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Head Injury Assessment in Juvenile Chinook Using the Alpha II-Spectrin Biomarker: Effects of Pressure Changes and Passage through a Removable Spillway Weir

Christine Jonason and Ann Miracle

ABSTRACT

The cytoskeletal protein alpha II-spectrin has specific neurodegenerative mechanisms that allow the necrotic (injury-induced) and apoptotic (non-injury-induced) pathways of proteolysis to be differentiated in an immunoblot. Consequently, αII-spectrin breakdown products (SBDPs) are potential biomarkers for diagnosing traumatic brain injury (TBI). The purpose of the following investigation, consisting of two studies, was to evaluate the utility of the spectrin biomarker in diagnosing TBI in fish that travel through hydroelectric dams in the Columbia and Snake Rivers. The first study used hyperbaric pressure chambers to simulate the pressure changes that affect fish during passage through a Federal Columbia River Power System (FCRPS) Kaplan turbine. The second study tested the effect of a removable spillway weir (RSW) on the passage of juvenile chinook (*Oncorhynchus tshawytscha*). This study was conducted in tandem with a balloon-tag study by the U.S. Army Corps of Engineers. Brain samples from fish were collected and analyzed using an immunoblot for SBDPs, and imaging software was used to quantify the protein band density and determine the ratio of cleaved protein to total protein. The biomarker analyses found higher SBDP expression levels in fish that were exposed to lower pressure nadirs and fish that passed through the RSW at a deep orientation. In general, the incidence of injuries observed after treatment positively correlated with expression levels, suggesting that the biomarker method of analysis is comparable to traditional methods of injury assessment. It was also found that, for some treatments, the 110 kDa spectrin fragment (SBDP 110) correlated more strongly with necrotic head injury incidence and mortality rates than did the total cleaved protein or the 120 kDa fragment. These studies will be informative in future decisions regarding the design of turbines and fish passage structures in hydroelectric dams and will hopefully contribute to the development of faster and more accurate techniques for diagnosing TBI in fish.

Introduction

Salmonid species passing through hydroelectric dams on the Columbia and Snake Rivers in Washington face the possibility of injury and death from these structures. Fish that cannot find a surface outlet at the forebay of a dam resort to swimming under the turbines at greater depths than they are accustomed to [1], [2]. This can have potentially lethal effects on their physiology and behavior, causing changes in buoyancy, swim bladder rupture, disorientation, cold stress and abnormal swimming behavior [2]. Additionally, the rotating turbines can cause injury and mortality through sheer stress and collisions sustained during passage [3], [4], [5].

In an attempt to decrease the frequency of injury and mortality from dam passage, the U.S. Army Corps of Engineers (USACE) has constructed fish passages, such as adult fish ladders and juvenile bypass systems, in eight of the 18 dams on the Columbia and Snake Rivers [6]–[8]. Evaluations of these structures usually involve observational studies in which the fish are tagged, released into the forebay and then recaptured and examined for injuries after swimming through the passage spillbay [4], [6], [9], [10]. These assessment methods are limited, though, in that they can diagnose only visible injuries, while fish may sustain subacute injuries, such as traumatic brain injuries, with no observable effects. Because of this limitation it is desirable to develop a molecular approach that goes beyond visible observations and produces a more quantitative assessment of the injuries that have occurred.

The solution to this problem may lie with a molecular marker of traumatic brain injury (TBI) known as the αII-spectrin biomarker, which has been studied extensively in mammals [11]–[17], but has

only recently been applied to research in fish [18], [19]. Alpha II-spectrin (αII-spectrin) is a subunit of the protein spectrin, which is found in axons and presynaptic terminals of the central nervous system [11]. The potential for using αII-spectrin as a biomarker for TBI emerges from the fact that proteolysis of the protein produces different-sized fragments when normal cell death occurs compared to when injury-induced cell death occurs. Proteolysis of αII-spectrin by means of a necrotic (injury-induced) pathway is mediated by calpains (calcium-dependent proteases with palpain activity) and is localized at the site of impact [11], [14]. In fish, this pathway produces 150 and 110 kDa protein fragments [18] (Figure 1). The apoptotic (non-injury-induced) pathway of cell death is mediated by calpains as well as by caspase-3, a cysteine protease. Apoptosis occurs in areas surrounding the impact site and results in 150, 120 and 110 kDa fragments [18]. These protein fragments, known as spectrin breakdown products (SBDPs), can be distinguished on an immunoblot and analyzed for density to determine their relative abundance in the brain tissue [11]–[19].

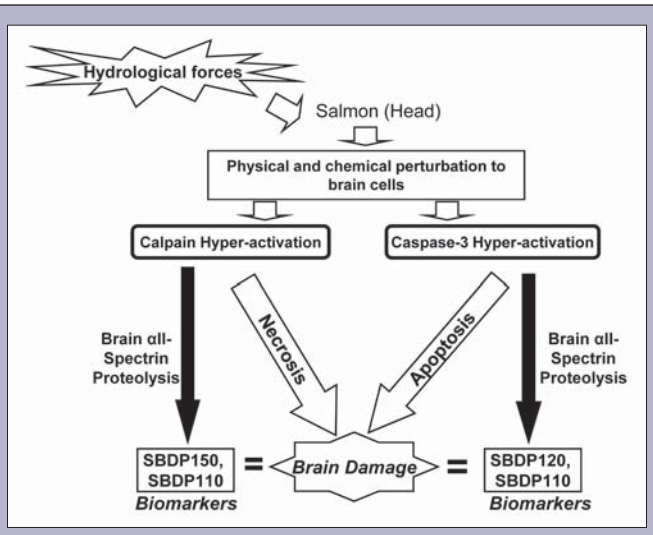


Figure 1. Injury-induced and non-injury-induced pathways of αII-spectrin proteolysis due to external forces [19].

