Studies to Establish Biological Design Criteria for Fish Passage Facilities: Separation by Size of Juvenile Salmonids using a High-Velocity Flume at Ice Harbor Dam in 2004

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EXECUTIVE SUMMARY

Juvenile salmonid separation for raceway holding and transportation is an ongoing objective of juvenile bypass facilities at hydroelectric dams on the Snake and Columbia Rivers. In the late 1970s to early 1980s, size separators were "dry," meaning fish were out of water during the separation process. Since 1983, size separation has been attempted through the use of wet separators. Wet separators use submerged bars with an attraction flow beneath the bars to induce volitional separation, where smaller fish dive between bars with smaller spacing.

Water depth in a wet separator is about 1 m, and fish exit through side outlets on the tank bottoms. These separators have two compartments. The first compartment has narrow gaps between the bars so that only the smaller fish can pass through. The second compartment has wider gaps for the larger smolts to pass through. A moderate downstream flow above the bars carries larger fish, such as adult salmon and other incidental species, into a separate flume for return to the river. Results with these separators have been mixed at best. Generally, it is difficult to maintain consistent attraction flows from beneath the separator bars; additionally, after separation, some smolts will remain in the compartments rather than exit.

Studies were initiated in 1996 to investigate methods to improve wet-separator performance and to explore alternative designs. A high-velocity-flume (HVF) concept was developed during these studies. In the HVF separator, submerged bars with differing gap sizes are again employed to achieve separation. However, instead of a quiet, “pool-type” environment both above and below the separator bars, a downstream flow of around 2 m/sec is maintained through the length of the separator. Water depth below the separator bars is about 40 cm, with “inline” exits at the downstream end of each compartment. Also, the entire system is enclosed, and uniform lighting is maintained to prevent shadows on the water surface.

In 2001, tests at Ice Harbor Dam using a prototype, high-velocity flume separator produced fairly high levels of separation (82% of the smaller fish entered the first compartment), with almost no delay in downstream passage. However, the numbers of smolts available for testing were limited, since many fish had been removed for transportation at collector dams upstream from this facility. Hence, nearly all tests were conducted when collection rates were low.
During 2004, a study design was implemented to test separator performance when numbers of fish or rates of fish passage more closely represent the actual conditions encountered at juvenile collection facilities during periods of peak passage. To accomplish this, juvenile salmon were collected from gatewells at Lower Granite Dam, transported to Ice Harbor Dam, and held overnight prior to testing. After holding, test fish were released through a 20.3-cm diameter hose directly into the 91-cm diameter pipe exiting the juvenile bypass channel. Passage through the pipe to the test separator (a distance of approximately 250 m) took just under 1 min. Results from this study indicated that juvenile salmonid separation efficiency of over 80% could be achieved with no delay in passage when nearly 2,000 smolts/min passed through the separator.
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INTRODUCTION

Bypass facilities at hydroelectric dams on the Snake and Columbia Rivers are used to collect juvenile Pacific salmon *Oncorhynchus* spp. for subsequent transport or release downriver. It is generally thought that juvenile Chinook salmon *O. tshawytscha* transported with juvenile steelhead *O. mykiss*, which are larger than the former, experience higher levels of stress than those transported with other conspecifics only (McCabe et al. 1979; Park et al. 1984; Schreck et al. 1995; Kelsey 1997). Therefore, separation of smolts by size has been an objective of operational juvenile bypass systems since shortly after their inception in the 1970s.

The first separators were a series of diverging, inclined PVC-covered pipes, wherein fish simply dropped through gaps between the pipes as they widened. Smaller fish separated first, while larger fish slid farther down the pipes prior to separation. Overhead spray bars kept the pipes wet, but the fish were out of water for most of the process; therefore, these systems were considered “dry” separators. This type of separator functioned quite well from a separation efficiency standpoint, but there was concern for the well-being of the fish, since some could remain out of the water for extended periods.

