Transactions of the American Fisheries Society 137:690–695, 2008

DOI: 10.1577/T07-014.1

[Note]

Hepatic Heat Shock Protein 70 and Plasma Cortisol Levels in Rainbow Trout after Tagging with a Passive Integrated Transponder

JOSEPH W. FELDHAUS*[1]

Oregon Cooperative Fish and Wildlife Research Unit, U.S. Geological Survey, and Department of Fisheries and Wildlife, Oregon State University, 104 Nash Hall, Corvallis, Oregon 97331-3803, USA

SCOTT A. HEPPELL

Department of Fisheries and Wildlife, Oregon State University, 104 Nash Hall, Corvallis, Oregon 97331-3803, USA

MATTHEW G. MESA

U.S. Geological Survey, Western Fisheries Research Center, Columbia River Research Laboratory, 5501-A Cook-Underwood Road, Cook, Washington 98605, USA

HIRAM LI

Oregon Cooperative Fish and Wildlife Research Unit, U.S. Geological Survey, and Department of Fisheries and Wildlife, Oregon State University, 104 Nash Hall, Corvallis, Oregon 97331-3803, USA

Abstract.—This study examined the potentially stressful effects of tagging juvenile rainbow trout *Oncorhynchus mykiss* with passive integrated transponder (PIT) tags by measuring short-term (<120-h) changes in plasma concentrations of cortisol and hepatic heat shock protein 70 (hsp70). In a laboratory experiment, plasma cortisol levels were measured in fish before they were tagged (0 h) and at 2, 6, 24, and 120 h after being tagged. Hepatic hsp70 levels were measured at 0, 24, and 120 h. All results were compared with those for fish that were handled but not tagged. Plasma cortisol levels were significantly higher in both treatment groups (tagged and handled but not tagged) at 2 h than in the pretreatment groups (0 h). Plasma cortisol levels in the treatment groups returned to near pretreatment levels by 6 h. However, there was a significant difference in plasma cortisol levels between treatment groups at 6 h. There were no significant differences in hepatic hsp70 levels among the two treatment groups, and hepatic hsp70 levels did not change through time. Our results suggest that PIT tagging is a low-impact tagging procedure for juvenile salmonids.

Passive integrated transponder (PIT) tags provide a method for uniquely identifying and tracking the movement of individual fish in streams (Prentice et al. 1990b; Ombredane et al. 1998; Roussel et al. 2000, 2004; Cucherousset et al. 2005). In the Columbia River basin, this technology has been used to estimate survival of juvenile salmonids and their travel times between hydroelectric dams equipped with PIT tag readers (Prentice et al. 1990a; Peterson et al. 1994; Muir et al. 2001; Hockersmith et al. 2003). Studies have reported that PIT tags have minimal effects on fish growth and survival (Prentice et al. 1990a; Peterson et al. 1994; Ombredane et al. 1998; Ruetz et al. 2006).

Understanding the physiological response of fish to PIT tagging is important when conducting in situ physiological experiments with PIT-tagged fish. To our knowledge, neither changes in plasma cortisol nor those in heat shock proteins (hsps) have been measured in rainbow trout *Oncorhynchus mykiss* after PIT tag insertion. Physiological stress (e.g., handling) can result in an elevated level of plasma cortisol (the primary stress hormone). Long-term elevation of cortisol can increase disease susceptibility, decrease growth rates, and inhibit reproduction (Hazon and Balment 1998; Schreck 2000; Schreck et al. 2001). Plasma cortisol is also important for energy metabolism and ion regulation and can interact with other hormonal systems (Hazon and Balment 1998).

Heat shock proteins, also called stress proteins, are molecular chaperone proteins that assist protein folding and are involved with the cellular immune response (Zugel and Kaufmann 1999). Constitutive heat shock protein isoforms are synthesized under normal cellular conditions, whereas inducible isoforms can be synthesized in response to both biotic and abiotic stressors

* Corresponding author: feldhauj@hotmail.com
[1] Present address: Coos Watershed Association, Post Office Box 5860, Charleston, Oregon 97420, USA.

Received January 14, 2007; accepted September 19, 2007
Published online April 10, 2008

(Hochachka and Somero 2002). As such, heat shock proteins are potential biomarkers for environmental stress in fish (Iwama et al. 1998).

