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Environmental Sciences Division

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

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SUMMARY

In 2005, Grant County Public Utility District No. 2 (GCPUD) replaced one of the 10 Kaplan turbines at Wanapum Dam with an advanced turbine that was developed with support from the U.S. Department of Energy's Advanced Hydropower Turbine System Program. Compared to a conventional Kaplan turbine, the advanced minimum gap runner (MGR) turbine is predicted to have lower values for several potential fish injury mechanisms, and therefore was expected to improve turbine-passage fish survival. Fish survival tests of the new turbine were carried out by GCPUD between February and April 2005. A total of 8,960 tagged juvenile summer Chinook salmon were used to quantify the differences in direct mortality associated with turbine passage for the new and old turbines.

In addition to direct mortality studies, the potential for indirect mortality (increased predation among uninjured but disoriented fish) was evaluated. Some of the fish that passed through the turbines or were introduced directly downstream from the dams as controls were examined for changes in behavior. We measured various attributes of C-start (burst) swimming performance typically used by prey fish to escape capture. Uninjured fish were retrieved from the tailwaters and rapidly transferred to the turbine deck, where their C-start behavior was recorded with a high-speed camera for later analysis. C-start reactions were recorded at 1, 5, and 15 minutes after return to the turbine deck to assess whether behavior changes are temporary. Aspects of C-start (escape) behavior that were compared among control and turbine-passed fish (and between the old and new turbines) were presence/absence of the C-start, time to onset of the reaction, duration of the reaction, and strength of the reaction. The C-start assay was used to test the hypothesis that the advanced turbine induces less sublethal stress (that might lead to increased predation mortality) than the conventional turbine.

Relatively few significant differences were detected in overall C-start behavior or its components that could be attributed to turbine passage conditions. In most cases, the behaviors of turbine-passed fish were not different from their respective tailrace-passed controls. Similarly, there were few test conditions in which we were able to distinguish between the old Kaplan turbine and the new MGR turbine on the basis of changes in the escape behavior of uninjured fish. These results are consistent with the findings of the coincident direct mortality studies, which found a high post-passage fish survival for both turbines ($\geq$ 94 percent) and no difference overall between the two turbines.

The absence of significant changes in escape behavior might have several explanations: (1) there may have been an insufficient number of replicates to detect small changes in behavior, especially given the complex, multifactor design of the overall experiment;

(2) the passage experience is not different enough between the two turbines or between the turbine and tailrace conditions to affect C-start behavior; (3) too much time elapsed between turbine passage and behavior testing, so that fish recovered from any temporary changes in escape behavior; and (4) some aspect of the tagging, release, and/or recovery procedure had an effect on C-start behavior that overwhelmed differences between treatments. Despite the few significant differences detected in the Wanapum Dam study, previous work has demonstrated that the types of stresses associated with hydroelectric turbine passage can alter C-start behavior, and these changes reflect similar changes in susceptibility of the fish to predators. If conditions allow for rapid recovery and testing of stressed fish, and other non-turbine-related stresses (e.g., tagging and handling procedures) can be minimized, measures of C-start behavior can be useful indicators of potential predation losses from sublethal physical and chemical stresses.

1. INTRODUCTION

Wanapum Dam is one of two dams that comprise the Priest Rapids Project (FERC Project No. 2114) on the Columbia River, Washington (Figure 1.1). The Wanapum development has 10 conventional Kaplan turbines that have been operating for over 40 years and are reaching the end of their useful life. Public Utility District No. 2 of Grant County (GCPUD) proposes to replace all 10 Kaplan turbines at Wanapum Dam with advanced turbines that were developed with support from the U.S. Department of Energy's Advanced Hydropower Turbine System (AHTS) Program.

The turbine replacements would be done sequentially between 2005 and 2012. In early 2005, GCPUD completed the installation of the first advanced turbine, a Minimum Gap Runner (MGR) design into Unit 8 (Figure 1.2). Compared to a conventional Kaplan turbine (Figure 1.3), the advanced turbine is predicted to have lower values for several potential fish injury mechanisms: shear stress, turbulence, cavitation, and grinding. On the other hand, the MGR has more blades (six vs. five) and more wicket gates (32 vs. 20) than the Kaplan turbines at Wanapum, which might increase the potential for strike injuries (Table 1.1). If fish passage survival through the advanced turbine meets decision criteria for environmental performance (i.e., fish survival through the advanced turbine is at least as good as through the existing turbine), then installation of the next nine advanced turbines will proceed as scheduled (Brown and Garnant 2006).

Installation and preliminary engineering performance testing of the MGR at Wanapum Unit 8 were completed by mid-February 2005. Fish survival tests using balloon-tagged and PIT-tagged fish were carried out in February, March, and April 2005 (Normandeau et al. 2006). Tagged fish were passed through two turbines (the new MGR in Unit 8 and the conventional Kaplan turbine in Unit 9), three intake slots (bays) in each turbine (A, B, and C), two intake release depths (10 ft [3 m] and 30 ft [9 m]), and five turbine flows (9, 11, 15, 17 and 18.5 kcfs). Releases of a total of 8,960 balloon-tagged fish were used to quantify direct mortality associated with turbine passage. Further, 1,000 releases of sensor fish (Carlson and Duncan 2003) provided information on passage conditions (velocities, accelerations, water pressures) within the turbines and draft tubes.

In addition to the evaluation of direct mortality (caused by injuries during passage), there is a need to assess potential indirect mortality. That is, even if the turbine passage stresses are not immediately lethal, the fish may nonetheless be physiologically stressed or disoriented, so that they are more susceptible to predation in the tailwaters or later succumb to disease. For example, Ferguson et al. (2006) quantified turbine passage fish mortality at McNary

Figure 1.1. Wanapum Dam, part of the Priest Rapids Project on the Columbia River.

Dam on the Columbia River. They estimated that delayed mortality comprised between 46 and 70% of total mortality, and suggested that this delayed mortality was caused by sublethal impacts to fish sensory systems that increased vulnerability to predation. Čada et al. (2003) developed an assay technique for measuring the likelihood of increased vulnerability to predation following turbine passage stresses that entails measuring various attributes of C-start (burst) swimming behavior typically used by prey to escape capture. In this study, the C-start swimming performance assay was used to test relative escape behaviors of juvenile summer Chinook salmon (*Oncorhynchus tshawytscha*) that were passed experimentally through the two Wanapum turbines and various control conditions. Uninjured fish were retrieved from the tailwaters and rapidly transferred to the turbine deck, where their C-start behavior was recorded with a high-speed camera. The C-start assay was used to test the hypothesis that the advanced turbine induces less sublethal stress (that might lead to increased predation mortality) than the conventional turbine.

Figure 1.2. Advanced hydroelectric turbine, the Minimum Gap Runner (MGR) that was installed into Unit 8 of Wanapum Dam in 2005.

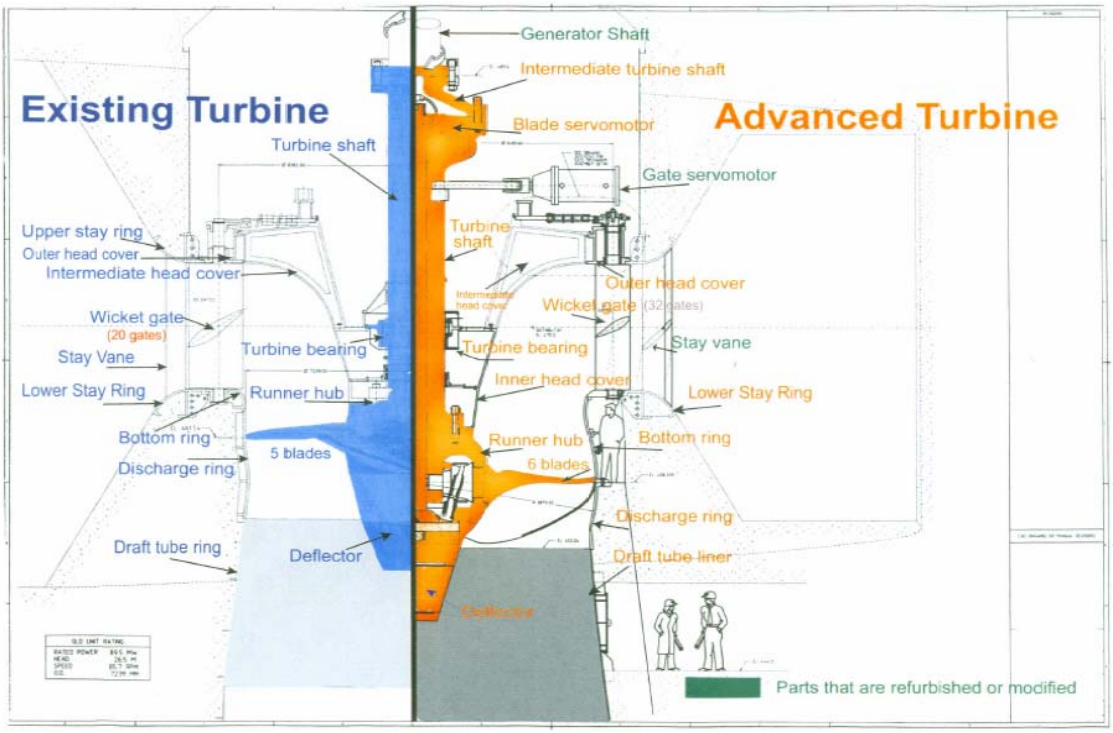

Figure 1.3. Comparison of the features of a conventional Kaplan turbine (Unit 9) and the advanced MGR turbine (Unit 8) at Wanapum Dam.

Table 1.1. Comparison of turbine design parameters that are relevant to fish passage in the old Kaplan and new MGR turbine at Wanapum Dam

Parameter	Old Kaplan (Unit 9)	New MGR (Unit 8)
Stay vane profile	Blunt - causes shear and trailing-edge turbulence	Reshaped to reduce shear and turbulence
Number of wicket gates	20	32
Size of wicket gates	Large	Smaller
Wicket gate spacing (in.)	33.54	21.48
Shape of wicket gates	Blunt - causes turbulence in flow entering runner	Thinner and re-shaped to reduce turbulence
Wicket gate overhang	Present - causes shear and turbulence	Eliminated - reduces severe hydraulic conditions
Alignment of stay vanes and wicket gates	Offset – when not aligned, potential for strike on leading edges of both vanes and gates	Aligned – reduces chance of strike
Hub gaps (in.) Leading edge Trailing edge	largest at lowest flows 0.12 to 4.89 0.12	 0.10 0.10
Blade tip gaps (in.) Leading edge Trailing edge	largest at highest flows 0.76 to 3.82 0.76 to 12.19	 0.31 0.31
Number of runner blades	5	6
Runner rotation rate (rpm)	85.7	85.7
Runner diameter (in)	283.48	304.37
Draft tube shape	Not optimized for fish survival; high turbulence and secondary flows (recirculation)	Flow section enlarged; velocities through elbow reduced

2. MATERIALS AND METHODS

2.1 Field Studies at Wanapum Dam

C-start reaction tests at the Wanapum Dam were carried out between March 30 and April 8, 2005. Test fish were yearling summer Chinook salmon smolts (*Oncorhynchus tshawytscha*) from the Wells Hatchery, Washington. Total lengths of the fish ranged from 147 to 201 mm. Fish were assigned at random to the treatments. Table 2.1 summarizes experimental conditions on the dates of the C-start tests.