In 1981, a study conducted at Little Goose Dam led to the installation of “wet” separators at collection/bypass sites (Gessel et al. 1985). Wet separators have two compartments, with each having its own set of submerged separator bars (Figure 1). The first compartment, or “A” side, has a narrower gap (18 mm) between the separator bars, and the second compartment, or “B” side, has a wider gap (38 mm). This type of separator requires an attraction flow from beneath the separator bars, which is used to induce fish to sound (i.e., dive) between bars, thereby separating of their own volition. This system works as follows:

Following partial dewatering, all fish are delivered to the "A" section of the separator, where smaller fish sound through the separator bars and are taken to a fish collection area. They eventually egress to a "small fish" holding area in the fish passage facility. Larger fish continue to the "B" section, where the next size class is removed in a similar manner. Fish too large to negotiate the bar gaps of the B-section pass into a flume at the end of the system for return to the river. For anadromous salmonids under ideal conditions, the A section is intended to segregate smaller smolts such as Chinook, coho *O. kisutch*, and sockeye *O. nerka* salmon from the larger, predominantly hatchery steelhead smolts. Large fish eliminated from the process are generally adult salmon fallbacks and non-salmonid incidental species.
Figure 1. Cross-sectional view of existing wet-separators in use at collector dams on the Snake and Columbia Rivers.
There are two primary problems with existing wet separators. First, using the standard operating conditions available, separation efficiency is generally poor. For example, the wet separator at McNary Dam exhibited poor performance in its A section, resulting in separation efficiency values of 41.4, 22.9, and 26.7% for yearling Chinook, coho, and sockeye salmon, respectively, in 1998 (Hurson et al. 1999). Possible reasons for these low separation rates were a) flow surges over the separator bars carried small fish past the A section with insufficient time to sound, and b) hydraulic conditions in the separator were inadequate to stimulate a sounding response.

The second problem is a tendency for the juveniles to remain within the holding tank rather than exiting. Some behavioral and physiological studies have indicated that fish that hold under the bars for extended periods are more stressed than those that exit quickly (James L. Congleton, University of Idaho, personal communication). This suggests that many fish exit only after they are fatigued as a result of swimming against certain hydraulic conditions within this type of unit.

In 1996, personnel from the National Marine Fisheries Service (NOAA Fisheries) in conjunction with the U.S. Army Corps of Engineers (COE), and with input from both state and other federal agencies, began a series of studies to investigate methods to improve wet separator performance and to explore alternatives to the existing design. One promising concept was the high–velocity flume (HVF) separator. This separator design required smolts to enter a section of open flume traveling at velocities higher than those normally present in conventional wet separators (>1 m/sec). Similar to the existing separators, the first section has narrow gaps between the bars, and the second section has wider gaps. However, the higher velocities allow both size groups of smolts to continue to different holding areas without delay, avoiding the stress and fatigue induced by combating flows within the separator.

Test results using an evaluation HVF separator at McNary Dam from 1998 through 2000 indicated that about 80% separation could be achieved for the total catch of all salmonid species combined. These tests used a transport velocity (through the separator) of 1 m/s combined with separation bars submerged 5 cm below and parallel to the water surface and spaced 19 mm apart (McComas et al. 2002). Based on these conclusions, a prototype HVF separator was constructed at Ice Harbor Dam for evaluation during the 1999 juvenile migration (Figure 2).
Figure 2. Overhead view of the prototype high-velocity flume wet separator tested at Ice Harbor Dam during the 2001 and 2004 field seasons.
This separator has only one set of separator bars, and the gap size between bars is set at 17 mm. Smaller smolts pass through the bars and into one flume, while larger smolts (and non-separated small smolts) stay above the bars and enter a different flume. Initial testing at Ice Harbor Dam, however, resulted in a preliminary estimate of less than 70% separation under the above conditions. Also, it appeared that some smaller smolts resisted sounding at the lower velocity tested in the prototype HVF separator.

