within a column of water and decelerating with the jet and deflected by a baffle.	Approximately 93% survival.
Fish traveling through a hydraulic jump or large stilling pool (single passage through stressor).	Approaches best conditions, 93-98% survival.
Fish striking a fixed baffle or object.	Approaching 0% survival.

Fish surviving impact is determined not only by the relative velocity between the fish and the object struck, but is also affected by the physical characteristics (e.g., hardness, sharpness, roughness, etc.) of the object struck. For example, at the same velocity, impact against solid objects caused higher mortality than entry into water (USACE 1991; Figure A-2).



**Figure A-2**  
Mortality of fish impacting a solid object vs. entry into water by (USACE 1991)

## Turbulence and Shear

Turbulence is a measurement of the fluctuation in velocity magnitude about a mean value. In general, it is difficult under both laboratory and field conditions to separate the injury resulting from turbulence and shear. Very little literature exists on the survival of fish exposed to different levels of turbulence, although proposed and on-going research is attempting to identify these effects (e.g., Odeh 2001).

Shear forces arise at the boundary between fast and slow moving water and is greatest in areas of rapid acceleration or deceleration. The magnitude of shear forces depends upon the relative net difference in velocity and direction between two masses of water at their interface. The differential between the velocity of the fish and the relative velocity of the surrounding water mass can lead to fish injury. There is a strong link between velocity, shear, and turbulence and in most cases it is impossible to separate the effects of shear from those of turbulence.

Groves (1972) examined the effects of shear using high speed cameras to observe juvenile salmon encountering a high velocity jet. Damage to fish was observed under conditions where

water moved at velocities greater than 9 m/s. During this study, localized areas of sharp velocity differences resulted in injuries that occurred within one millisecond of exposure and in a 2.5 cm square area.

Johnson conducted experiments in the late 1960s and early 1970s on fingerling salmon exposed to shear forces generated by a water jet entering still water at different velocities (Johnson 1970; 1972 as cited in Ruggles and Murray 1983). Fingerling salmon (18 to 20 cm) were jetted into a pool of water through a 15 cm nozzle at a velocity of 17.5 m/s. No mortality was observed (immediate or delayed) during the seven-day post test holding period. Tests using a 10-cm nozzle and velocities of 20.3 cm/s resulted in low mortality (0 to 5.4%). Johnson concluded that the critical threshold velocity for smolts 18-20 cm length was near 20.3 m/s.

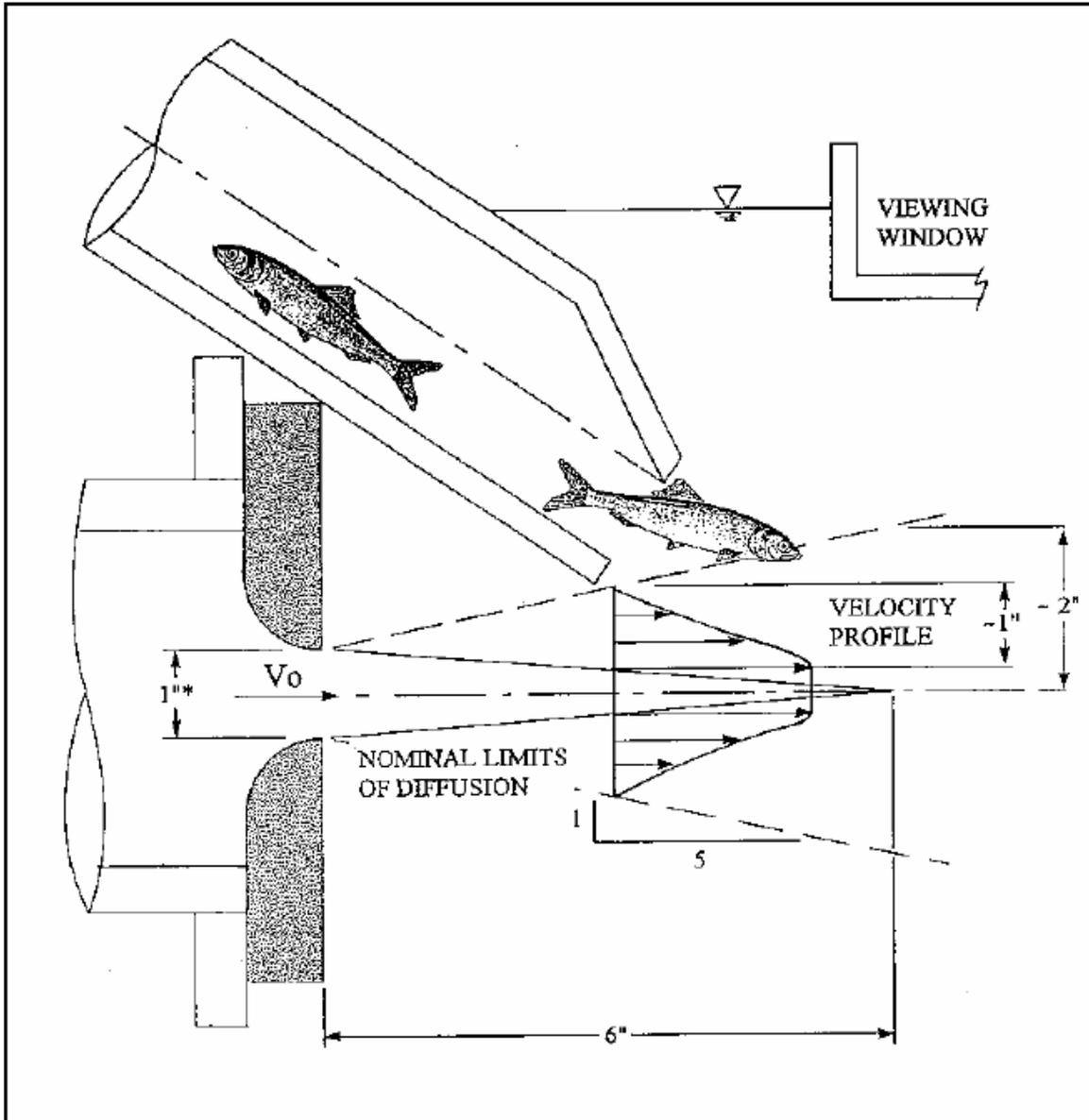
In subsequent tests, Johnson (1972 as cited in Ruggles and Murray 1983) exposed salmon and steelhead (*O. mykiss*) to a wide range of velocities (Table A-11). Johnson concluded that velocities exceeding 20 m/s can cause injury to fish and that the rate of fish injury rises sharply at velocities greater than 24 m/s.