Prior to release into the turbines or tailwaters, fish were anaesthetized with MS-222, fitted with a radio tag and two balloon tags, and allowed to recover in clean water for at least 20 minutes (Figure 2.1). Turbine-passed (treatment) fish were released into the intakes of Units 8 and 9 in batches of 10 through a pre-positioned pipe (Figure 2.2 and Normandeau et al. 2006). After passing through the turbine, treatment fish were buoyed to the surface by

Table 2.1. Test conditions at Wanapum Dam, March 30-April 8, 2005. Turbine Unit in parentheses

Date	Water temperature, C^o	Length of fish used in C-start tests, mm	Time between release and recovery, min	Turbine discharge, kcfs	Wicket gate opening, % open	Blade angle, % open	Hydraulic head, ft
3-30-05	4.5	163-187	3-7	11 (8) 11 (9)	72-73 (8) 71-72 (9)	16-19 (8) 29-32 (9)	76.7-77.4
3-31-05	4.4	147-186	4-10	15 (8) 15 (9)	85-86 (8) 83-84 (9)	49-52 (8) 74-78 (9)	77.0-77.6
4-1-05	4.3	158-194	4-13	18. 3-18.8 (8) 17.6-18.2 (9)	93-94 (8) 93-96 (9)	81-83 (8) 97-98 (9)	76.8-77.4
4-5-05	4.8	153-193	4-17	17 (8) 17 (9)	90-91 (8) 88 (9)	67-71 (8) 92-95 (9)	76.5-77.7
4-6-05	5.1	153-201	3-9	15 (8) 15 (9)	84-85 (8) 85-86 (9)	50-54 (8) 50-54 (9)	76.4-77.1
4-7-05	5.1	160-193	4-9	11 (8) 11 (9)	71-73 (8) 71-72 (9)	16-19 (8) 28-29 (9)	76.4-77.3
4-8-05	5.1	163-190	3-8	9 (8) 9 (9)	62-65 (8) 62 (9)	2-3 (8) 1-2 (9)	76.3-77.6

Figure 2.1. Juvenile summer Chinook salmon fitted with two Hi-Z (balloon) tags and a radio tag, prior to release.

the inflated balloon tags and dipnetted into 20-L (5-gal) plastic buckets. Tags were removed in the boat immediately after capture. The first three live fish that had no obvious external injuries were immediately brought to the turbine deck for transfer to the C-start filming tank; each group of three fish was considered an experimental unit. Total time from release of the fish into the turbines, recovery below the dam, and transfer into the filming tank averaged 12 minutes (range: 7 to 17 minutes).

In addition to treatment groups of turbine-passed fish, three control groups of fish were analyzed to evaluate the effects of pre-treatment handling and tagging on C-start behavior: (1) hatchery controls; (2) tagging controls; and (3) tailrace controls. Hatchery controls were used to evaluate the effects of normal dipnetting and handling on C-start behavior; they were not anaesthetized, tagged, or introduced into a turbine or the river. For each hatchery control replicate, three untested hatchery fish were removed from the holding tanks, placed

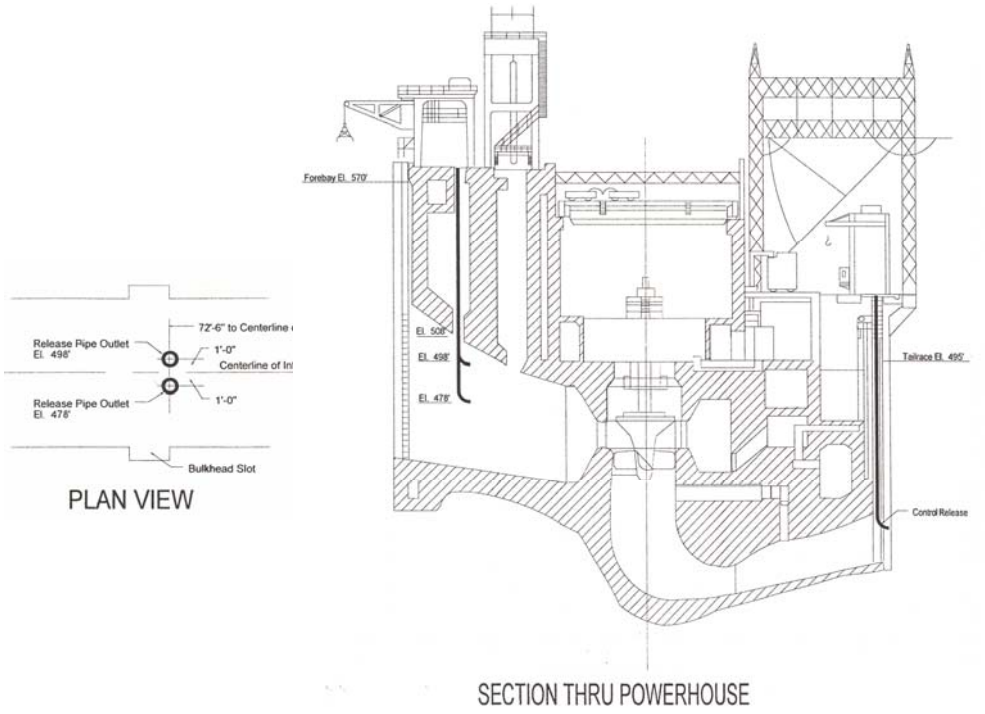

Figure 2.2. Section view of the Wanapum powerhouse and turbine. Dark lines show release points for juvenile Chinook salmon at the intake (10' and 30' depths) and at the draft tube exit.

in a 20-L plastic bucket, transported to the turbine deck, and poured into the water-filled filming tank. Tagging controls were used to assess the effects of dipnetting, handling, anaesthetization, and tagging on C-start behavior. For each tagging control replicate, three fish were removed from the holding tank, anaesthetized, and tagged with a radio tag and two balloon tags. After being allowed to recover in fresh water (at least 20 minutes), the balloon tags were inflated in the holding tank. Several minutes later, the radio tags and balloon tags were removed and the tagging control fish were transported to the filming tank in a 20-L plastic bucket. Tailrace control fish were treated in the same manner as turbine-passed fish, except that they were released directly into the tailrace below the Unit 8 and Unit 9 turbines. That is, these fish were anaesthetized, tagged, allowed to recover, transported to the turbine deck, and released into the river below the dam through the same type of fixed release pipes as used for turbine-passed fish (Normandeau et al. 2006). Boats were used to recover tailrace controls in the same way as turbine-passed (treatment) fish. As with treatment fish, the first three uninjured tailrace control fish that were collected were immediately transported to the turbine deck and transferred to the filming tank.

The cylindrical filming tank was composed of translucent, white, high-density Nalgene© High Density Polyethylene (HDPE) (Figure 2.3) and measured 55 cm in diameter and 80 cm high. An aluminum strike plate was clamped to the side of the filming tank. An aluminum rod welded to the strike plate extended down into the water nearly to the bottom of the tank. The rod ensured that vibrations from the hammer strike were transmitted rapidly into the water to create a startle stimulus for the fish. During tests, water in the tank was approximately 25 cm deep (60 L). A Phototron Fastcam® PCI black and white, high-speed video camera was positioned directly over the filming tank, 140 cm above the tank bottom. The observation area was illuminated by a single 250-W tungsten halogen overhead lamp. Fresh water was supplied from the tailrace for each test, and ranged in temperature from 4.3 to 5.1 ^{o}C. Water in the filming tank was replaced after each test to prevent changes in temperature or buildup of chemicals excreted by previously tested fish that might affect fish behavior in subsequent tests.

All treatment and control fish were filmed in the same manner. Contents of the 20-L transfer bucket containing three fish were gently poured into the cylindrical filming tank, which contained fresh river water at the same temperature as that in the transfer bucket. Addition of the fish and transfer bucket water increased the volume of water in the filming tank to a volume of about 60 L. Fish were allowed to acclimate to the tank for one minute, at which point the camera was turned on and the strike plate was struck sharply with the hammer to provide the C-start stimulus (the investigator stood away from the tank to avoid being seen by the fish). The fishes' reactions to the stimulus were recorded for about 3 seconds. To evaluate possible longer-term changes in C-start behavior, the same fishes' reactions to the strike stimulus also were recorded at 5 and 15 minutes after being placed in the tank. Fish were allowed to rest undisturbed between each stimulus. After the 15-minute test, fish were dipnetted out of the filming tank and placed in a holding tank with the other test fish for observation of 48-h post-test survival.

2.2 Quantification of Fish Behavior

Fish reactions were digitally recorded at 500 frames per second and a shutter speed of 3,000 s^{-1}. Video recordings were saved to the hard disk of a computer and also copied to DVDs in standard audio video interleave (AVI) format for later analysis. The movements of individual test fish were analyzed with a frame-by-frame visual review (0.002 s per frame) by an observer. The observer noted if the fish moved in response to the hammer-strike stimulus and, if so, the extent of movement. Fish were tracked manually by identifying the most anterior part of the head and the most posterior part of the tail from their darkened silhouette and measuring the changes in distance between these points as the escape behavior progressed.

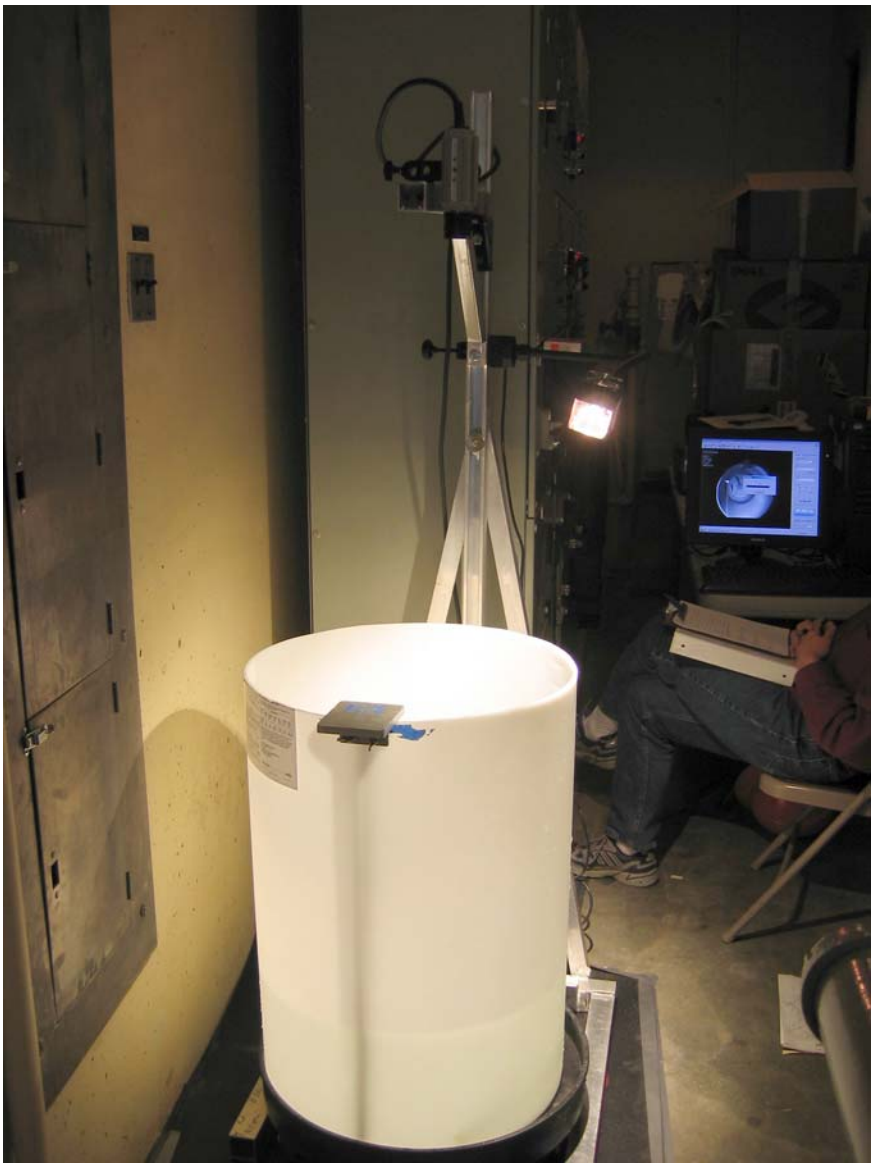

Figure 2.3. C-start filming apparatus used for the Wanapum tests. A high-speed digital video camera recorded movements of fish in the white, 80-cm-high filming tank. The strike plate and rod extending into the water are clamped on the front of the tank. The computer monitor that displayed the video images can be seen in the background.