Further analysis showed that total catch separation efficiency was higher at a transport velocity of 2 m/s (72%) than 1 m/s (65%). With incremental improvements in separation bar conditions, separation efficiency in 2000 increased to 80%, and analysis of data from the Ice Harbor Dam HVF for 2001 indicated a total catch separation value of 82% using the most advantageous light and substrate treatments with a 2 m/s transport velocity.

Although these results were satisfactory, the fish available for tests during the 1999-2001 evaluations of the HVF were limited to those exiting the Ice Harbor bypass channel. Most replicates lasted 30-60 min, and replicate sizes ranged from about 50 to 300 smolts. These densities were ideal for establishing physical criteria for a working separator because they impacted the fewest possible numbers of fish. However, since separators are operated continuously throughout the migration season, fish densities can far exceed the densities tested.

Following separation studies in 2001, a preliminary separator redesign effort was undertaken to identify the suitability of separator types for installation at lower Snake River dams. Given the consistent separation efficiency achieved using the HVF separator, the study identified Lower Granite and Lower Monumental Dams as prospective sites to install high–velocity flume separators.

To adequately evaluate the HVF separator prior to installation at either of these dams, additional testing was required to simulate the actual fish densities expected to occur during any juvenile migration. Thus, our objective in 2004 was to evaluate the effects of fish density on volitional sounding response (resulting in size class separation), exit efficiency, and fish condition of salmonid smolts in a high–velocity flume separator.
METHODS

Prototype Separator

We used the prototype wet-separator at Ice Harbor Dam to evaluate treatments under the study objective (Figure 2). The adjustable-slope channel and test separator were 1 m wide by 1.5 m high. The separation-bar array was comprised of four interconnecting 3-m long panels (12-m overall length). The high–density test configuration (flume angle, makeup water requirements, and dewatering settings) were the same as those used in the prototype tests at Ice Harbor Dam (see McComas et al. 2003, Appendix A). Test conditions included a transport water velocity of 2 m/s, pedestal-style separation bars spaced 17 mm apart, 5-cm water depth over the separation bars, flow through the separator parallel to the separation-bar array, controlled full-spectrum artificial light over the entire separator with no shadows, and flat black substrate (which included separation bars and the area under the separation-bar array).

The 17-mm bar spacing was intended to segregate small fish (<180 mm fork length) from larger fish (≥180 mm FL). Fish exiting the separator section were routed to one of the two holding tanks, dependent on whether they had separated or not. Fish from each holding tank and from the test separator were anesthetized with MS-222 and checked for descaling. Fork lengths were tallied by length group (<180 mm or ≥180 mm) for each species, and data were recorded by species for each replicate. Salmonids were inspected for condition using descaling criteria of the Fish Transportation Oversight Team (Ceballos et al. 1992). Following a suitable period for recovery from the effects of anesthesia, all fish were released into the existing facility bypass flume for return to the river.

Separation efficiency values were estimated for both separated and non-separated fish as the fraction of a given length group negotiating the separation bars divided by the total number of fish in that group captured from both holding tanks. Similarly, exit-efficiency values were estimated as the fraction having exited the test separator divided by the total number of fish entering the unit during the test.
Fish Density Evaluations

To test the prototype high–velocity separator under “high density” smolt passage conditions, we first determined the numbers of fish that might be present during peak passage periods at dams. Daily estimates of smolt passage were provided by the Fish Passage Center (www.fpc.com). These estimates were computed from different sample rates projected over a 24-h period. For example, a 10–min sample rate of 50 yearling Chinook smolts would be extrapolated to 300 fish/h and to 7,200 fish/24 h (6 × 50 × 24 = 7,200). Hourly counts, in conjunction with a 24-h multiplier and an additional discrepancy factor, provided the 24-h fish count.

A review of daily counts from past years showed that high 24-h counts at Lower Granite Dam generally ranged from 200,000 to 400,000 smolts, but in some years counts were as high as 900,000. In fact, on 6 May 2004, the total daily count exceeded 800,000 smolts. These totals were derived using hourly estimates that at times exceeded 90,000 fish. Therefore, it is probable that smolt collection exceeded 1,500 fish/min at Lower Granite Dam for a few hours during the 2004 juvenile migration.