Previous studies involving mammalian systems have found that SBDP levels correlate with different types and severities of head injuries [14]–[17]. Based on these studies and preliminary results from Pacific Northwest National Laboratory (PNNL) [18], it was proposed that TBIs sustained by fish could be quantified based on differential analysis of expression of the 110 and 120 kDa SBDPs in brain tissue. The purpose of both studies described in this paper was to evaluate the effectiveness of the αII-spectrin biomarker at diagnosing incidences of TBI in juvenile chinook salmon (*Oncorhynchus tshawytscha*) and to determine if the biomarker can be used to quantify the severity of head injuries sustained during dam passage.

The first study was designed to simulate the rapid decompression experienced by fish swimming through a Federal Columbia River Power System (FCRPS) Kaplan turbine. Hyperbaric pressure chambers were used to acclimate fish to a standard pressure, representative of normal river conditions, and then subject them to a rapid decompression down to a typical nadir pressure. It was expected that lower nadir pressures would result in a higher incidence of injury and mortality in the fish. Observations during necropsies of the fish were compared to molecular analysis to evaluate the effectiveness of the biomarker at detecting and quantifying these injuries.

The second study was conducted to evaluate the effects of a removable spillway weir (RSW) at Lower Monumental Dam on the Snake River. The RSW is considered more efficient than a traditional spillway in reducing migration delays [1]. The goal of this study was to quantify the SBDP expression levels among two treatment groups involving the RSW and one control group, in order to ultimately determine if TBI incidence was higher in fish that passed through the RSW. Additionally, visible signs of injury in the test fish were recorded, and the rate of observable passage-related injuries was compared to the molecular analysis order to assess the accuracy of the biomarker.

Materials And Methods

Treatments and Tissue Collection, Pressure Study

The pressure study was conducted at PNNL from January 4 to February 4, 2008. Test fish ranged from 120 to 140 mm in length and came from a single cohort of hatchery-raised fish. The test fish were randomly assigned to seven treatment groups. Each group was placed in a hyperbaric pressure chamber and acclimated to a standard pressure of 21.2 psi in 123–125% total dissolved gas (TDG) for at least 16 hours. Control groups were acclimated along with treatment groups. These test conditions simulated passage at 15 feet below the surface of the water, with TDG exceeding 100% to simulate the turbulent, hyperoxygenated conditions of water moving through a turbine. After acclimation, a pressure spike was applied throughout all of the chambers except for those with control fish. The pressure started at 58 psi and decreased to a nadir between 2.1 and 14.31 psi, according to the designated treatment (Table 1). After testing, the fish were euthanized using 250 mg of

Test	Acclimation depth (ft)	Nadir (psi)	Total Dissolved Gas (%)	Class
997	15	2.1	124.5	extreme
989	15	2.59	124.76	extreme
978	15	3.32	125.22	extreme
1000	15	3.66	123.92	extreme
1005	15	12.21	124.25	intermediate
994	15	12.36	124.18	intermediate
1015	15	12.36	123.74	intermediate
981	15	12.75	123.61	intermediate
979	15	14.31	125.42	intermediate
995	15	33.13	124.47	intermediate
980	15	-999	123.75	control
1036	15	-999	125.39	control
1037	15	-999	124.98	control
1039	15	-999	125.4	control
1043	15	-999	123.38	control

Table 1. Pressure study tests, organized by classification. Acclimation pressure for all groups was 21.2 psi (15 ft).

MS-222 (tricaine methanesulfonate)/L of river water and necropsied. Afterwards each fish was decapitated and the brain was surgically extracted. Brain samples were transferred to cryotubes, flash-frozen in liquid nitrogen and stored at -80 °C.

Lower Monumental Dam Study

The USACE contracted with Normandeau Associates, Inc. (Normandeau) to conduct a balloon-tag study involving a new removable spillway weir (RSW) at Lower Monumental Dam (Kahlotus, Washington) during March 24–31, 2008. Juvenile hatchery chinook were obtained from Kooskia National Hatchery (mean fork length x 114 mm). They were tagged according to Normandeau's balloon-tag method [21] and sent through the RSW in groups of ten to fifteen at either "deep" or "mid" intake levels. After being captured downstream of the dam, the fish were held in tanks for 48 hours and then were briefly anaesthetized with clove oil and examined by Normandeau personnel for signs of injury. Control fish were tagged and released at the tailrace of the juvenile bypass system, captured downstream and then handled in the same manner. After observations were complete, a subset of these fish (30 per passage type) was euthanized and brain samples were extracted using the same procedure described for the pressure study.

Assessment of Head Trauma

Each fish in the pressure study was examined for swim bladder rupture and signs of embolism, hemorrhaging and hematoma in the gills, eyes and fins during necropsy. Injuries that were considered signs of head trauma included hemorrhaging and embolism in the eyes and exothalmia (eye popping). Head injuries were documented and later correlated with expression data obtained from the biomarker analysis. For the Lower Monumental study, the percent of fish sustaining passage-related maladies from each treatment was calculated from field data provided by Normandeau.