The following C-start response indicators were evaluated for each test:

1. presence or absence of any response to the stimulus, including C-start;
2. presence or absence of a C-start reaction (i.e., sometimes the response was limited to straight ahead swimming);
3. Time (milliseconds) between the strike stimulus and the first reaction;
4. Duration of Stage 1 of the C-start reaction, the initial behavior in which the fish bends rapidly to one side of the body, forming the C shape);
5. Duration of Stage 2 of the C-start reaction (the initial propulsive stage, beginning with the fish moving its body in the opposite direction from the Stage 1 bend and ending when the tail stroke reaches its maximum excursion on the opposite side of the body);
6. Duration of escape reaction (the length of time the fish displayed a continuous, non-stop movement after the strike stimulus);
7. Completeness of C-shape ratio (closest distance between the head and tail at the end of Stage 1 divided by the total body length).

2.3 Statistical Analyses

General Statistical Considerations

The C-start indicators (Section 2.2) included categorical and measured behavioral responses. Categorical responses to a post-stress stimulus were counts of (1) presence/absence of any response; and (2) presence/absence of C-start behavior. Measured responses to the stimulus following an experimental treatment included (1) time to the onset of a C-start reaction; (2) duration of Stage 1 of the C-start reaction; (3) duration of Stage 2 of the C-start reaction; (4) duration of the escape reaction; and (5) magnitude of the C-shape formation (C-shape ratio). Categorical and measured responses (i.e., dependent or response variables) were analyzed with logistic regression and analysis of variance (ANOVA), respectively, for main effects (i.e., independent or predictor variables) of turbine, slot, depth, flow, and the post-stress startle stimulus time. Statistical Analysis System (SAS) software and procedures were used for all statistical analyses (SAS Institute Incorporated, Cary, North Carolina, PC SAS Version 9.1.3).

Because there were no controls specifically for turbine intake slot and depth, a statistical test of treatment vs. controls for all factors was not possible (Tables 2.2 and 2. 3). To maximize information for each main treatment factor (i.e. predictor variable), subsets of various combinations of treatment and control data were used. Results from the analyses of data subsets were used to determine if data pooling was appropriate. For example, because a

treatment for slot was not included in all possible treatment combinations, a subset of data for Units 8 and 9 was analyzed that included the maximum number of combinations of turbine flows (i.e., 15 and 18.5 kfs) and depths (10 and 30 ft) that were tested in both intake slots B and C. If the analysis of such a data subset indicated no significant intake slot effect, then further analyses could proceed without regard to slot. However, because intake slot was tested only in Unit 8 and 9 treatments (and not controls), this test could only determine if slot effects were important in Unit 8 vs. Unit 9, but not if slot effects were important to fish behavior in general. This constraint existed for all treatments for which there was no control. Fully saturated multifactoral models (i.e., main effects and all interactions terms) were used for each data subset for logistic regression and ANOVA, and the same progression of analysis of data subsets was followed for testing each response variable and the appropriateness of data pooling (Table 2.4).

Table 2.2. Number of experimental units[a] in each treatment for categorical data analysis

Intake slot	Turbine discharge (kcfs)	Depth (ft)	Turbine Unit 8			Turbine Unit 9			Turbine 8 control			Turbine 9 control			Hatchery control			Tagging control		
			Post-recovery stimulus time																	
			1	5	15	1	5	15	1	5	15	1	5	15	1	5	15	1	5	15
A	17	10	4	4	4	4	4	4												
		30	4	4	4	4	4	4												
B	11	10	3	3	3	3	3	3												
		30	3	3	3	5	5	5												
	15	10	4	4	4	3	3	3												
		30	4	4	4	3	3	3												
	18.5	10	2	2	2	3	3	3												
		30	3	3	3	3	3	3												
C	9	10	4	4	4	4	4	4												
		30	4	4	4	4	4	4												
	11	10				3	3	3												
		30				1	2	2												
	15	10	4	4	4	3	3	3												
		30	4	4	4	3	3	3												
	18.5	10	2	2	2	3	3	3												
		30	4	4	4	3	3	3												
NT[b]	9	NT							2	2	2	2	2	2						
NT	11	NT							2	2	2	2	2	2						
NT	15	NT							2	2	2	2	2	2						
NT	17	NT							1	1	1	1	1	1						
NT	18.5	NT							2	2	2	2	2	2						
NT	NT	NT													10	10	10	7	7	7

[a]Each experimental unit was comprised of three fish.

[b]NT = not tested.

Table 2.3. Number of experimental units[a] in each treatment for measured responses

			Turbine Unit 8			Turbine Unit 9			Turbine 8 control			Turbine 9 control			Hatchery control			Tagging control		
			Post-recovery stimulus time																	
Intake slot	Turbine discharge (kcfs)	Depth (ft)	1	5	15	1	5	15	1	5	15	1	5	15	1	5	15	1	5	15
A	17	10	4	4	3	3	3	4												
		30	2	4	4	3	4	4												
B	11	10	2	3	3	2	3	3												
		30	4	3	4	4	5	5												
		10	2	3	2	3	4	5												
		30	2	4	3	3	3	3												
		10	1	1	2	2	2	3												
		30	3	3	2	2	3	2												
C	9	10	4	4	4	3	2	4												
		30	4	4	4	3	4	3												
18.5		10				0	2	2												
		30				0	1	2												
		10	3	4	3	3	3	1												
		30	3	4	4	2	2	2												
11		10	3	3	3	3	3	3												
		30	3	3	4	3	2	3												
NT[b]	9	NT							2	2	2	1	1	1						
NT	11	NT							2	2	1	2	2	2						
NT	15	NT							2	2	2	1	2	1						
NT	17	NT							1	1	1	1	1	1						
NT	18.5	NT							2	2	2	1	0	1						
NT	NT	NT													10	8	10	5	4	5

[a]Only fish exhibiting a C-start response were included in the measured response analysis; thus, experimental units consisted of one to three fish.

[b]NT = not tested.

Table 2.4. Data analysis scheme for logistic regression and analysis of variance

Factor assessed (all factors were treated as fixed)	Additional factors included	Combination of treatments used	
		Logistic regression	ANOVA
Intake slot	Turbine, depth, flow, time	Slot (B, C) Turbine (Units 8, 9) Depth (10, 30) Flow (15, 18.5) Time (1, 5, 15)	Slot (B, C) Turbine (Units 8, 9) Depth (10, 30) Flow (15, 18.5) Time (1, 5, 15)
Flow (11, 15, 18.5)	Turbine, depth, time	Slot (B) Turbine (Units 8, 9) Depth (10, 30) Time (1, 5, 15)	Slot (B) Turbine (Units 8, 9) Depth (10, 30) Time (1, 5, 15)
Flow (9, 15, 18.5)	Turbine, depth, time	Slot (C) Turbine (8, 9) Depth (10, 30) Time (1, 5, 15)	Slot (C) Turbine (8, 9) Depth (10, 30) Time (1, 5, 15)
Depth (10, 30)	Turbine, time	Turbine (Units 8, 9) Slot (A) Flow (17) Time (1, 5, 15)	[a]
Depth (10, 30)	Turbine, time	Slot (B) Flow (11, 15, 18.5)[b] Time (1, 5, 15)	Slot (B) Flow (11, 15, 18.5)[b] Time (1, 5, 15)
Depth (10, 30)	Turbine, time	Slot (C) Flow (9, 15, 18.5)[b] Time (1, 5, 15)	Slot (B) Flow (9, 15, 18.5)[b] Time (1, 5, 15)
Turbine (Units 8, 9)	Time	Turbine Slot (A) Flow (17) Depth (10, 30)[c] Time (1, 5, 15)	[a]
Turbine (Units 8, 9)	Time	Slot (B) Flow (11, 15, 18.5)[c] Depth (10, 30)[c] Time (1, 5, 15)	Slot (B) Flow (11, 15, 18.5)[c] Depth (10, 30)[c] Time (1, 5, 15)
Turbine (Units 8, 9)	Time	Slot (C) Flow (9, 15, 18.5)[c] Depth (10, 30)[c] Time (1, 5, 15)	Slot (C) Flow (9, 15, 18.5)[c] Depth (10, 30)[c] Time (1, 5, 15)

Table 2.4. (continued)

Factor assessed (all factors were treated as fixed)	Additional factors included	Combination of treatments used	
		Logistic regression	ANOVA
Turbine (Unit 8) *vs* turbine 8 tailrace	Flow, time	Slot (A)[d] Flow (17) Depth (10, 30)[d] Time (1, 5, 15)	[a]
Turbine (Unit 8) *vs* turbine 8 tailrace	Flow, time	Slot (B)[d] Flow (11, 15, 18.5)[d] Depth (10, 30)[d] Time (1, 5, 15)	Slot (B)[d] Flow (11, 15, 18.5)[d] Depth (10, 30)[d] Time (1, 5, 15)
Turbine (Unit 8) *vs* turbine 8 tailrace	Flow, time	Slot (C) Flow (9, 15, 18.5)[d] Depth (10, 30)[d] Time (1, 5, 15)	Slot (C) Flow (9, 15, 18.5)[d] Depth (10, 30)[d] Time (1, 5, 15)
Turbine (Unit 9) *vs* turbine 9 tailrace	Flow, time	Slot (A)[d] Flow (17) Depth (10, 30)[d] Time (1, 5, 15)	[a]
Turbine (Unit 9) *vs* turbine 9 tailrace	Flow, time	Slot (B)[d] Flow (11, 15, 18.5)[d] Depth (10, 30)[d] Time (1, 5, 15)	Slot (B)[d] Flow (11, 15, 18.5)[d] Depth (10, 30)[d] Time (1, 5, 15)
Turbine (Unit 9) *vs* turbine 9 tailrace	Flow, time	Slot (C) Flow (9, 15, 18.5)[d] Depth (10, 30)[d] Time (1, 5, 15)	Slot (C) Flow (9, 15, 18.5)[d] Depth (10, 30)[d] Time (1, 5, 15)
Turbine *vs* controls[e]	Time	Turbine (Units 8, 9, T8, T9, H, T) Time (1, 5, 15)	Turbine (Units 8, 9, T8, T9, H, T) Time (1, 5, 15)

[a]Insufficient data to test measured responses for flow = 17.
[b]Separate analysis performed for each flow.
[c]Separate analysis performed for each depth and flow.
[d]Separate analysis performed for each depth and flow for Turbine Units 8 and 9; since depth was not a control-treatment, same set of controls was used for each combination of turbine and depth.
[e]This test was an overall two-factor analysis of turbine treatment and time.