Smolts were collected from gatewells at Lower Granite Dam using a dipbasket and crane similar to those described by Swan et al. (1979). Collecting smolts from the gatewells provided test fish that had not encountered a juvenile fish bypass channel or separator unit, and in this regard, were considered naïve.

Gatewell dipping began each morning around 0800 PST and continued until 1000-1200 PST, depending upon fish numbers. Four 200-gallon insulated aluminum tanks were used to collect and then transport the smolts to Ice Harbor Dam. Each tank was provided with fresh river water during the collection period, and auxiliary oxygen was supplied via airstones during the trip from Lower Granite Dam to Ice Harbor Dam.

After transport, the fish were held in fresh river water overnight and then transferred (water-to-water) into a larger release tank that was situated at the downstream end of the juvenile bypass collection channel. The release tank was 2.1-m long, 0.6-m wide, and 1.5-m high (650 gallons). A clear plastic 20.3-cm diameter hose was used to release the fish directly into the 91.4-cm diameter pipe that carries smolts to the juvenile collection facility at Ice Harbor Dam. After each release, the tank was refilled and emptied twice to ensure that all released fish had entered the bypass pipe.

Prior to beginning a replicate, flow was initiated in the prototype separator using system flush lines and the auxiliary water supply described in McComas et al. (in prep). The drop gate was then opened, and flow from the juvenile bypass system was directed into
the test separator facility. Flow adjustments were then made at the prototype test separator to ensure that test conditions were maintained. Once these procedures had been completed, personnel operating the release tank were radioed and directed to release the test fish.

Generally, the setup procedure required about 1 h, so fish were given a short acclimation period between transfer to the large release tank and actual release into the bypass pipe. Opening the drop gate meant that all smolts volitionally passing via the bypass system at Ice Harbor Dam would then be directed into the prototype test separator. There was initial concern that these fish might somehow “bias” the test results because they could have previously encountered separation facilities upstream. However, this concern was short-lived simply there were so few smolts passing via the Ice Harbor juvenile bypass system during the study.

Prior to any fish testing, flows through the system were monitored by placing neutrally buoyant particles into the transport pipe. Passage rate was just over 4 m/sec for these particles.

Distance from the release tank to the high–velocity separator was about 250 m, and fish passage time (from point of release to the separator bars) was just less than 1 min for all replicates.
RESULTS AND DISCUSSION

The test schedule required collection of smolts dipped from the gatewells at Lower Granite Dam one day per week during the 2004 field season (late-April to mid-June). Smolts were then transported to Ice Harbor Dam and used to test the high–velocity separator the next day. However, setup problems, followed by large numbers of smolts in the gatewells at Lower Granite Dam in early May, delayed the start of evaluations from late April to mid-May.

Also, the study proposal originally called for replicates using high, medium, and low densities of smolts. Low–density tests were meant to be conducted using fish collected from the Ice Harbor Dam juvenile bypass system during standard facility operations. However, there were high spill volumes at the dam, and a large proportion of juvenile fish had been removed for transportation at upriver collection sites (Lower Granite, Little Goose, and Lower Monumental Dams). Therefore, the anticipated smolt collection numbers at Ice Harbor Dam were drastically reduced. We attempted to run low-density tests throughout the field season, but we never collected more than 35 fish for a test, even though each test was run for nearly 2 h (most tests had between 10-20 smolts total collection).

We released a total of seven replicates of juvenile salmonids collected and transported from Lower Granite Dam. The total number of smolts transported from Lower Granite Dam for the study was 9,484 (Table 1). A fairly good species mix was available during the tests, with juvenile sockeye the only species lacking. As evident from Table 1, the last two replicates were composed almost entirely of subyearling Chinook salmon.

Separation percentages for small (<180 mm FL) and large smolts (≥180 mm FL) are shown in Table 2. During the high–velocity wet separator tests conducted at Ice Harbor Dam in 2001, total separation ranged from 65-92 % (McComas et al. in prep) which was similar to the range for the high–density tests conducted here (76-95%).