Protein Homogenization

Brain samples were homogenized using a MiniBeadBeater-8 and 1.0-mm Zirconia/Silica beads (BioSpec Products, Inc.). A 1X Triton protein extraction buffer (20 mM tris, pH x 7.4, 150 mM NaCl, 5 mM EDTA, 5 mM EGTA, 1% TritonX-100) with protease inhibitors and 1.0 mM DTT was added to the sample before homogenization. The lysates were incubated on ice for 90 minutes and then centrifuged at 14000 rpm for 30 minutes at 4 °C. The protein concentrations of the supernatants were determined using the direct current protein assay (Bio-Rad) and a Genesys 10 UV scanning spectrophotometer (Thermo Scientific). After determining the protein concentrations, 20-µg aliquots of each sample were made and stored at -80 °C.

Immunoblot Technique

All samples were boiled for five minutes with loading buffer containing sodium dodecyl sulfate (SDS). Samples were loaded on polyacrylamide gels (4–15% Tris HCl, Criterion) and gel electrophoresis was conducted in a 10% Tris-glycine-SDS buffer (Sigma) at 125 V for two hours. After separation, the proteins were transferred onto a 0.45-µm polyvinylidene fluoride (PVDF) membrane (Pall Corporation) at 20 V for two hours using a blotter assembly (Criterion). The membrane was then blocked in 5% milk in Tris-buffered saline containing 0.05% Tween 20 (TBST, Sigma-Aldrich) for one hour and incubated for one hour with a mouse polyclonal antibody for αII-spectrin (Banyan Biomarkers). Next the membrane was washed in TBST three times for five minutes each time and probed with a biotinylated secondary antibody (Banyan Biomarkers) for one hour. After completing the TBST washing procedure again, protein bands were detected by incubating the membrane with 5 mL of BCIP-NBT reagent (Banyan Biomarkers) for one to two minutes. The membranes were washed in deionized water and air-dried.

Protein Band Visualization

A digital picture of each gel was taken, and densitometric analysis of the bands was conducted using ImageJ software (version 1.6, National Institutes of Health), which quantifies the bands based on their relative intensities [13]. Densitometry values were recorded for the 280 kDa band (the intact protein) and the 120 and 110 kDa bands (the breakdown products). A final densitometric ratio was figured by determining the ratio of breakdown products (SBDP 120+SBDP 110) to total protein (intact 280 kDa+SBDP 120+SBDP 110). Additionally, the expression of each individual breakdown product was calculated by dividing each SBDP expression level by the sum of intact protein and that SBDP. For example, density values for the SBDP 110 band were divided by density values for intact 280 kDa+SBDP 110. Densitometry measurements were taken twice and averaged for each fish.

Statistical Analyses

After densitometric values for each sample were configured, a *t*-test (two-tailed, $\alpha = 0.05$) was used to detect statistically significant variations in SBDP levels. The correlation of SBDP levels to observed injuries was calculated using a regression analysis in SigmaPlot 10.0 (Systat).

Results

Pressure Study Results

Out of 161 fish that were exposed to the pressure treatments, 115 brain samples were assayed for the αII-spectrin biomarker. Figure 2 describes the range of densitometric values obtained from the biomarker analyses. Overall, the fish exposed to pressure treatments showed a higher level of expression of SBDP 110 and SBDP 120+SBDP 110 versus the control fish. The individual 110 and 120 kDa bands were able to distinguish between treatment groups with significant results for some, but not all, of the tests (Table 2). The level of observed head injuries was highest in fish that were exposed to lower pressure nadirs (the "extreme" treatments), and this trend correlates with the higher SBDP expression levels that were detected in the molecular analysis.

Lower Monumental Study Results

A total of 108 brain samples were collected from the Lower Monumental site: 90 mid-release, deep-release and control fish at 48 hours post-release on March 28, 2008, and 18 deep-release fish at 48 hours post-release on April 1, 2008 (Table 3). Figures 3 and 4 describe the range of densitometric values obtained from the biomarker analyses. There were two separate sampling days for fish from the deep treatments, indicated as deep day 1 and deep day 2. Statistical tests, as described above, compared the 120+110 densitometric values (i.e., the ratio of cleaved protein to total protein) and revealed significant differences between the control treatments