Categorical Response Data Analysis

For testing categorical binary responses (e.g., either presence or absence of a response) for the effects of two or more independent (predictor) variables, a logistic regression is generally recommended because it is more efficient than chi-square or exact tests (Quinn and Keough 2002; Sokal and Rohlf 1995). Logistic regression uses a logit transformation (i.e., the natural log) of the dependent variable to model against the independent variables. Because the error terms of a logistic model will not be normally distributed, maximum likelihood estimation of model parameters is considered more appropriate than ordinary least squares estimation (Agresti 2002).

Parameter estimates for logistic regression are odds ratios (Quinn and Keough 2002). For fish behavior responses, odds ratios provided an estimate of the chance of a fish responding to a stimulus. An odds ratio of 1.0 would indicate that the odds of a fish responding were equal to the odds of a fish not responding. An odds ratio >1.0 would indicate that the odds of a fish responding to a stimulus were greater than the odds of no response, whereas an odds ratio of <1.0 would indicate that the odds of not responding were greater than those of responding.

Because the precision of parameter estimates is generally improved with fewer predictor variables, it is often of interest to try to identify those variables that best explain the response variable (Agresti 2002; Quinn and Keough 2002; Sokal and Rohlf 1995). Stepwise procedures provide a useful initial step for screening predictor variables. However, because they are multiple testing procedures, there is an increased risk for Type I errors (i.e., declaring an effect when none exists); thus, the use of higher p-values is generally recommended. The backward selection procedure starts with the full (i.e., saturated) model and then systematically removes terms based on a given criterion (e.g., model term with highest p-value). When the criterion is no longer satisfied, the selection process stops. We used the backward elimination approach available with the SAS procedure, PROC LOGISTIC, to analyze categorical responses and to identify the most influential independent variables. We assessed overall model fit with a likelihood ratio test. When poor fit was indicated, the Pearson and deviance statistics were used to assess the residuals for evidence of under- or over-dispersion (i.e., the variance is less than or greater than the mean; Agresti 2002). When over-dispersion was indicated, the Williams method of correction was applied (Agresti 2002). All independent variables were treated as fixed and discrete categorical variables. Although the variables flow, depth, and time could have been classified as continuous, the number of flows tested across combinations of other factors and the small range of depths and times tested were insufficient to be representative of all possible conditions potentially experienced by fish. To account for multiple testing in this analysis, a criterion of $p \geq 0.2$ was used for eliminating terms from the model.

Measured Response Data Analysis

Measured responses considered in the analysis of behavioral effects included head-to-tail ratio (H:T), time to onset of response (OR), duration of C-start response (DR), time to reach Stage 1 of C-shape formation (D1), and time of stage 2 of C-shape formation (D2). Because we were interested in the effects of treatments on startle response, only those fish that responded in some way (either with a distinct C-start or with some other type of escape behavior) were included in the analysis. The C-start behavior was considered the most predictive of predator avoidance; thus, the group that comprised all fish that responded in any way was not analyzed further unless clear patterns of treatment effects on overall response were detected. Assumptions of normality and homogeneity of the variance of each response variable were initially tested with Shapiro-Wilks and Levene's tests, respectively (Zar 1999); results of the tests indicated that the assumptions were met.

There were no *a priori* assumptions about which measured responses would be most informative as indicators of stress. Furthermore, this was a new approach for assessing effects on fish behavior, and it was not known if any of the responses were correlated and thus, potentially providing redundant information. By identifying which responses are correlated, future studies of fish behavior could be streamlined to focus more effort on those responses that provide maximum information. As an initial step in the analysis of the five behavioral response metrics, they were examined with a Pearson correlation analysis (SAS CORR procedure) for possible correlation (Sokal and Rohlf 1995). Responses found to be uncorrelated ($p > 0.05$) were further tested individually with a multifactor ANOVA using the SAS procedure, PROC GLM; correlated responses were dropped from further consideration. Following the same rationale used for classifying independent variables in the logistic regression as discrete, all independent variables for the ANOVAs were considered fixed. The level of testing of main-factor effects was based on results for interaction terms in the saturated model. A significant interaction indicates a lack of independence between factors. In such cases, testing of main-factor effects is generally not recommended, or if sufficient data exists, it is normally recommended that the effects of one factor should be tested at all levels of the other (Quinn and Keough 2002). However, even in the presence of a significant interaction, tests for main effects can still be useful provided the limitations of such analyses are considered in the interpretation of the results. If a significant main effect still exists when there is an interaction, the effect is likely to be present, but non-significant effects could be misleading because effects can still be present at some levels. Given that significant ($p < 0.05$) interactions were commonly present in analyses of subsets, overall statistical analyses (i.e., ANOVA) were performed for turbine differences within each post-stimulus time and across all post-stimulus times. In this analysis, a significant ($p <0.05$) effect of turbine would provide strong evidence that responses in at least

two fish treatments were different. However, because of the possible influence of the interaction effect of turbine with one or more other factors (i.e., intake slot, flow, or depth, as well as post-recovery stimulus time in the analysis of overall turbine effects), no definitive conclusions of effect could be made in the absence of statistical significance (i.e., $p > 0.05$).

3. RESULTS

Some of the fish from all of the test and control groups reacted when startled by the hammer-strike stimulus. In some groups all three fish in the observation tank reacted to the stimulus, whereas in others only two or one or none reacted. Not every fish responded to the hammer strike stimulus in a strong C-shape; in some instances, the C-start reaction was only a slight curvature. A small number of fish reacted to the stimulus with relatively slow, straight-ahead swimming, without formation of a C-shape. We believe that this response was a less effective predator avoidance response than the rapid, reflexive C-start, which occurs in a fraction of a second and realigns the fish in an unpredictable direction.

We describe in Section 3.1 the effects of treatments on the ability of fish to exhibit a startle response, as indicated by (1) any kind of movement or (2) a C-start behavior. In Section 3.2 we focus on measurements associated with the C-start formation for only those fish that exhibited that reaction, on the assumption that C-start is a more effective predator avoidance mechanism than simple swimming.

3.1 Presence/Absence of Reaction

There were a total of 1080 fish observations characterized for a reaction to the startling stimulus, i.e., 360 juvenile salmon filmed at three times after exposure to the stress. Of the 1080 observations, there was some type of reaction in 708; of these, there was a C-start reaction in 578 observations.

Table 3.1 summarizes the odds ratios or chances of fish responding to the stimulus in some fashion (including C-start). If an odds ratio = 1.0 for a particular comparison, say response of fish in Turbine Unit 8 vs Unit 8 Tailrace, then the chances of a fish responding from either group would be equal. If the odds ratio is not 1.0, and the 95% Wald confidence limits do not include the value of 1.0, then we are 95% confident that the chance of a fish responding was less or more for one treatment versus the other. When all three test times were combined, differences were detected between hatchery controls and tailrace controls, tagging controls and turbine-passed fish, and hatchery controls and tagging controls. For example, the hatchery controls (those fish that were tested directly from the holding tank and not anaesthetized, tagged, or exposed to the turbine/tailrace) were 2.1 times more likely to react to the stimulus than fish that had been exposed to the Unit 9 tailrace. Hatchery controls were 2.8 times more likely to react than tagging controls. Fish that passed through Units 8 and 9 were nearly twice as likely to respond to the startling stimulus as fish in the tagging control groups. Similar results were seen at a time of 5 and 15 minutes; tagging controls were less likely to react than other fish. The only

Table 3.1. Odds ratios for comparisons among test and control groups of yearling summer Chinook salmon exhibiting any reaction (including C-start) in response to the stimulus. Comparisons that were significantly different ($P \leq 0.05$) are in bold.

Comparison	Odds ratio estimates	95% Wald confidence limits	
All post-recovery filming times combined (1, 5, and 15 minutes)			
Unit 9 vs Unit 9 tailrace	1.464	0.844	2.541
Hatchery control vs Unit 9 tailrace	**2.107**	**1.018**	**4.360**
Tagging control vs Unit 9 tailrace	0.747	0.355	1.572
Unit 8 vs Unit 8 tailrace	1.103	0.628	1.935
Hatchery control vs Unit 8 tailrace	1.624	0.777	3.395
Tagging control vs Unit 8 tailrace	0.576	0.271	1.224
Unit 8 vs tagging control	**1.915**	**1.047**	**3.502**
Unit 9 vs tagging control	**1.961**	**1.071**	**3.590**
Hatchery control vs tagging control	**2.821**	**1.308**	**6.086**
Unit 8 vs hatchery control	0.679	0.379	1.215
Unit 9 vs hatchery control	0.695	0.388	1.246
Post-recovery filming time = 1 minute			
Unit 9 vs Unit 9 tailrace	1.345	0.497	3.641
Hatchery control vs Unit 9 tailrace	1.600	0.448	5.709
Tagging control vs Unit 9 tailrace	0.880	0.227	3.415
Unit 8 vs Unit 8 tailrace	0.533	0.168	1.697
Hatchery control vs Unit 8 tailrace	0.571	0.141	2.321
Tagging control vs Unit 8 tailrace	0.314	0.072	1.378
Unit 8 vs tagging control	1.697	0.564	5.107
Unit 9 vs tagging control	1.528	0.508	4.596
Hatchery control vs tagging control	1.818	0.468	7.056
Unit 8 vs hatchery control	0.933	0.345	2.528
Unit 9 vs hatchery control	0.840	0.310	2.276
Post-recovery filming time = 5 minutes			
Unit 9 vs Unit 9 tailrace	0.726	0.261	2.016
Hatchery control vs Unit 9 tailrace	0.963	0.266	3.485
Tagging control vs Unit 9 tailrace	**0.263**	**0.070**	**0.991**
Unit 8 vs Unit 8 tailrace	1.463	0.567	3.770
Hatchery control vs Unit 8 tailrace	1.618	0.475	5.507
Tagging control vs Unit 8 tailrace	0.441	0.124	1.569
Unit 8 vs tagging control	**3.315**	**1.185**	**9.277**
Unit 9 vs tagging control	2.764	0.992	7.705
Hatchery control vs tagging control	**3.667**	**1.010**	**13.315**
Unit 8 vs hatchery control	0.904	0.341	2.396
Unit 9 vs hatchery control	0.754	0.286	1.989
Post-recovery filming time = 15 minutes			
Unit 9 vs Unit 9 tailrace	**3.007**	**1.171**	**7.720**
Hatchery control vs Unit 9 tailrace	**6.250**	**1.609**	**24.282**
Tagging control vs Unit 9 tailrace	1.667	0.466	5.967
Unit 8 vs Unit 8 tailrace	1.567	0.621	3.955
Hatchery control vs Unit 8 tailrace	**4.643**	**1.198**	**17.986**
Tagging control vs Unit 8 tailrace	1.238	0.347	4.419
Unit 8 vs tagging control	1.266	0.449	3.569
Unit 9 vs tagging control	1.804	0.632	5.147
Hatchery control vs tagging control	3.750	0.895	15.708
Unit 8 vs hatchery control	0.338	0.108	1.051
Unit 9 vs hatchery control	0.481	0.153	1.514

differences detected between turbine-passed fish (either unit) and their respective tailrace controls was at a time of 15 minutes, where Unit 9-passed fish were 3.0 times more likely to react than Unit 9 tailrace fish.