Combining the separation data for all sizes of fish by replicate produced an average total separation efficiency estimate of roughly 80%. We did not consider this estimate to be highly precise due to a lack of sufficient replication. However, we were only attempting to determine if an overall separation efficiency value similar to that measured at Ice Harbor Dam in 2001 was attainable when fish densities through the separator were very high. It appeared that the separator performed quite well in this regard, since conservative estimates of passage rate during the different replicates ranged from about 300 to 2,100 fish/min.
Table 1. Species and numbers of juvenile salmonids transported from Lower Granite Dam during the prototype high–velocity wet separator tests conducted at Ice Harbor Dam during the spring and early summer juvenile migration season, 2004.

<table>
<thead>
<tr>
<th>Date</th>
<th>Yearling Chinook</th>
<th>Coho</th>
<th>Steelhead</th>
<th>Sockeye</th>
<th>Subyearling Chinook</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 May</td>
<td>378</td>
<td>0</td>
<td>215</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>21 May</td>
<td>811</td>
<td>461</td>
<td>624</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>26 May</td>
<td>292</td>
<td>245</td>
<td>731</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2 June</td>
<td>244</td>
<td>91</td>
<td>430</td>
<td>1</td>
<td>189</td>
</tr>
<tr>
<td>4 June</td>
<td>122</td>
<td>23</td>
<td>101</td>
<td>4</td>
<td>193</td>
</tr>
<tr>
<td>9 June</td>
<td>141</td>
<td>19</td>
<td>54</td>
<td>3</td>
<td>1,993</td>
</tr>
<tr>
<td>15 June</td>
<td>77</td>
<td>16</td>
<td>4</td>
<td>4</td>
<td>2,008</td>
</tr>
<tr>
<td>Totals</td>
<td>2,065</td>
<td>855</td>
<td>2,159</td>
<td>22</td>
<td>4,383</td>
</tr>
</tbody>
</table>

Table 2. Numbers of fish and separation percentages for all juvenile salmonids (<180 mm and ≥180 mm fork length) used during the high–velocity separator tests conducted at Ice Harbor Dam during the spring and early summer juvenile migration season, 2004.

<table>
<thead>
<tr>
<th>Date</th>
<th>Small fish (&lt;180 mm FL)</th>
<th>Separation (%)</th>
<th>Large fish (≥180 mm FL)</th>
<th>Separation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 May</td>
<td>429</td>
<td>94</td>
<td>164</td>
<td>100</td>
</tr>
<tr>
<td>21 May</td>
<td>1,337</td>
<td>74</td>
<td>566</td>
<td>99</td>
</tr>
<tr>
<td>26 May</td>
<td>619</td>
<td>71</td>
<td>549</td>
<td>98</td>
</tr>
<tr>
<td>2 June</td>
<td>594</td>
<td>64</td>
<td>317</td>
<td>99</td>
</tr>
<tr>
<td>4 June</td>
<td>266</td>
<td>82</td>
<td>75</td>
<td>95</td>
</tr>
<tr>
<td>9 June</td>
<td>1,943</td>
<td>89</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>15 June</td>
<td>2,105</td>
<td>93</td>
<td>4</td>
<td>100</td>
</tr>
</tbody>
</table>
Expanding these values produced estimated hourly smolt passage rates of 26,400 to 126,000 during our study. One qualifier of this estimate is that from a logistical standpoint, our study could only test separation efficiencies at these rates for short periods. In each of the individual replicates, the vast majority of smolts entered and exited the test area (the 12-m section of separator bars) in less than 1 min. A working separator at Lower Granite Dam can expect to incur similar passage rates, but over much longer time intervals.

There was essentially no delay in fish passage through the separator. Nearly all smolts passed through the system. A total of ten smolts were removed from under the separator bars at the end of the tests (three yearling Chinook on May 14, two yearling Chinook and one wild steelhead on May 21, one yearling Chinook and one wild steelhead on May 26, and two wild steelhead on June 2).