**Table A-10**  
**Survival of salmon and steelhead fingerlings fired from a jet at different velocities (Johnson 1972 as cited in Ruggles and Murray 1983)**

Jet Velocity (m/s)	17.5	20.3	23.5	28.0
Survival (%)	100.0	97.6	92.8	69.0

The study of most relevance to examining shear effects was conducted at Pacific Northwest National Laboratory (PNNL). In previous research, velocity distributions and time series associated with the test conditions had not been reported in all of the studies. Because of this, a definitive comparison between the results of the previous studies cannot be made. A notable exception to this is the Nietzel et al. (2000) report titled, “Laboratory Studies on the Effects of Shear on Fish.” This report provides a complete description of the experimental methods and analysis procedures used in their study.

The primary objective of the Nietzel et al. (2000) study was to, “specify an index describing the hydraulic force that fish experience when subjected to a shear environment.” In this study, fish were exposed to a shear environment produced by a submerged jet (Figure A-3) with velocities ranging from 0 to 70 ft/sec. Fish were released, in either a head-first or tail-first orientation, at the edge of the jet stream or within the jet stream and injury was caused by the flow patterns developed by the expanding jet. Test fish included juvenile rainbow trout, spring and fall Chinook salmon, and American shad.



**Figure A-3**  
**Shear environment testing apparatus (modified from Nietzel et al. 2000)**

Strain rate was used as the index of physical force that fish experienced when subjected to the shear environment in the test facility. In their report, Nietzel et al. referred to this force as the *exposure strain rate* ( $\Sigma$ ) calculated by equation 1:

$$\text{Exposure strain rate} = \epsilon = du/dy = (u_1 - u_2) / 1.8 \text{ cm/s/cm} \quad (\text{eq. 1})$$

where:

$u_1$  = the jet velocity ( $V_0$  in Figure 2-3)