When the analysis was limited to juvenile salmon that exhibited C-start reaction, similar results were observed. For example, differences were detected in comparisons between hatchery controls and tailrace controls and between hatchery controls and tagging controls (Table 3.2). When all post-recovery filming times are combined, the hatchery controls were 3.1 times more likely to react to the stimulus than fish that had been exposed to the Unit 9 tailrace. Hatchery controls were 3.0 times more likely to exhibit a C-start reaction than tagging controls. There were no significant differences among groups at a time of 1 minute. The only differences detected between turbine-passed fish and their respective controls were for Unit 9; salmon that passed through Unit 9 were 1.8 times more likely to exhibit a C-start than Unit 9 tailrace control fish. Whether in terms of overall reaction or C-start reaction, hatchery control fish tended to be more reactive than other groups (i.e., odds ratios > 1.0), and tagging control fish tended to be less reactive than other groups (odds ratios < 1.0).

3.2 Trends in Measured Responses

A diminished C-start reaction (and presumably a reduced predator avoidance ability) might be revealed not only by a reduced incidence of C-starts, but also by increased time between the startling stimulus and the onset of the C-start reaction, increased duration of Stage 1 or Stage 2, and/or a smaller H:T ratio, i.e., the fish did not bend into a strong C shape, resulting in less power when swimming out in Stage 2. In addition to the categorical response variables (presence or absence of a reaction to the startling stimulus) discussed in Section 3.1, several measured components of the C-start reaction were analyzed: the time in seconds from the hammer strike stimulus until the onset of the C-start reaction, the time in seconds from the onset of the reaction until the maximum C shape (duration of Stage 1), and the magnitude of the C-shape formation. This initial evaluation of measured responses for redundancy indicated that two time-related measurement variables (duration of Stage 2 and duration of the total response) had significant positive correlations with the other time-related variables (time to onset and duration of Stage 1; Table 3.3). Because they would be expected to respond to the treatment factors in the same way as other time-related variables, the duration of total response and duration of Stage 2 were not analyzed further.

Table 3.2. Odds ratios for comparisons among test and control groups of yearling summer Chinook salmon exhibiting a C-start reaction in response to the stimulus. Comparisons that were significantly different (P < 0.05) are in bold.

Comparison	Odds ratio estimates	95% Wald confidence limits	
All post-recovery filming times combined (1, 5, and 15 minutes)			
Unit 9 vs Unit 9 tailrace	**1.814**	**1.045**	**3.150**
Hatchery control vs Unit 9 tailrace	**3.062**	**1.513**	**6.196**
Tagging control vs Unit 9 tailrace	1.007	0.472	2.152
Unit 8 vs Unit 8 tailrace	0.959	0.557	1.651
Hatchery control vs Unit 8 tailrace	1.600	0.795	3.218
Tagging control vs Unit 8 tailrace	0.526	0.248	1.118
Unit 8 vs tagging control	1.822	0.988	3.361
Unit 9 vs tagging control	1.801	0.976	3.326
Hatchery control vs tagging control	**3.040**	**1.430**	**6.460**
Unit 8 vs hatchery control	0.599	0.348	1.032
Unit 9 vs hatchery control	0.593	0.344	1.021
Post-recovery filming time = 1 minute			
Unit 9 vs Unit 9 tailrace	1.652	0.600	4.544
Hatchery control vs Unit 9 tailrace	1.902	0.537	6.734
Tagging control vs Unit 9 tailrace	0.727	0.175	3.026
Unit 8 vs Unit 8 tailrace	0.579	0.208	1.613
Hatchery control vs Unit 8 tailrace	0.769	0.215	2.756
Tagging control vs Unit 8 tailrace	0.294	0.070	1.237
Unit 8 vs tagging control	1.969	0.615	6.308
Unit 9 vs tagging control	2.271	0.707	7.291
Hatchery control vs tagging control	2.615	0.651	10.509
Unit 8 vs hatchery control	0.753	0.288	1.967
Unit 9 vs hatchery control	0.868	0.332	2.274
Post-recovery filming time = 5 minutes			
Unit 9 vs Unit 9 tailrace	1.501	0.601	3.753
Hatchery control vs Unit 9 tailrace	2.512	0.787	8.016
Tagging control vs Unit 9 tailrace	0.727	0.200	2.648
Unit 8 vs Unit 8 tailrace	1.224	0.493	3.038
Hatchery control vs Unit 8 tailrace	1.382	0.436	4.379
Tagging control vs Unit 8 tailrace	0.400	0.111	1.446
Unit 8 vs tagging control	**3.059**	**1.062**	**8.806**
Unit 9 vs tagging control	2.064	0.718	5.935
Hatchery control vs tagging control	3.454	0.967	12.344
Unit 8 vs hatchery control	0.885	0.362	2.163
Unit 9 vs hatchery control	0.598	0.245	1.457
Post-recovery filming time = 15 minutes			
Unit 9 vs Unit 9 tailrace	2.419	0.928	6.307
Hatchery control vs Unit 9 tailrace	**6.800**	**1.805**	**25.624**
Tagging control vs Unit 9 tailrace	1.870	0.512	6.826
Unit 8 vs Unit 8 tailrace	1.238	0.490	3.131
Hatchery control vs Unit 8 tailrace	**4.308**	**1.165**	**15.926**
Tagging control vs Unit 8 tailrace	1.185	0.331	4.240
Unit 8 vs tagging control	1.046	0.373	2.933
Unit 9 vs tagging control	1.294	0.459	3.643
Hatchery control vs tagging control	3.636	0.912	14.503
Unit 8 vs hatchery control	**0.288**	**0.098**	**0.839**
Unit 9 vs hatchery control	0.356	0.121	1.042

Table 3.3. Pearson correlation analysis results for measured parameters.
Values include correlation coefficients (Rho) with probability values in parentheses. Significant correlations ($p < 0.05$) are in bold.

Measured variable	H:T ratio	Time to onset of reaction	Duration of reaction	Duration of stage 1
Time to onset of reaction	0.04407 (0.4104)			
Duration of reaction	**-0.12062 (0.0236)**	**0.58616 (<0.0001)**		
Duration of stage 1	-0.10225 (0.0553)	-0.06417 (0.2304)	**0.12349 (0.0205)**	
Duration of stage 2	0.05233 (0.3276)	**0.13636 (0.0105)**	**0.30182 (<0.0001)**	**0.22584 (<0.0001)**

The components of C-start behavior were analyzed for various combinations of treatment effects (turbine, slot, introduction depth, turbine flow, and time; Section 2.3). Because the C-start studies were conducted during only a portion of the overall Wanapum fish testing program (that ran from February 18 to April 29, 2005), a balanced dataset was not available for all independent variables related to fish behavior. The number of behavioral observations was sufficient to apply a full statistical model to only Units 8 and 9, two slots (B and C) and two flows (15 and 18.5 kcfs). The main effects of other operating conditions were tested with the subset models.

3.2.1 Time to Onset of C-Start Reaction

For the dependent variable of time to onset of C-start reaction, neither the 5-way interaction nor 4-way interactions were significant ($P < 0.09$). One of the 10 possible 3-way interactions was significant (Turbine*Slot*Depth), so various combinations of those factor levels were tested to identify which had affected behavior. Most comparisons revealed no significant effects on the onset of C-start behavior that could be related to specific slots, turbines, or flows. However, the statistical analyses revealed the following significant differences ($P < 0.05$):

- The time to onset of reaction was significantly greater for slot C, Unit 8, 30’ depth than for (1) slot C, Unit 8, 10’depth; (2) slot B, Unit 8, 30’depth, (3) slot B, Unit 9, 30’ depth; and (4) slot B, Unit 9, 10’depth

- For Unit 8, slot B, depth of 10', and flow of 11 kcfs, time to onset of reaction was significantly greater than for all other combinations

- For slot C, onset of reaction was significantly greater at a flow of 9 kcfs than for other flows. This applies to both turbines and both introduction depths

- For slot B, flow =11 kcfs, depth of 10', time to onset of reaction was significantly greater for Unit 8 than for Unit 9

- For new turbine 8, slot B, and flow = 11 kcfs, time to onset of reaction was significantly greater for fish introduced at 10' than at 30'

- For slot C, flow = 18.5 kcfs, depth of 10', time to onset of reaction was greater for existing turbine 9 than for new turbine 8

- For slot C, flow = 18.5 kcfs, depth of 30', time to onset of reaction was greater for new turbine 8 than for existing turbine 9

- For slot C, flow = 18.5 kcfs, new turbine 8, time of onset of reaction was greater for fish introduced at 30' than for those at 10'

- For slot B, flow = 11 kcfs, depth = 10', time to onset of reaction was greater for new turbine 8 than for existing turbine 9

- For new turbine 8, slot B, depth = 10', time to onset of reaction was greater at 11 kcfs than at 15 kcfs

- For new turbine 8, slot C, depth = 30', onset of reaction was greater at 9 kcfs than at 15 or 18.5 kcfs

- For existing turbine 9, slot C, depth = 30', and flow = 9 kcfs, time to onset of reaction was greater than for most other combinations

- For many of the combinations, time to onset of C-start reaction was greatest at 9 kcfs

3.2.2 Duration of Stage 1

For the dependent variable duration of Stage 1, the 5-way interaction was not significant. However, two of the 4-way interactions were significant, so various combinations of those factor levels were tested to identify which affected this behavioral response. Most comparisons revealed no significant effects on the duration of Stage 1 behavior that could be related to specific slots, turbines, or flows. However, the analyses detected the following differences ($P < 0.05$):

- Duration of Stage 1 was significantly greater for fish that passed through new turbine 8, slot B, depth = 30, and flow = 15 kcfs than for (1) turbine 8, slot C, depth = 10', flow = 18.5 kcfs; and (2) turbine 8, slot C, depth = 30', flow = 15 kcfs

- For slot B, flow = 18.5 kcfs, depth = 30', duration of Stage 1 was greater for existing turbine 9 than for new turbine 8

- For new turbine 8, slot B, and depth = 30', fish that passed through the turbine at 15 kcfs had a significantly greater Stage 1 duration than fish that passed through at 18.5 kcfs

- For the new turbine 8, slot B, depth = 30', and flow = 11 or 15 kcfs, turbine-passed fish had significantly greater Stage 1 durations than tailrace-released fish

- For existing turbine 9, slot B, and depth = 30', turbine-passed fish had a significantly longer duration of Stage 1 than tailrace-released fish.