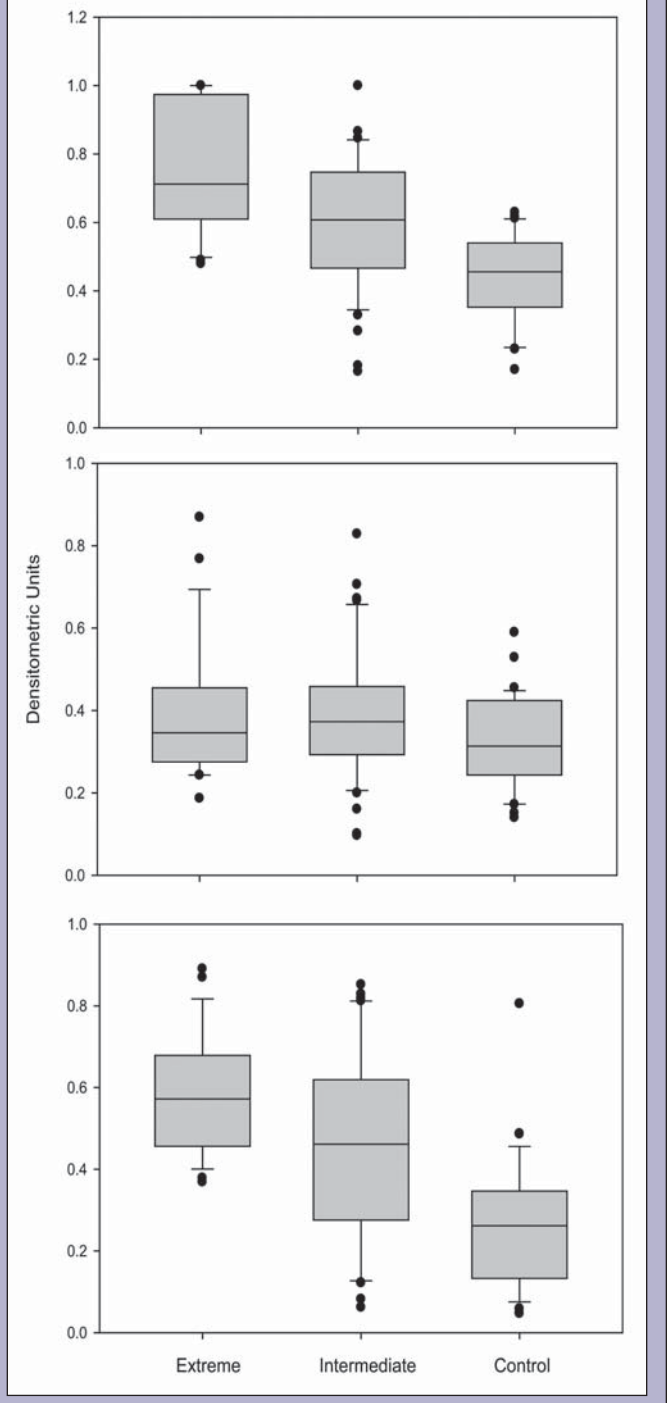


Figure 2. Pressure study results: a) SBDP 120+SBDP 110; b) SBDP 120; and c) SBDP 110 expression levels for extreme treatments (nadir range 2.1–3.66 psi), intermediate treatments (nadir range 12.21–33.13 psi) and control groups. Units are densitometric values for the SBDPs divided by intact protein+SBDP value.

and deep treatments (Table 4). The SBDP 110 densitometric ratios were significantly different between the control and mid groups, control and deep (separately and combined) and mid and deep (day 2 and both days combined) (Figure 4). Finally, the SBDP 120 densitometric values were significantly different between control and mid groups, control and deep day 2 and mid and deep (separately and combined) (Figure 4).

The rate of observed injury in the test fish positively correlated with expression levels of the SBDP 120+SBDP 110 biomarkers (i.e., the relative amount of cleaved protein compared to total protein). However, this correlation was not significant (r^2 x 0.78, Figure 5).

Discussion and Conclusion

These studies evaluated the effectiveness of the αII-spectrin biomarker at quantifying the severity of head injuries sustained during two dam passage scenarios. The ratio of cleaved total protein (SBDP 120+SBDP 110) to intact protein (280 kDa) is indicative of αII-spectrin proteolysis as the result of either the apoptotic or necrotic pathway. Based on past work [18], [19], it is known that, in fish, the SBDP 120 fragment results from apoptotic pathway of cell death, while the SBDP 110 fragment results from both pathways.

For both studies, the trends in SBDP expression levels accurately reflect the incidence of injuries observed in the fish after treatment. Additionally, in both studies at least one of the biomarker metrics (SBDP 110, SBDP 120, or total cleaved protein) was able to distinguish extreme treatments (low pressure nadirs or "deep" RSW level) from intermediate and control treatments, although not all results were significant. The results support a concept that emerged from research at McNary Dam [18], [19], that the αII-spectrin biomarker method is comparable to observational methods of injury assessment used for dam passage evaluations.

While biomarker expression from Lower Monumental passage treatments is positively correlated with observed injuries (Figure 5), the correlation is not as strong (r^2 x 0.78) as it was in a 2007 study conducted at McNary Dam (r^2 x 0.91) [18], [19]. Several factors may have contributed to this discrepancy. First, the biomarker detected a much higher incidence of subacute injury in the control fish from Lower Monumental than from the study at McNary. Whether this is due to differences in the passage type or to an actual increase in subacute injuries is unknown. The fish used for the Lower Monumental study were obtained from Kooskia National Fish Hatchery, had a mean length of 114 mm, and were generally in poor condition. Comparatively, the fish from McNary were from Little White Salmon hatchery, had a mean length of 140 mm, and were in relatively good condition [18], [19]. Either of these differences could have been a factor in the higher incidence of injuries. In any event, the higher levels of SBDP expression in control fish from Lower Monumental demonstrate that these fish were generally in poor health compared to the fish in the McNary study.