One heat shock protein that has been used as a biomarker is the hsp70 chaperone protein family. This protein family is highly conserved, correlates with thermal tolerance (Feder and Hofmann 1999; Basu et al. 2002), and is highly temperature responsive (Parsell and Lindquist 1993; Sanders 1993). Measuring hsp70 levels has advantages over measuring stress hormones (e.g., plasma cortisol) because the levels of hsp70 in rainbow trout are not altered by handling stress (Vijayan et al. 1997; Washburn et al. 2002). However, previous studies examining hsp70 in response to handling stress have not examined the effect of placing an object (e.g., a PIT tag) in the body cavity of a fish.

The objectives of this study were to (1) determine whether PIT tagging induces a stress response, measured as a difference in plasma cortisol levels between tagged and untagged juvenile rainbow trout, (2) determine whether PIT tagging alters hepatic (liver) hsp70 levels in juvenile rainbow trout, and (3) examine the relation between cortisol and hsp70 levels in juvenile rainbow trout.

Methods

Fish and rearing conditions.—The experiment was conducted between 12 August and 14 September 2004 at Oregon State University's Fish Performance and Genetics Laboratory in Corvallis. Fish were age-0 hatchery rainbow trout from the Roaring River Fish Hatchery on the McKenzie River, Oregon. On 12 August 2004, 180 rainbow trout were divided among four outdoor 1-m-diameter circular fiberglass tanks (45 fish/tank) and exposed to natural photoperiod. Tanks were supplied with 13–14°C well water adjusted to1 L/min flow. The fish were acclimated to the experimental tanks for 21 d before the beginning of the experiment. During the acclimation period, fish were fed by hand to satiation twice per day during the week and once per day on weekends with BioDiet Grower, a semimoist commercial pellet manufactured by Bio-Oregon. Fish were considered satiated when feeding slowed and uneaten pellets remained on the tank bottom. The fish were fasted for 48 h before the start of the experiment. For 3 days after the start of the experiment, all fish were fed 9–10 g of food per tank each day and then fed to satiation one time each day for the duration of the study. This study was conducted under the auspices of the Oregon State University Institutional Animal Care and Use Committee permit 3055.

Experimental design and sampling procedures.—Fish in two tanks were randomly assigned as handled controls. The remaining two tanks of fish were designated as the treatment group. We injected a 11.5-mm × 2.1-mm PIT tag into the body cavity of each fish in the treatment group using a modified handheld syringe following the methods of Prentice et al. (1990a). The PIT tagging took 3–5 s per fish. Handled control fish were held out of the water for 3–5 s to simulate handling, but the body cavity was not pierced.

Before processing each tank of fish, a fresh solution of buffered tricaine methanesulfonate (MS-222) was prepared in 5 L of water for both light anesthesia (50 mg MS-222/L + 125 mg $NaHCO_3$/L) and lethal dose solutions (200 mg MS-222/L + 500 mg $NaHCO_3$/L). The light anesthesia was used during tagging and handling. At 0 h, six fish from each tank were rapidly netted and given the lethal dose of MS-222. These fish provided baseline resting values for cortisol and hsp70 before the start of the experiment.

Immediately after the initial samples were taken, the remaining fish were rapidly netted from a tank, placed in light anesthesia, and given the appropriate treatment (i.e., either handled and PIT-tagged or just handled). Fish were considered anesthetized and ready for handling after equilibrium was lost and opercular movements became irregular. After the fish were handled, all fish were placed back in the experimental tank and allowed to recover. Processing each tank of fish ($N = 39$/tank) took between 8 and 10 min.

After the preexperiment sampling (0 h), six fish from each tank were lethally sampled at 2, 6, 24, and 120 h posthandling. Fork length (mm) and mass (0.1 g) were recorded for all fish. From each fish, we collected blood and liver tissue. Blood was collected into ammonium heparin–containing capillary tubes by severing the caudal peduncle with a razor blade. Blood was then transferred into microcentrifuge tubes and centrifuged, and plasma was separated and stored on ice. Next, fish livers were removed, wrapped in aluminum foil, and flash-frozen in liquid nitrogen. Plasma and liver samples were stored at −80°C for later analysis.