3.2.3 Completeness of C-Shape Formation (H:T Ratio)

For the dependent variable H:T ratio (magnitude of C shape), none of the 5-way or 4-way interactions was significant. Six of the 10 possible 3-way interactions were significant, so various combinations of those factor levels were tested to identify which affected H:T ratios. Most comparisons revealed no significant differences in the H:T ratio that could be related to specific slots, turbines, or flows. However, the analyses revealed the following differences ($P < 0.05$):

- At 30' the mean H:T was smaller (i.e., C-start reaction is stronger) for the existing turbine 9 than for the new turbine 8

- Within new turbine 8, fish exhibited a smaller H:T ratio at 15 kcfs than at 18 kcfs

- Within existing turbine 9, fish exhibited smaller H:T ratio at 11 kcfs than at 15 kcfs

- For slot B, flow =18.5 kcfs, fish exhibited smaller H:T ratio in existing turbine 9 than in new turbine 8

- For slot C, flow =9 kcfs, fish exhibited a smaller H:T ratio in existing turbine 9 than in new turbine 8

- For slot C, flow =9 kcfs, fish exhibited a smaller H:T ratio when introduced at 30 feet than at 10 feet

- For slot B, flow = 15 kcfs, depth = 10', fish exhibited a smaller H:T ratio for new turbine 8 than existing turbine 9

- For slot B, flow = 15 kcfs, existing turbine 9, fish exhibited a smaller H:T ratio when introduced at 30 feet than when introduced at 10 feet

- For slot B, flow = 15 kcfs, both turbines, the fish introduced at 30 feet had a smaller H:T ratio at 1 minute than at 5 and 15 minutes

- For slot C, flow = 18.5 kcfs, new turbine 8, fish introduced at 30 feet had a smaller H:T ratio than fish introduced at 10 feet

- For slot B, flow = 18.5 kcfs, and depth = 30', fish exhibited a smaller H:T ratio from existing turbine 9 than from new turbine 8

- For slot C, flow = 9 kcfs, and depth = 10', fish exhibited a smaller H:T ratio from existing turbine 9 than from new turbine 8

- For existing turbine 9, slot C, depth = 30', fish released at 9 kcfs had a smaller H:T ratio than fish released at 15 or 18.5 kcfs

- For existing turbine 9, slot C, depth = 30', and flow = 9 kcfs, control fish released into the Unit 9 tailrace had a smaller H:T ratio than Unit 9-passed fish

- For existing turbine 9, slot B, depth = 30', fish released at 11 kcfs had a smaller H:T ratio than fish released at 15 or 18.5 kcfs

- For existing turbine 9, slot B, depth = 10', fish released at 15 kcfs had a smaller H:T ratio than fish released at 18.5 kcfs

- For new turbine 8, slot C, depth = 10', fish released at 15 kcfs had a smaller H:T ratio than fish released at 9 or 18.5 kcfs. At both 5 and 15 minutes, Unit 8 tailrace control fish had a smaller H:T ratio than Unit 8 turbine-passed fish

- For new turbine 8, slot B, depth = 30', fish released at 15 kcfs had a smaller H:T ratio than fish released at 18.5 kcfs. Fish released at 11 kcfs had a smaller H:T ratio than fish released at 18.5 kcfs. At a time of 5 minutes, Unit 8 tailrace control fish had a smaller H:T ratio than Unit 8 turbine-passed fish

- For new turbine 8, slot B, depth = 10', fish released at 15 kcfs had a smaller H:T ratio than fish released at 18.5 kcfs.

3.3 General Averages/Trends in Measured Responses

Overall mean values for these components of the behavior were compared among the test and control groups. Figure 3.1 shows the means for all test conditions and all post-exposure filming times (time = 1, 5 and 15 minutes combined), a total of 346 video files. The average times to onset of reaction varied from 0.25 s to 0.57 s. The fish that were introduced into the Unit 9 tailrace had a significantly longer time to onset of reaction than the hatchery control fish ($P \leq 0.04$). There was a weakly significant difference between Unit 9 turbine-passed fish and Unit 9 tailrace fish ($P \leq 0.10$), with turbine-passed fish showing a faster response than the tailrace controls. Mean durations of Stage 1 ranged from 0.17 s to 0.28 s; the mean times were significantly longer ($P \leq 0.05$) for turbine-passed salmon than for either their respective tailrace controls or hatchery controls. The mean H:T ratios (distance between head and tail at the point of maximum C shape divided by the fish's total length) ranged from 0.63 to 0.72. H:T ratios for Unit 8 turbine-passed and tailrace fish were significantly greater than for the tagging and hatchery controls.

Different patterns for these components of C-start behavior were seen when examined at specific post-stress stimulus times. At time = 1 (one minute after the fish were placed into the observation tank), no significant differences ($P \leq 0.05$) were detected among the test and control groups in the mean times to onset of reaction; the decreased times for onset of reaction

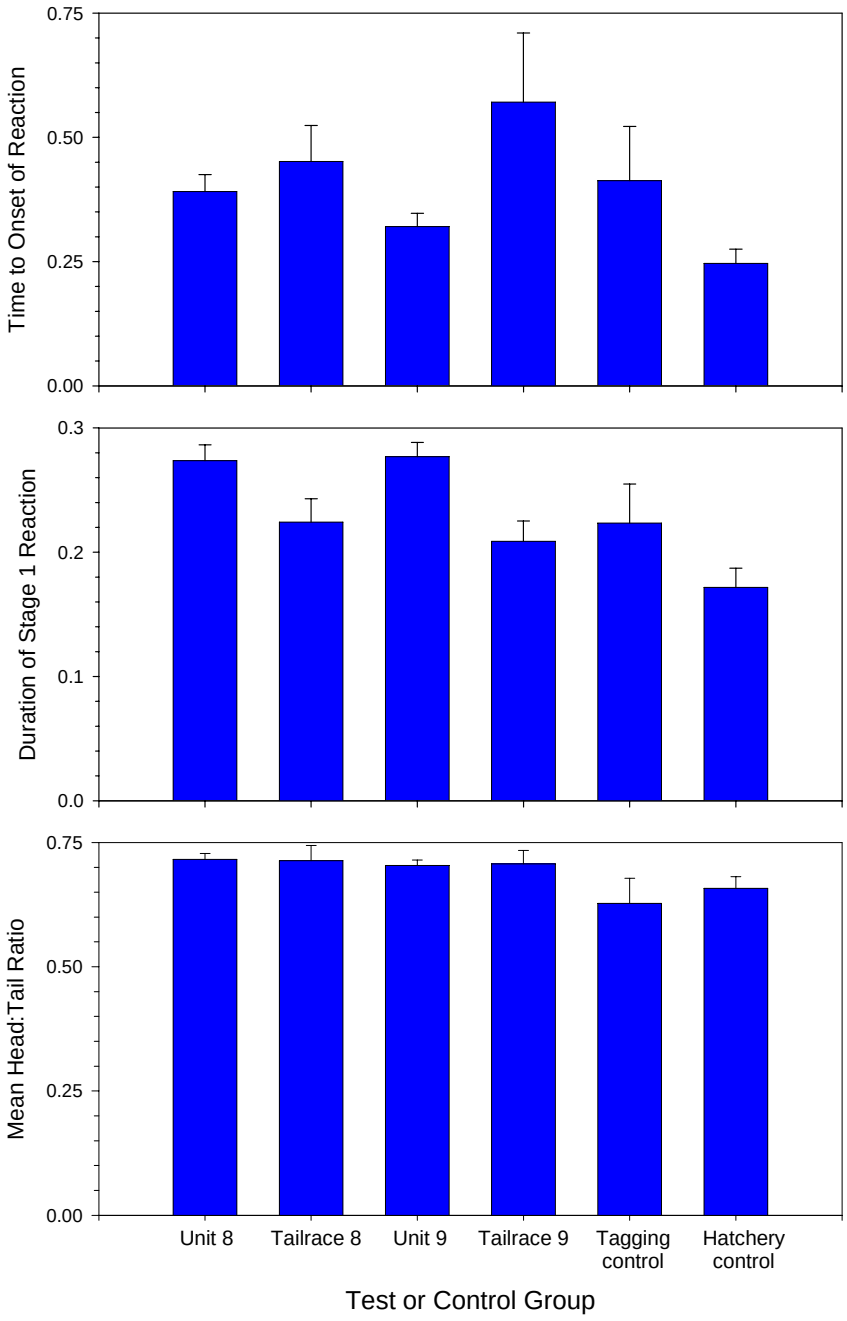

Figure 3.1. Overall mean values (± 1 SE) by treatment (across all other main factors including time) for measured responses of yearling summer Chinook salmon to a post-stress startle stimulus. Time to onset of reaction and duration of reaction are measured in seconds.

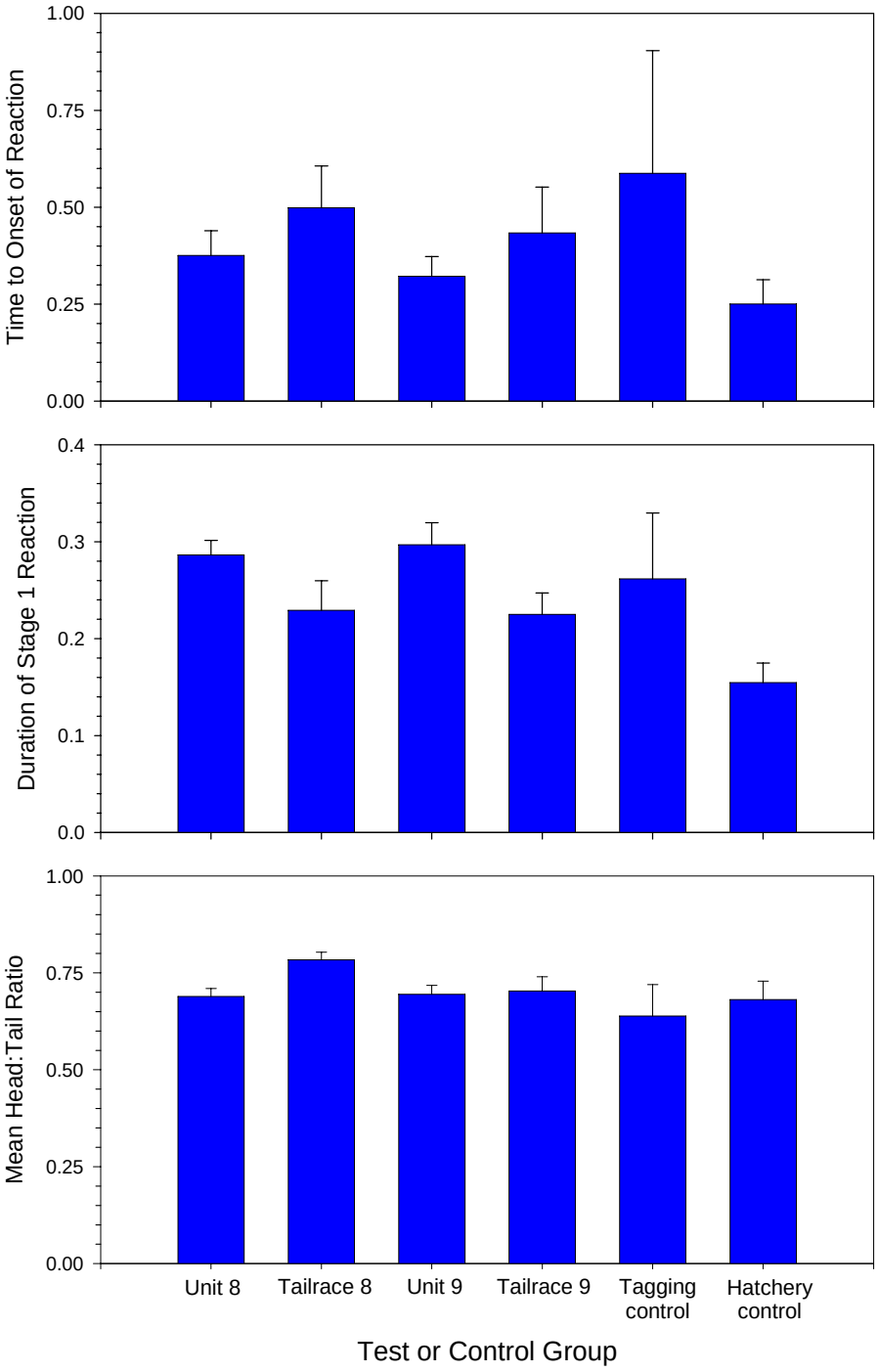

Figure 3.2. Overall mean values (± 1 SE) by treatment at a post-recovery stimulus time of 1 minute for measured responses of yearling summer Chinook salmon to a startle stimulus. Time to onset of reaction and duration of reaction are measured in seconds.