A second factor to consider when interpreting the Lower Monumental data is that the observed incidence of injury was very low for the day of the first deep release (deep day 1). Injury and malady data provided by Normandeau indicate a 5.5% injury in test fish from deep day 1, compared to a 17% injury in test fish from deep day 2 (Figure 5). When the second deep release date is considered separately, there is a marked increase in expression levels detected by the molecular assay which corresponds to the observed increase in injuries. Therefore, the incidence of injuries observed for deep day 1 correlate with the levels of SBDP expression on that day. For future studies it would be beneficial to sample fish from multiple release dates to ensure that a variety of passage-related injuries and maladies will be included in the analysis. Additionally, more consideration should be given to maintaining uninjured control fish to ensure an

SBDP 110		Extreme			Intermediate						Control				
		989	978	1000	1005	994	1015	981	979	995	980	1036	1037	1039	1043
Extreme	997	**0.0135**	**0.0215**	**0.0371**	0.1224	**0.0222**	**0.0054**	0.8866	0.2657	**0.0401**	0.5693	**3.59E-05**	**0.0011**	**0.0037**	**0.0012**
	989		0.4768	0.6937	**0.0015**	0.8549	**9.54E-05**	0.0595	0.7389	0.7898	0.1628	**2.40E-06**	**3.14E-05**	**6.94E-05**	**5.23E-05**
	978			0.3193	**0.0020**	0.6004	**1.88E-04**	**0.0389**	0.4243	0.6921	0.0930	**1.58E-05**	**1.25E-04**	**1.94E-04**	**2.54E-04**
	1000				**0.0037**	0.5933	**2.09E-04**	0.1057	0.9340	0.5640	0.2756	**6.10E-06**	**8.48E-05**	**1.74E-04**	**1.66E-04**
Intermediate	1005					**0.0021**	0.0731	0.3539	**0.0474**	**0.0039**	0.1069	**0.0036**	0.0989	0.1322	0.3732
	994						**1.53E-04**	0.0592	0.6609	0.9219	0.1529	**6.73E-06**	**7.30E-05**	**1.35E-04**	**1.42E-04**
	1015							**0.0335**	**2.99E-04**	**5.17E-04**	**0.0079**	0.2042	0.6019	0.3872	0.1870
	981								0.2632	0.0694	0.5803	**0.0039**	**0.0396**	0.0507	0.1079
	979									0.6259	0.4885	**8.65E-04**	**0.0058**	**0.0072**	**0.0137**
	995										0.1661	**3.42E-05**	**5.57E-04**	**9.19E-04**	**0.0011**
Control	980											**6.38E-04**	**0.0071**	**0.0103**	**0.0189**
	1036												0.0550	0.0733	**0.0024**
	1037													0.9621	0.2159
	1039														0.2979

SBDP 120		Extreme			Intermediate						Control				
		989	978	1000	1005	994	1015	981	979	995	980	1036	1037	1039	1043
Extreme	997	0.2955	0.0707	0.0604	0.1113	0.0806	0.8071	0.2274	0.0597	0.1066	0.3458	0.2374	0.1542	0.5453	0.0548
	989		0.0703	0.1313	0.1986	0.1033	0.3539	0.6903	0.1542	0.1897	0.9692	**0.0213**	0.4134	0.9510	0.2097
	978			0.2843	0.3193	0.6236	**0.0413**	0.1238	0.2594	0.3254	0.0989	**0.0128**	0.1488	0.1118	0.1599
	1000				0.9694	0.5144	0.0786	0.2737	0.8254	0.9516	0.1813	**0.0048**	0.4796	0.3184	0.5403
Intermediate	1005					0.5652	0.1025	0.3450	0.8238	0.9841	0.2519	**0.0139**	0.5206	0.3510	0.5847
	994						0.0583	0.1852	0.4373	0.5761	0.1405	**0.0120**	0.2554	0.1838	0.2782
	1015							0.2971	0.1096	0.1461	0.3893	0.6603	0.2675	0.2833	0.1560
	981								0.3330	0.3329	0.7512	**0.0212**	0.6738	0.8665	0.4612
	979									0.8060	0.2143	**0.0045**	0.6060	0.4077	0.6873
	995										0.2422	**0.0222**	0.4422	0.5509	0.5880
Control	980											**0.0371**	0.4823	0.9726	0.2824
	1036												**0.0140**	0.1480	**0.0028**
	1037													0.6355	0.8362
	1039														0.5107

Table 2. P-values from t-tests comparing pressure study results (two-tailed, $\alpha \times 0.05$, assuming equal variance). Bold values indicate significant differences in SBDP 110 or SBDP 120 expression levels between treatment groups. Extreme and intermediate treatments are in order of increasing pressure nadir.