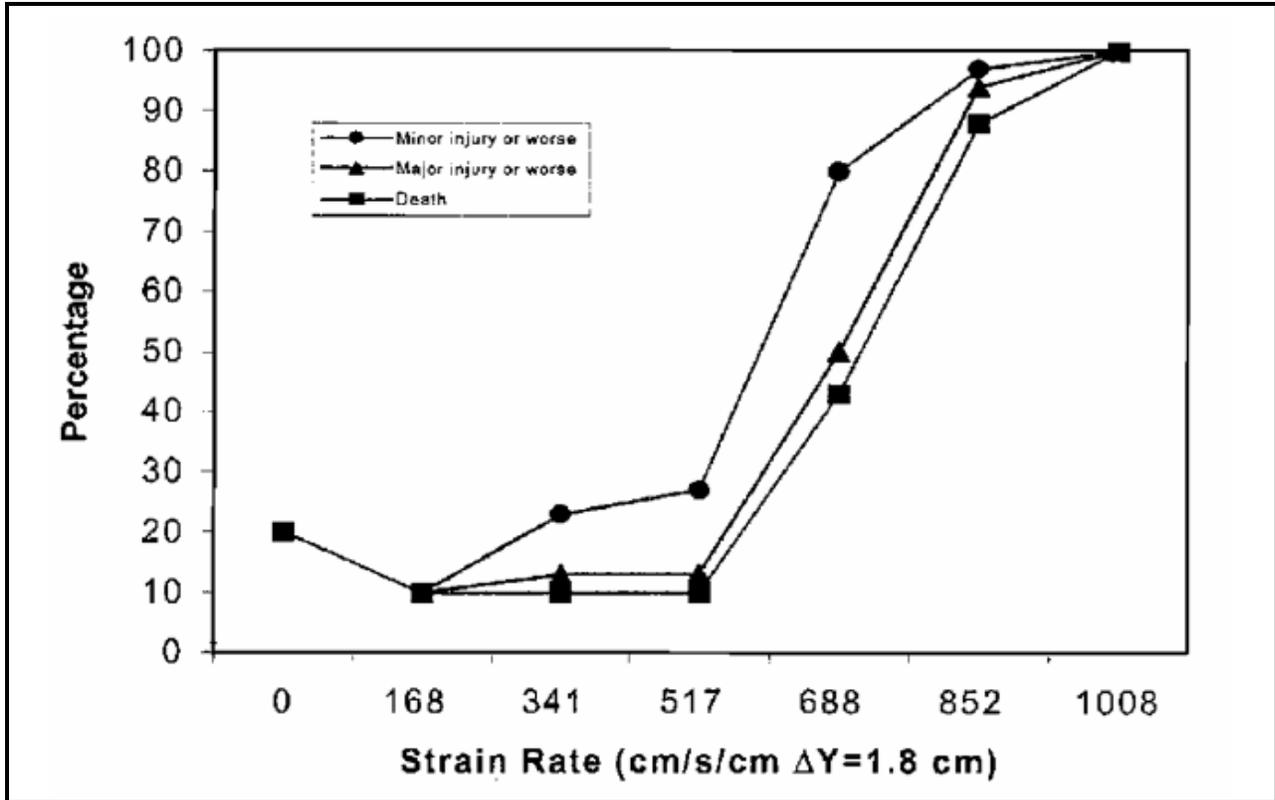
$u_2$  = a velocity measured a small distance away from  $u_1$  (1.8 cm)

1.8 cm = the spatial resolution of the velocity measurements (also the minimum width of salmonids tested)

The dy distance (1.8 cm) was set because it matched the length of the fish used during the tests. In the experiment, Nietzel et al. released fish into the shear environment of the test facility (as shown in Figure A-3) and recorded the amount of injury sustained by the fish. The exposure strain rate for the test was equal to the maximum strain rate developed by the jet (velocity measurements, used for the calculation of exposure strain rate, were made in the close vicinity of the nozzle where fish were exposed to the most severe and least variable shear environment). The rate of strain experienced by test fish varied from 0 cm/s/cm<sup>2</sup> to 1185 cm/s/cm.<sup>2</sup>

Injuries to the test fish were categorized as minor or major. Minor injuries were those that were visible, but not life-threatening, and tended to heal and disappear during the post-exposure period. Small bruises (< 0.5 cm in diameter) with minor discoloration were also given a minor injury rating. Major injuries were those that resulted in prolonged loss of equilibrium and the more severe injuries that persisted throughout the post-exposure observation; for example, large bruises (> 0.5 cm in diameter), damage to spinal column, cuts with bleeding, injured eyeballs, gill damage, and de-scaling.

For each test, the percentage of test fish with minor injury, major injury, or death was calculated. The results of tests with American shad are shown in Figure A-4. In these tests, as with other Nietzel et al. (2000) tests, the percentage of injured fish and the severity of injury increased as the exposure strain rate rose.



**Figure A-4**  
The percentage of American shad (mean FL = 10 cm) injured or killed during headfirst exposure to different strain rates (N = 150) (modified from Nietzel et al. 2000)

As a result of their testing, Nietzel et al. (2000) concluded “that juvenile salmonids and American shad should survive shear environments where strain rates do not exceed 500 cm/s/cm at a dy of 1.8 cm.” In Figure A-4, for example, major injury or death was not observed when exposure strain rates were less than 500 cm/s/cm (when adjusted for control mortality). When strain rates were less than 341 cm/s/cm no significant injuries to any fish were reported. However, when strain rates were greater than 1,008 cm/s/cm, no fish survived.