in turbine-passed fish compared to their respective controls were only weakly significant ($P \leq 0.18$ and $P \leq 0.16$ for Units 8 and 9, respectively) (Figure 3.2). On the other hand, durations of Stage 1 were longer for turbine-passed fish than for the hatchery controls ($P \leq 0.01$). Salmon that passed through Unit 8 had a smaller H:T ratio than Unit 8 tailrace controls ($P \leq 0.06$).

At time = 5 (5 minutes after fish were placed into the observation tank), no significant differences were detected among the test and control groups in the mean times to onset of reaction (Figure 3.3). Unit 9-passed fish had a longer duration of Stage 1 than Unit 9 tailrace fish ($P \leq 0.09$). Salmon that passed through Units 8 and 9 had significantly longer Stage 1 durations than hatchery controls ($P < 0.03$). Mean H:T ratios tended to be larger for turbine-passed and tailrace-passed fish than for the tagging and hatchery controls.

At time = 15 (15 minutes after fish were placed into the observation tank), time to onset of reaction was over 0.9 s for fish introduced to the tailrace of Unit 9; times for all other groups were significantly shorter and not significantly different from each other (Figure 3.4). Mean durations of Stage 1 were significantly longer for turbine-passed fish than for hatchery controls. Similarly, Unit 9-passed fish had a significantly greater H:T ratio than hatchery controls ($P \leq 0.09$).

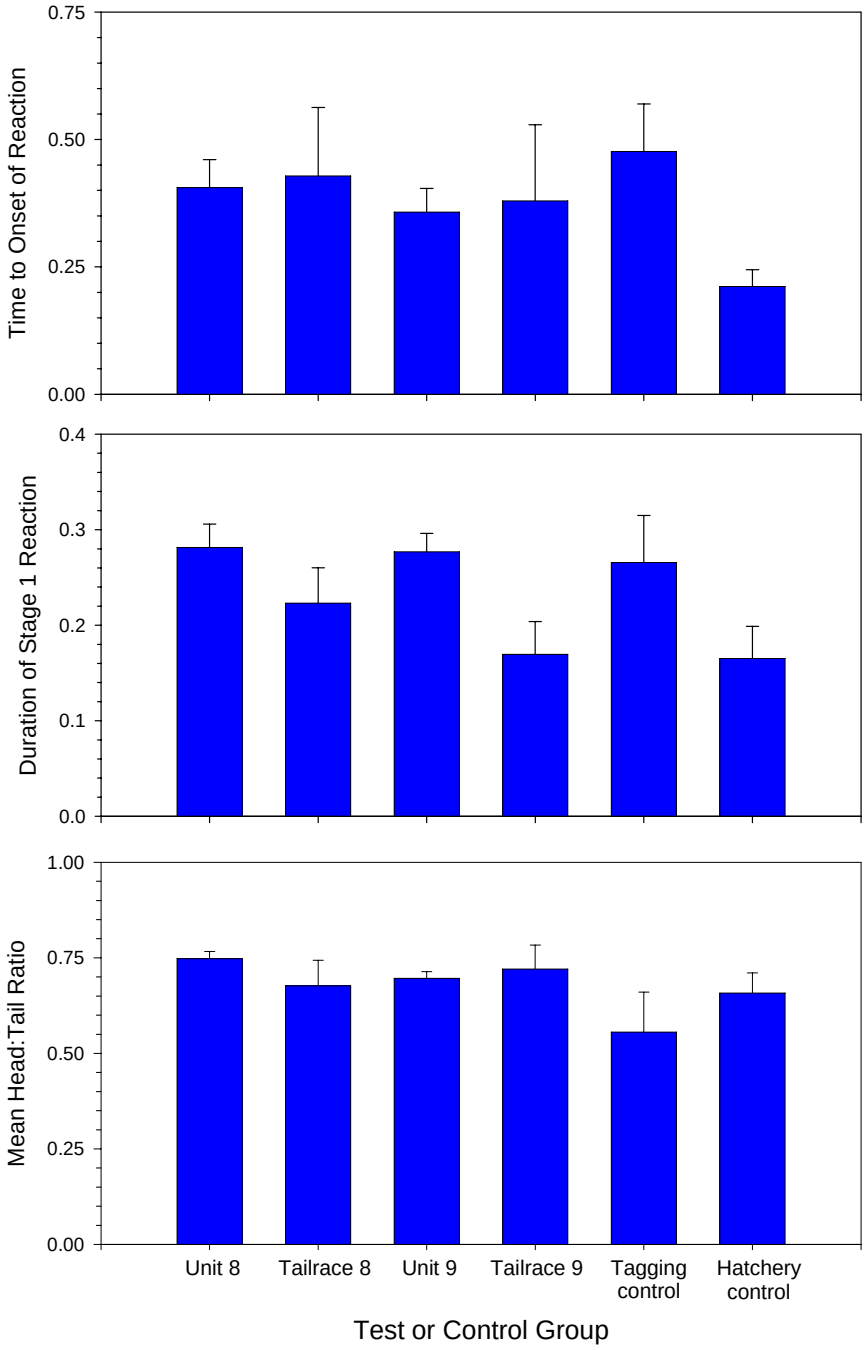

Figure 3.3. Overall mean values (± 1 SE) by treatment at a post-recovery stimulus time of 5 minutes for measured responses of yearling summer Chinook salmon to a startle stimulus. Time to onset of reaction and duration of reaction are measured in seconds.

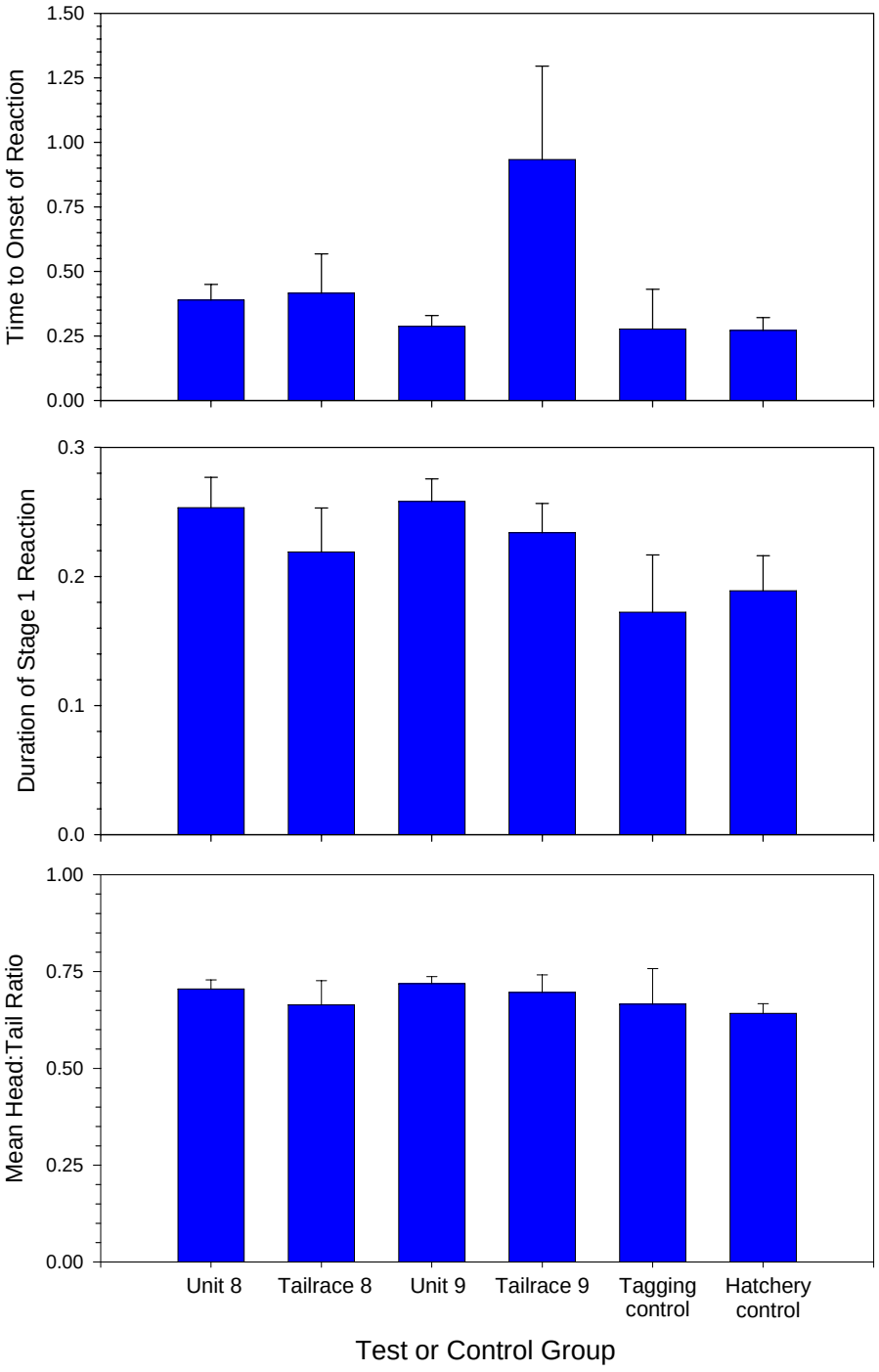

Figure 3.4. Overall mean values (± 1 SE) by treatment at a post-recovery stimulus time of 15 minutes for measured responses of yearling summer Chinook salmon to a startle stimulus. Time to onset of reaction and duration of reaction are measured in seconds.

4. DISCUSSION

Relatively few significant differences were detected in overall C-start behavior or its components that could be attributed to turbine passage conditions. Furthermore, few differences were found in fish responses at the 1-, 5-, and 15-minute post-recovery stimulus times. In most cases, the behaviors of turbine-passed fish were no different from their respective tailrace-passed controls.