Tissue sample preparation and Western blotting.—Liver samples were lysed and homogenized with a tissue tearer in ice-cold lysis buffer (50 mM tris base, 20 mM NaCl, 5 mM EDTA; pH 7.5) containing protease inhibitors (leupeptin, 0.5 mg/mL; phenylmethylsulfonyl fluoride, 2 mM; aprotinin, 1 mg/mL; and pepstatin, 0.7 mg/mL). Lysates were centrifuged at 0°C for 30 min at 2,700 × gravity. The resulting supernatant was divided into aliquots and stored at −80°C. Protein concentrations in lysates were mea-

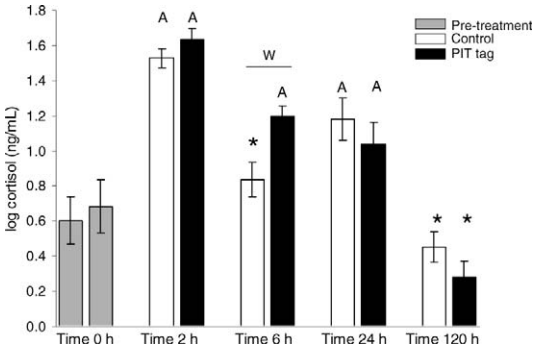

FIGURE 1.—Logarithmic plasma cortisol levels ± SEs prior to treatment (0 h) and 2, 6, 24, and 120 h posttreatment. The letter A indicates a significant difference between a treatment value and the corresponding pretreatment value, the letter W a significant difference between treatment values at a particular sampling time, and an asterisk a significant difference from other posttreatment (2–120 h) fish; $N = 12$ for each bar.

sured in duplicate with the bicinchoninic acid assay method (Smith et al. 1985).

Western blotting followed methods outlined in Towbin et al. (1979). Samples were mixed with an equal amount of sodium dodecyl sulfate buffer (Laemmli 1970) and heated at 95°C for 3 min. Twenty-five micrograms of liver protein was electrophoretically separated on 8% tris–glycine gels (Invitrogen Corporation) for 2 h at 125 V. A calibrated molecular weight marker (Biorad) and 52 ng of recombinant Chinook salmon hsp70 protein (SPP-763; StressGen Biotechnologies, Victoria, British Columbia) were applied to each gel to serve as internal standards for determining molecular weight and blotting efficiency. Proteins were transferred to a polyvinylidene difluoride membrane at 100 V for 1 h, then blocked overnight at 4°C in blocking solution (5% nonfat dry milk, 20 mM tris buffer, and 0.01% Tween-20).

All incubations were performed at room temperature for 1 h. The commercially available polyclonal hsp70 antibody (StressGen; SPA-758) and the secondary antibody, an alkaline phosphatase conjugated goat–anti-rabbit IgG (StressGen; SAB-301) were both diluted 1:5000 in blocking solution. After incubation with the primary antibody, the membranes were washed three times at 10 min per wash. The first and third washes used tris-buffered saline solution (TBS) and the second wash used TBS with 0.5% Tween-20. After washing, membranes were incubated with the secondary antibody, and membranes were rinsed as previously described. Proteins were visualized colorimetrically by using an alkaline phosphatase conjugate substrate kit (Biorad; 170-6432). Blots were developed for 15 min. The reaction was stopped by rinsing with distilled water for 10 min. Relative hsp70 band density was measured by using ImageQuant 6.1 (Amersham Biosciences) densitometry software. Protein band density is expressed by subtracting the background and dividing the band density of the unknown sample by the density of the hsp70 protein standard band.

Cortisol assay.—Plasma cortisol levels were analyzed by radioimmunoassay as described in Foster and Dunn (1974) and adapted by Redding et al. (1984). All values below the lowest standard (3.9 ng/mL) were designated to contain 1.95 ng/mL cortisol.

Statistics.—Plasma cortisol data were log transformed to increase homogeneity of variance. For the plasma cortisol analysis, fish from similar treatments within a time block were pooled. Within a time block, treatment groups were compared with a two-sample *t*-test. Hsp70 data were analyzed with one- and two-way analysis of variance (ANOVA) on nontransformed data. The significance level was 0.05. Multiple comparisons through time were analyzed with a Bonferroni multiple comparison test. Analyses were conducted with the statistical software package SAS (SAS Institute 2003).