Nietzel et al. (2000) studied conditions beyond those found within a typical power facility return system. Site-specific studies can give a more realistic understanding of these stressors in the field. In 2001, the PSEG Energy Group conducted a series of laboratory and field studies to assess mortality associated with the fish collection and return system at the Salem Nuclear Generating Station (Salem). Computational Fluid Dynamics (CFD) models of the existing Salem fish return system were developed and the results compared to literature values. A test facility was built at Alden Research Laboratory to evaluate the effect of changing the fish return system point-of-discharge from a subsurface discharge to one with a 1.3 ft drop and 6.0 ft drop. The facility simulated the end-of-pipe discharge, the return troughs, and the fish collection pools to quantify stressors within these system components.

Alewife, a relatively fragile species, was selected for testing. The alewife that were evaluated ranged from 48-142 mm FL (mean 79.4 mm). Six replicate control-treatment releases were

performed under each of three different test conditions: existing configuration and 1.3 ft and 6.0 ft freefalls. The results indicate that survival was nearly 100% under all conditions tested. Survival rates ranged from 99.5% to 101.4% when adjusted for control mortality (Note: survival rates over 100% are the result of adjusting for control mortality that is higher than treatment mortality).

The results indicated that mortality was minimal for alewife exposed to the existing hydraulic conditions in the end-of-pipe portion of the Salem fish return system. Analysis of the potential effect of shear on fish passing through the existing end-of-pipe demonstrated that conditions within the pipe were not a significant source of mortality for alewife. Based on the results of EOP testing, it was concluded that fish exiting the Salem fish return system experience minimal injury and mortality associated with the end-of-pipe portion of the fish return system (99.5% survival). There was no discernable difference in survival potential between the existing and alternative conditions (PSEG 2002).

### **Existing Guidelines for Fish Return Systems**

Guidelines for the fish returns and other fish handling systems (e.g., fish bypasses, spillways, intakes, fish screens) have been developed by federal, state, and professional organizations (e.g., American Society of Civil Engineers (ASCE), NOAA Fisheries, U.S. Fish and Wildlife Service, Canadian Department of Fisheries and Oceans). These guidelines were developed through an understanding of the hydraulic conditions likely to produce fish injury and how to avoid them, common sense, and the best professional judgment of fish passage experts. While guidelines do not specifically address individual stressors, they do provide criteria for the design of fish-friendly passage.

With all guidelines, site-specific factors will dictate the applicability of any one of the criteria. Reviews of factors influencing fish return system design led to the development of criteria for sluiceway and pipeline design (Table A-12; ASCE 1982). In general, the goal of the ASCE guidelines was to create conditions that allow for efficient and safe transport of fish back to their natural environment. In reviewing the conditions recommended (e.g. water depth and transport velocities) it is apparent that these guidelines were developed for juvenile and adult fish.

**Table A-11**  
**Criteria for the design of fish conveyance structures (modified from ASCE 1982)**

Criteria	Stressor(s)
All surfaces of conveyance structures must be smooth to prevent abrasion to fish. Suitable materials include fiberglass, polyethylene or coated steel to prevent injury to fish.	Abrasion
The system size must be based on the number and size of fish. Use a minimum water depth of 6 in. (15.2 cm), minimum width 18 in. (45.7cm). Appropriate free board must be provided based on the jumping capability of the strongest fish to be transported.	Abrasion and impact. Maximizes the number of fish returned to water body
Transport velocities must be greater than the sustained cruising speed of the fish, often 2 to 4 fps (0.61 to 1.22 m/sec).	Limits physiological exhaustion to fish
Materials used for the structures must minimize biofouling.	Abrasion and reduction in impediments to fish movement
Long radius ( $r/d > 2.5$ ) bends must be provided so that fish do not abrade on the sides of the bend.	Abrasion
Pipe joints must be constructed carefully so that all edges match and there are no jagged protuberances.	Abrasion and impact
Valves, meters, etc. must provide clear passage for the fish and create as little obstruction as possible.	Impact and reduction in impediments to fish movement
All transitions must be gradual to prevent flow separation and rapid changes in velocity.	Shear and turbulence
Smooth transitions must be provided where flow from several pipes or channels combine.	Abrasion and impact
In Northern latitudes, above ground sluiceways or pipes must be protected from freezing. Buried pipes must be located below the frost depth.	Impact and reduction in impediments to fish movement
Velocity control weirs must have drainage orifices to reduce entrapment of fish and debris when the water supply is shut down for cleaning screens. The sluiceway must completely drain following shut down.	Minimize stranding fish





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