Table 4.1 displays the particular test conditions under which the new MGR turbine performed better than the old Kaplan turbine (from the standpoint of effects on fish C-start behavior), and vice versa. At a turbine flow rate of 18.5 kcfs, Unit 8 performed better under two conditions and Unit 9 performed better under two conditions. In one instance (flow = 18.5 kcfs; depth = 30 feet; Slot B), fish passing through Unit 8 had a shorter duration of Stage 1 (faster escape response) but also a larger H:T ratio (weaker C-start reaction). Overall, there were few test conditions in which we were able to distinguish between the old Kaplan turbine (Unit 9) and the new MGR turbine (Unit 8) on the basis of changes in the escape behavior of uninjured fish. This result is consistent with the findings of the direct mortality studies, which found a high post-passage survival for both turbines ($\geq$ 94 percent under all conditions) and overall no difference between the two turbines (Normandeau et al. 2006).

Table 4.1. Comparison of new MGR (Unit 8) and old Kaplan (Unit 9) turbines based on expressions of C-start behavior among turbine-passed yearling summer Chinook salmon. All differences are statistically significant ($P \leq 0.05$).

MGR (Unit 8) is better	Kaplan (Unit 9) is better
	At 30' the mean Head:Tail ratio was smaller for turbine 9 than for turbine 8. That is, C-start reaction is stronger for mid-blade passed fish in the Kaplan than for the other depth-turbine combinations
For slot B, flow = 18.5 kcfs, depth = 30', duration of Stage 1 was greater for turbine 9 than for turbine 8	
For slot C, flow = 18.5 kcfs, depth = 10', time to onset of reaction was greater for turbine 9 than for turbine 8	For slot B, flow =18.5 kcfs, depth = 30', H:T ratio was smaller in turbine 9 than in turbine 8. No significant effects due to depth or time
For slot B, flow = 15 kcfs, depth = 10', the H:T ratio was smaller for turbine 8 than for turbine 9	For slot C, flow =9 kcfs, H:T ratio was smaller in turbine 9 than in turbine 8
	For slot B, flow = 11 kcfs, depth = 10', time to onset of reaction was greater for turbine 8 than for turbine 9
	For slot C, flow = 18.5 kcfs, depth = 30', time to onset of reaction was greater for turbine 8 than for turbine 9

The absence of significant changes in escape behavior might have several explanations: (1) insufficient replication of test conditions may have limited the power of the statistical tests to detect differences; (2) the hydraulic stresses associated with passage were not different enough between the two turbines or between the turbine and tailrace release controls to affect C-start behavior; (3) too much time elapsed between turbine passage and behavior testing, so that fish recovered from any temporary changes in escape behavior; and (4) some aspect of the tagging, release, and/or recovery procedure had an effect on escape behavior that overwhelmed differences between treatments. Each of these possibilities is discussed below.

It is well known that the detection of differences between treatments is dependent in part on the number of replicates. This constraint is particularly significant if the differences between treatments are small, and it may have been a factor in our study. Although we made 1080 observations of behavior, the test fish were partitioned among a variety of conditions (turbines/controls, depths, intake slots, and turbine flow rates) in an unbalanced design. Assigning more fish to fewer experimental treatments might have allowed the detection of smaller changes in fish behavior.

Regarding the hydraulic stresses associated with turbine passage, we assume that, like many stressors, the potential for turbulence to disorient fish is a function of magnitude (intensity) and exposure time. There are three reasons why demonstrating this assumption at Wanapum Dam is difficult. First, turbulent stresses associated with passage through the MGR runner, the Kaplan runner, or the draft tubes have not been quantified. Instead, the best surrogate we have for turbulence is the turbine flow rate. Because of the characteristics of the Kaplan and MGR turbines and their draft tubes, it was predicted that flows in the middle range (11 and 15 kcfs) would be the least turbulent, and that flows at the outer range of operating conditions (9, 17, and 18.5 kcfs) would be the most turbulent. Whether that is true or not remains to be measured. Second, each fish takes a different path through the turbine, and the level of turbulence within the flow path is highly variable over time and space (Čada et al. 2006). It is possible that a fish passing through at turbine flow rates that are expected to be damaging (e.g., 18.5 kcfs) may nonetheless happen to travel in a path through the turbine that has low turbulence. Conversely, a fish entrained at moderate flow rates could experience a high level of turbulence if it followed a different path, e.g. close to fixed or moving surfaces or in vortices associated with the runner hub. Finally, exposure time for both turbines is similar and relatively small, on the order of 1-2 minutes between the fish's introduction upstream from the turbines and its passage out the draft tube. It is possible that over the range of flow conditions studied, the turbulence time-exposure histories in the two turbines were not sufficiently different either from each other or from tailrace controls to be reflected in measurable changes in fish behavior. Consequently, in terms of

effects on fish behavior, there may not be significant differences between the two turbines, or it may not be possible to distinguish effects without detailed knowledge of the fluid stresses associated with each fish passage event.

Previous studies of the effects of turbulence on fish escape behavior were carried out in the laboratory or pilot-scale turbine tests; the tests at Wanapum are the first application of C-start analyses in the field. In the laboratory, Čada et al. (2003) exposed striped shiners (*Luxilus chrysolcephalus*) and fathead minnows (*Pimephales promelas*) to 10-, 20-, or 30-minutes of turbulence in laboratory tanks, then examined for C-start behavior beginning 1 min after the end of the turbulence stress. Temporary, but significant loss of C-start behavior occurred immediately after the 20- and 30-minute exposures. Turbulence was quantified in the laboratory tanks, but it is not known how those values compare to the unknown levels of fluid stresses experienced by turbine-passed fish at the Wanapum Dam. Subsequently, Ryon et al. (2004) examined changes in C-start behavior among rainbow trout (*Oncorhynchus mykiss*) that passed through a pilot-scale turbine runner that was tested at the Alden Research Laboratory. In these laboratory tests, there were no significant differences among the treatment and control groups in terms of presence/absence of C-start response, but the time to first reaction, duration of the reaction, and the duration of Stage 1 among turbine-passed fish were all significantly different from the controls. No measurements of turbulence are available for the Alden runner tests, so we cannot determine whether the fluid stresses are comparable to those associated with the Wanapum tests. Whether or not the turbulence intensities in the laboratory and field tests were comparable, it is certain that the duration of exposure to strong turbulence was lower for Wanapum-passed fish than for the minnows tested by Čada et al. (2003).

It is possible that too much time elapsed between a fish's passage through the turbine and our measurement of its behavior. Earlier studies demonstrated that turbulence-induced effects on C-start behavior diminished with time when the fish were allowed to recover in quiet water (Čada et al. 2003; Ryon et al. 2004). The recovery time between application of turbulent stresses and behavior testing was greater in the present Wanapum tests than in the previous laboratory studies. Passage through the Wanapum turbines was completed within 1-2 minutes of introduction, after which the Hi-Z (balloon) tagged fish drifted in the tailwaters until retrieved by the recovery boats and placed in holding tanks. In all cases the salmon had been recovered from the tailwaters and transported to the behavior observation tank within 20 minutes of introduction (mean time = 12 minutes). During the tailrace collection and transferal to the onshore observation tank, potentially disorienting turbulence was minimized. Thus, the periods of exposure to turbulence used in the laboratory by Čada et al. (2003; 20 or 30 minutes of exposure to turbulence followed by behavior testing within 1 minute) were much longer than

those experienced by fish at Wanapum Dam (1-2 minutes of exposure to turbulence followed by behavior testing 10-20 minutes later). Further, the additional recovery time in the present study may have been enough to diminish temporary effects on predator avoidance behavior.

Finally, it is possible that the elaborate tagging, introduction, and recovery procedures needed to estimate direct injuries and mortality at Wanapum Dam may have influenced C-start behavior in a way that reduced the differences between treatment groups. Turbine- and tailrace-released fish were anaesthetized with MS-222, tagged with 2 Hi-Z balloon tags and a radio tag, and allowed to recover in a holding tank for about 20 minutes before being introduced into the turbines or tailrace (Normandeau et al. 2005). The three tags penetrate the dorsal musculature of the fish, trail behind the fish during its time in the river, and buoy it to the surface after inflation. Although the tags were removed from recaptured fish before behavior testing, the wounds could have affected the strength of C-start reaction. In addition, the anesthesia used in the Wanapum tests to reduce the stress of tagging, handling, and recovery could have lowered the sensitivity of fish to the startle stimulus in our tests. For example, Čada et al. (2003) found that exposure to MS-222 significantly (but temporarily) reduced C-start behaviors in minnows. In anticipation of this possibility, we conducted a series of C-start behavior tests using juvenile Chinook salmon that were not anaesthetized, tagged or handled (except to transfer them from Normandeau's holding tank to the C-start observation tank). These were the hatchery control group. Often, the hatchery controls exhibited more-pronounced C-start behaviors (most likely to react; smallest time to onset of reaction; shortest duration of Stage 1 reaction; smallest H:T ratio) than the other test groups. On the other hand, tagging control fish, which were anaesthetized, tagged, and had tags removed but no exposure to the river, often had the poorest C-start behaviors. These observations suggest that some aspect of the introduction and recovery procedures, most likely insufficient recovery from anesthesia, may have reduced the C-start behaviors of fish in the turbine-passage and tailrace control groups.

The usefulness of the C-start behavior test as an indicator of indirect mortality is predicated on how well the selected measurements reflect actual actions taken to avoid predation. Predation upon sublethally stressed fish is very difficult to measure in a river, but in the laboratory it has been examined by means of predator-preference tests (Coutant 1973; Mesa et al. 1994; Neitzel et al. 2000). In these tests, mixtures of equal numbers of stressed and unstressed (control) fish are offered to predators, and the proportions of the two groups remaining at the end of a feeding period are used to determine whether predators preferentially preyed on stressed fish. We were not able to carry out predator-preference tests during the Wanapum C-start tests. However, in our earlier laboratory studies we compared our C-start behavior tests to simultaneous predator-preference tests. The first study used minnows stressed by laboratory-generated turbulence or anesthetic (Čada et al. 2003), and the second study used juvenile striped bass (*Morone saxatilis*) stressed by elevated water temperatures (Cloe Luckett, ORNL, 2005 unpublished

data). In both laboratory studies, the C-start and predator-preference tests gave the same results – levels of stress that were sufficient to cause a change in C-start behavior also increased the susceptibility to predators.

Measures of C-start behavior can be useful indicators of potential predation losses in a field setting if conditions allow for proper application of the technique. The effects of some sublethal stresses on behavior may be relatively long-lasting, e.g., the influence of chemical contaminants on resident fish. In such cases, recovery time may not be an overriding consideration when designing C-start tests. In the case of effluent plumes, caged fish could be exposed to the stress and easily retrieved for testing. However, some types of sublethal stresses may cause only a temporary change in behavior (e.g., temporary disorientation from turbulence), so it is often important to retrieve and test the stressed fish as quickly as possible. Other coincident stresses, such as anesthesia, handling, and tagging, must be minimized; while these may not affect the results of direct survival studies, they could significantly alter behavior and obscure the effects of the treatments. Finally, it would be valuable to conduct at least some predator-preference studies concurrently with tests of C-start behavior to establish that observed effects reflect a real potential for increased indirect fish mortality.

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