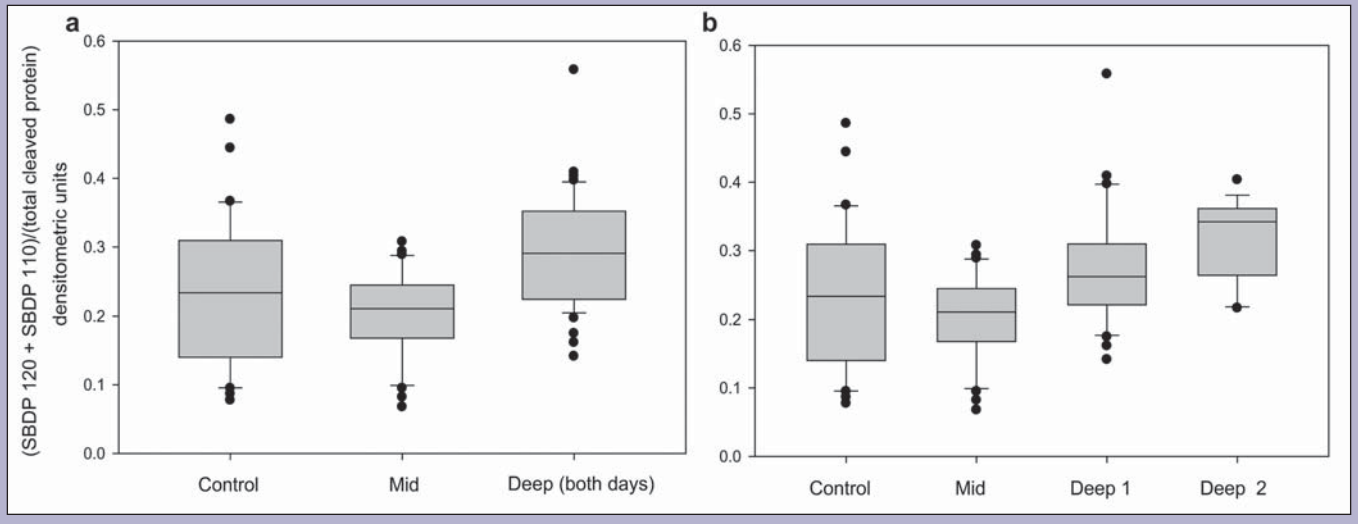
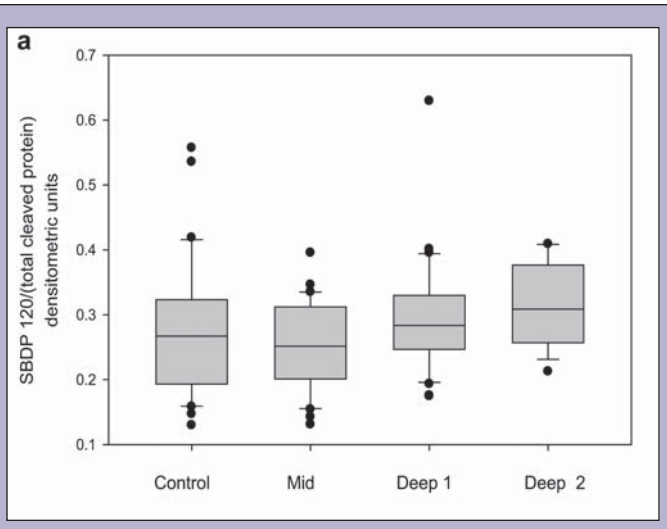


Figure 3. Lower Monumental Dam study results — SBDP 120 + SBDP 110 densitometric values according to passage condition, a) analyzing both deep sampling events together and b) analyzing the deep sampling events separately.



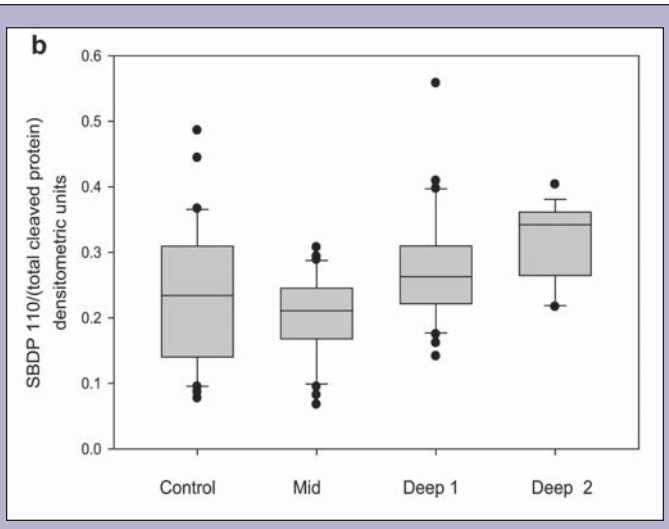


Figure 4. a) SBDP 120 and b) SBDP 110 densitometric values for treatment groups from the Lower Monumental Dam study at 48 hr post-release. SBDP 120 expression is indicative of the apoptotic pathway, while SBDP 110 indicates injury-induced (necrotic) cell death.

Route	Treatment	RSW discharge (cfs)	No. of fish collected at 48-hrs
RSW	Mid	8.7	30
RSW	Deep	8.7	48
Juvenile bypass pipe	Control	8.7	30

Table 3. Lower Monumental Dam study treatments.

SBDP120+110	Mid	Deep day 1	Deep day 2	Deep (both days)
Control	0.38	0.12	**0.01**	**0.02**
Mid		**3.25E-03**	**2.15E-05**	**5.97E-05**

SBDP 110	Mid	Deep day 1	Deep day 2	Deep (both days)
Control	0.21	0.08	**4.10E-03**	**0.01**
Mid		0.69	**3.07E-03**	**1.20E-06**

SBDP 120	Mid	Deep day 1	Deep day 2	Deep (both days)
Control	0.36	0.38	1.46E-01	0.17
Mid		**0.04**	**2.03E-03**	**5.42E-03**

Table 4. P-values from t-tests comparing densitometric values across treatment groups in the Lower Monumental Dam study (two-tailed, $\alpha \times 0.05$, assuming equal variance).

accurate background expression of biomarker levels for comparison to the treatments.