A few smolts (<20 total for all tests combined) did lodge between the separator bars, but these were easily moved downstream by the attendants. Although we saw no immediate mortality when we examined the test fish, it is possible that the fish that lodged between the separator bars may have died later. The high rate of flow over and through the separator bars would make it difficult for these fish to escape, especially if previous fatigue was a contributing factor. We did not attempt to monitor delayed mortality, nor did the study design call for stress evaluation tests. Although it appears that few smolts would be affected by wedging between the separator bars, it would be prudent to consider evaluations of both stress and delayed mortality when designing and installing a working high-velocity separator.

Descaling estimates were very low with only 0.4% (26/6203) of the fish descaled in the small fish tank and 1.6% (46/2917) descaled in the large fish tank. We saw no indication of any other external injuries (eye damage, torn opercles, or torn fins) that fish might have incurred given the high flow conditions used during these tests. However, we caution that no debris was present during testing. This situation (no debris) is not likely to be extant in a working high-velocity wet separator installed at a dam.

As we expected from the descaling data, mortality was also very low. We saw no immediate mortality after subjecting the smolts to the prototype tests. A total of 22 mortalities could be attributed to gatewell dipping and/or subsequent transport. All of these fish were removed from the holding tanks or the release tank prior to testing.
CONCLUSIONS

1. Total separation (the percent of fish <180 mm FL in the “A” section and the percent of fish ≥180 mm FL in the “B” section) averaged over 80% for all replicates combined.

2. No appreciable delay in passage through the separator was evident. Fewer than 10 fish (total for all replicates combined) remained under the separator bars after the tests were completed. No fish remained above the separator bars during any of the tests.

3. No descaling or injury problems were evident.

RECOMMENDATIONS

The high-velocity flume wet separator appears to be an efficient method of separating juvenile salmonids by size, and therefore by species (i.e., Chinook salmon from hatchery steelhead). However, to date it has not been possible to test a “complete” separator system (i.e., one that includes all the components required of an actual working separator). Size and space limitations in the prototype flume at Ice Harbor Dam have limited us to testing specific groups of fish based on size, passage rate, and removal of adult fish and debris from the system. We have not had the capability to test a complete system that includes both adult/debris and juvenile fish separator bars.

Both the numbers of fish passing through the current separator system at Lower Granite Dam (passage estimates exceed 1,500 fish/min during periods of peak passage) and the flow conditions (2 m/s) that are expected to be used in a “working separator” underline the need for prudence when “separator re-design” is considered. A set of debris/adult fish separator bars must be included in an actual working separator. The prototype design requires that these bars be upstream from the juvenile separator bars, and whether these additional bars would affect juvenile separation efficiency is unknown.

Also, regarding the lodging or wedging of some smolts between separator bars, exactly why this occurred is unknown. Smolts wedging between the separator bars was not a problem during the earlier high velocity separator tests when small numbers of fish were used (Lynn McComas, NMFS, personal communication). It is possible that during the high density 2004 study the transported smolts were fatigued or injured in some way that
was not evident to us; however, it is also possible that the large number of fish being tested as well as flow conditions within the prototype separator may have contributed to the problem. In the prototype the make-up water for the flume beneath the separator bars is introduced at the entrance to this flume (a mesh screen was placed over this opening to block any upstream smolt movement). Since this is the only point for introducing make-up flow, it was impossible to maintain a consistent 5-cm depth over the entire length of separator bars. Standing waves were created at each of the cross-supports that maintain separator bar spacing. The 2 m/s downstream flow made it difficult to determine where the smolts were initially being wedged, but it is possible that areas with less than the 5-cm of water over the separator bars were a contributing factor. To alleviate this condition, a working separator should be designed so that additional water (to maintain the 5-cm depth) can be introduced at any point along the complete length of the separator and from either side of the separator bars.

ACKNOWLEDGMENTS

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REFERENCES