One development from these studies that was not previously observed is the utility of the 110 kDa breakdown product (SBDP 110), which in some treatments correlated more strongly with necrotic head injury incidence than the total cleaved protein or SBDP 120 alone. Results of the pressure study (Figure 2) demonstrate that total cleaved protein (SBDP 120+SBP110) is higher in extreme treatments. However, it is clear from the plots of the individual biomarkers that this trend is driven almost completely by an increase in SBDP 110 expression rather than an increase in both SBDP 110 and SBDP 120. Furthermore, more of the extreme

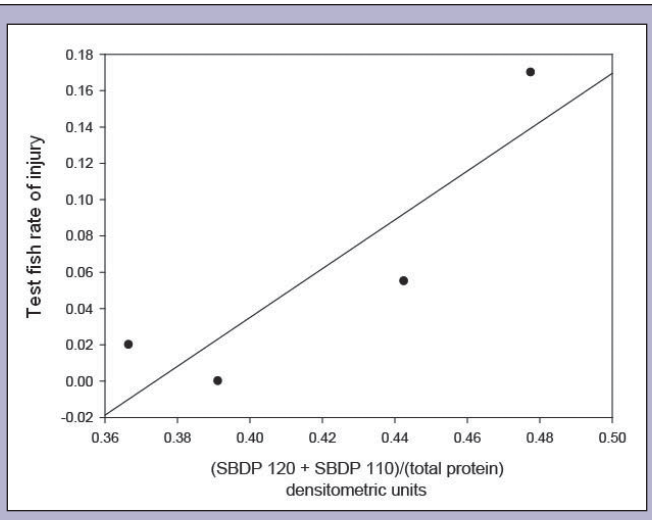


Figure 5. Correlation of protein densitometry (SBDP 120+SBDP 110) to observed incidence of injury across all treatment groups in the Lower Monumental Dam study ($R^2 \times 0.78$, $P \times 0.05$).

and intermediate pressure study treatments had significantly higher expression levels compared to control treatments when the SBDP 110 metric was used (Table 2). Also, in the Lower Monumental study, biomarker expression levels from the "deep" RSW treatment are higher when the SBDP 110 metric is used (Figure 5).

Since the 110 kDa fragment is produced from the calpain-mediated necrotic pathway, this result is perhaps not surprising. Wang [22] found that calpain-mediated pathways are overactivated as a result of both necrotic and apoptotic pathways, so a higher expression of calpain-cleaved fragments would be expected after injury. Also, Pike, et al. [23] demonstrated that traumatic brain injury can increase calpain activity without increasing caspase activity, and according to Warren [16], the site of the injury may also contribute to the level of expression of certain SBDPs. From the current studies, there is no way of knowing if there is a threshold severity of injury that induces SBDP 110 expression in chinook, or if expression is dependent

on the type of injury, but these research questions would be worth exploring in future studies involving the αII-spectrin biomarker in fish.

These studies are some of the first to apply the use of a molecular biomarker to issues in wildlife management. Fish mitigation on the Columbia and Snake Rivers has been a goal of the USACE since construction of the dams began in the 1930s [6], and there are undeniable ecological and economic benefits to maintaining the populations of salmonid species in these waterways. Previous tests of an RSW at Lower Granite Dam showed a 98% survival rate in fish that used the RSW [1], and results from the study described here suggest that operating the RSW at a mid-level orientation will result in fewer incidences of head trauma and overall injury to juvenile fish. In terms of turbine design, past research [2], [3], [20] has demonstrated that fish that pass through a turbine on the suction side of the blade experience the lowest possible pressure nadirs from this trajectory. The observational and molecular results from the pressure study both demonstrate that low pressure profiles cause more internal injuries (swim bladder rupture, embolisms and hemorrhaging) and head trauma in fish. More information is needed about the distribution of fish entering turbines and the probability of exposure to a low pressure nadir and subsequent barotrauma before definitive guidelines can be developed [20], but results from this study imply that it would be best to design turbines that maintain high pressure profiles, or to increase altogether the use of fish screens, spillbays and bypass systems that divert fish from turbines.

There is great potential for development of the αII-spectrin biomarker as a method for assessing head injury in fish. The biomarker assay holds an advantage over traditional assessment methods because it can detect sublethal injuries and is a more cost-effective approach than using live fish for injury analysis. The studies described here essentially analyzed only short-term head injury impacts, but further research into this method may allow a threshold level of biomarker expression to be established, from which the probability of long-term survival for migrating fish populations could be estimated. Prospective research will also focus on developing rapid and non-lethal techniques for diagnosing TBI in fish by using the biomarker assay on blood samples rather than brain tissue samples. Finally, the observational studies and biomarker analyses from these studies have clearly demonstrated that low pressure nadirs and the "deep" RSW orientation result in higher incidences of injury in juvenile chinook salmon, and these results will be informative in future decisions regarding the design of turbines and fish passage structures in hydroelectric dams on the Columbia and Snake Rivers.

Note

All handling and processing of animals for this study was done in compliance with the Battelle Institutional Animal Care and Use Committee guidelines

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References

[1] U.S. Army Corps of Engineers, "Spillway Weir" Sept. 2006, http://www.nww.usace.army.mil/spillway_weir/default.html.

[2] C.C. Coutant, "Fish behavior in relation to passage through hydropower turbines: a review," *Transactions of the American Fisheries Society.,* vol. 129, no. 2, pp. 351–380, March 2000.

[3] G.F. Cada, "The development of advanced hydroelectric turbines to improve fish passage survival," *Fisheries,* vol. 26, no. 9, pp. 14–23, September 2001.

[4] J.W. Ferguson, R.F. Absalon, T.J. Carlson and B.P. Sandford, "Evidence of delayed mortality on juvenile Pacific salmon passing through turbines at Columbia River dams," *Transactions of the American Fisheries Society*, vol. 135, no. 1, pp. 139–150, January 2006.

[5] W.D. Muir, S.G. Smith, G.J. Williams and B.P. Sandford, "Survival of juvenile salmonids passing through bypass systems, turbines and spillways with and without flow deflectors at Snake River dams," *North American Journal of Fisheries Management,* vol. 21, no. 1, pp. 135–146, February 2001.

[6] L. Mighetto and W.J. Ebel, *Saving the Salmon: A History of the U.S. Army Corps' Efforts to Protect Anadromous Fish on the Columbia and Snake Rivers*, Seattle: Historical Research Associates, Inc., 1994.

[7] U.S. Army Corps of Engineers, "Columbia River Fish Mitigation" Feb. 23, 2006, http://www.nwd.usace.army.mil/ps/fishmit.asp.

[8] U.S. Army Corps of Engineers, "Salmon Survival: Surface Bypass Systems" Feb. 5, 2008 http://www.nww.usace.army.mil/html/OFFICES/PA/FactSheets/SalmonSurvival_SurfaceBypassStructuresNOV07.pdf.

[9] G.E. Johnson, B.D. Ebberts, D.D. Dauble, A.E. Giorgi, P.G. Heisy, R.P. Mueller and D.A. Neitzel, "Effects of jet entry at high-flow outfalls on juvenile pacific salmon," *North American Journal of Fisheries Management,.* vol. 23, no. 2, pp. 441–449, May 2003.

[10] J.R. Skalski, D. Mathur and P.G. Heisey, "Effects of turbine operating efficiency on smolt passage survival," *North American Journal of Fisheries Management,,* vol. 22, no. 4, pp. 1193–1200, November 2002.

[11] A. Czogalla and A.F. Sikorski, "Spectrin and calpain: a 'target' and a 'sniper' in the pathology of neuronal cells," *Cellular and Molecular Life Sciences.*, vol. 62, no. 17, pp. 1913–1924, June 2005.

[12] O. Farkas and B. Polgar, J. Szekeres-Bartho, T.Doczi, J.T. Povlishock and A. Buki, "Spectrin breakdown products in the cerebrospinal fluid in severe head injury — preliminary observations," *Acta Neurochirurgica,* vol. 147, no. 8, pp. 855–861, August 2005.

[13] F.H. Kobeissy, A.K. Ottens, Z. Zhang, M.C. Liu, N.D. Denslow, J.R. Dave, F.C. Tortella, R.L. Hayes and K.K.W. Wang, "Novel differential neuroproteomics analysis of traumatic brain injury in rats," *Molecular and Cellular Proteomics*, vol. 5, no. 10, pp. 1887–1898, June 2006.

[14] S.B. Lewis, G.J. Velat, L. Miralla, L. Papa, J.D. Aikman, R.A. Wolper, C.S.Firment, M.C. Liu, J.A. Pineda, K.K.W. Wang and R.L. Hayes, "Alpha-II spectrin breakdown products in aneurysmal subarachnoid hemorrhage: A novel biomarker of proteolytic injury," *Journal of Neurosurgery,* vol. 107, pp. 792–796, October 2007.

[15] R. Nath, M. Scott, R. Nadimpalli, R. Gupta and K.K.W. Wang, "Activation of apoptosis-linked caspase(s) in NMDA-injured brains in neonatal rats," *Neurochemistry International,* vol. 36, no. 2, pp. 119–126, February 1999.

[16] M.W. Warren, F.H. Kobeissy, M.C. Liu, R.L. Hayes, M.S. Gold and K.K.W. Wang, "Concurrent calpain and caspase-3 mediated proteolysis of aII-spectrin and tau in rat brain after methamphetamine exposure: A similar profile to traumatic brain injury," *Life Sciences.,* vol. 78, no. 3, pp. 301–309, December 2005.

[17] D. Zhou, J.A. Ursitti and R.J. Bloch, "Developmental expression of spectrins in rat skeletal muscle," *Molecular Biology of the Cell,*, vol. 9, pp. 47–61, January 1998.

[18] A. Miracle, N.D. Denslow, K.J. Kroll, M.C. Liu and K.K.W. Wang, "Spillway-induced salmon head injury triggers degradation of brain αII-spectrin similar to mammalian traumatic brain injury," *PLoS:ONE*, Feb. 2009 http://www.plosone.org/article/info:doi/10.1371/journal.pone.0004491.

[19] A.L. Miracle, "Head Injury Assessment of Juvenile Chinook Salmon Spill Passage Routes at McNary Dam, 2007," Battelle Pacific Northwest Division, Tech. Rep. PNWD-3954, Aug. 2008.

[20] R.S. Brown, T.J. Carlson, A.E. Welch, J.R. Stephenson, C.S. Abernethy, C.A. McKinstry and M.H. Theriault, "Assessment of barotrauma resulting from rapid decompression of depth-acclimated juvenile chinook salmon bearing radio telemetry transmitters," Pacific Northwest National Laboratory, Tech. Rep. PNNL-16790, 2007.

[21] P. Heisey, D. Mathur and T. Rineer, "A reliable tag-recapture technique for estimating turbine passage survival: application to young-of-the-year American shad (*Alosa sapidissima*)," *Canadian Journal of Fisheries and Aquatic Sciences,* vol. 49, no. 9, pp. 1826–1834, September 1992.

[22] K.K.W. Wang, "Calpain and caspase: Can you tell the difference?" *Trends in Neurosciences*, vol. 23, pp. 20–26, 2000.

[23] B. Pike, X. Zhao, J. Newcomb, R.M. Posmantur, K.K.W. Wang and R.L. Hayes, "Regional calpain and caspase-3 proteolysis of alpha-spectrin after traumatic brain injury," *Neuroreport,* vol. 9, no. 110, pp. 2437–2442, 1998.