Results

Retention of PIT tags during this experiment was 100%. The mean ± SE fork length of fish sampled in this experiment was 110 ± 0.8 mm (range, 79–142 mm). The average mass was 16.7 ± 0.4 g (range, 7.1–34.6 g). Within each time block, there was no difference in fish length among the experimental tanks (ANOVA; $P > 0.05$).

Plasma Cortisol

Through time, there was a significant change in plasma cortisol levels ($F_{4,\ 114} = 38.5$, $P < 0.001$; Figure 1). Differences between treatment groups were not detected at 0 ($P = 0.69$), 2 ($P = 0.24$), 24 ($P = 0.42$), or 120 h ($P = 0.20$). At 6 h, however, there was a significant difference between treatment groups ($P = 0.004$), plasma cortisol levels being significantly greater than the pretreatment levels in the PIT-tagged group but not the handled control group.

Compared with that in fish at 0 h (i.e., pretreatment), plasma cortisol levels increased significantly between 0 and 2 h in both treatment groups ($P < 0.05$; Bonferroni multiple-comparison test). Between 2 and 6 h, plasma cortisol levels decreased significantly in the handled control, but not the PIT-tagged group. At 24 h, the handled control group had significantly higher plasma cortisol levels than the pretreatment group, but the PIT-tagged group did not. Plasma cortisol levels in

both treatment groups dropped significantly between 24 and 120 h; by 120 h, plasma cortisol levels were similar to pretreatment levels in both treatment groups.

Hepatic hsp70 Levels

Hepatic hsp70 levels were measured at 0, 24, and 120 h after treatment (Figure 2). One hsp70 band of approximately 70–75 kDa was detected. Hepatic hsp70 levels of fish were similar in all tanks at 0 h ($F_{3, 20} = 0.68$, $P = 0.57$) and did not change through time ($F_{2, 69} = 2.41$, $P = 0.097$). There was no evidence of a treatment effect at 24 ($F_{1, 22} = 0.148$, $P = 0.70$) or 120 h ($F_{1, 22} = 0.028$, $P = 0.87$). Hepatic hsp70 levels were not correlated with fish length ($F_{1, 70} = 0.86$, $P = 0.356$, $r^2 = 0.046$) or mass ($F_{1, 70} = 0.837$, $P = 0.36$, $r^2 = 0.012$).

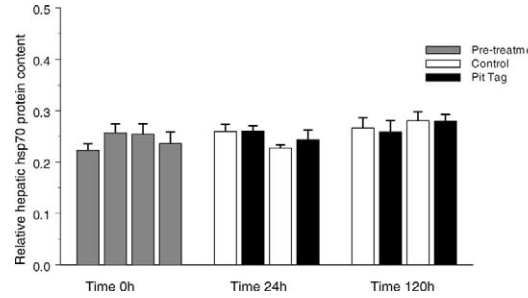

FIGURE 2.—Relative heat shock protein 70 (hsp70) levels before (0 h) and 24 and 120 h after experimental treatment. The values shown are the mean densitometry values of protein bands expressed as density relative to the positive control in the Western blot band density ± SE; $N = 6$ for each bar.

Discussion

Both handling and PIT tagging were stressful events, as indicated by the increases in plasma cortisol levels between 0 and 2 h. The increase at 2 h followed by a decrease at 6 h indicates a quick response to these stressors followed by rapid recovery. This type of response to handling has been well documented (Barton et al. 1988; Mesa and Schreck 1989; Sharpe et al. 1998).

Despite the decrease in plasma cortisol levels in both groups between 2 and 6 h, there were significant differences in plasma cortisol levels between treatment groups at 6 h. The decrease in plasma cortisol levels was not significant for the PIT-tagged group, suggesting that this group did not recover as quickly from the effects of PIT tagging. At 24 h, plasma cortisol levels in both treatment groups were significantly elevated over pretreatment levels and were similar to the levels measured in fish sampled at 6 h. We do not know why plasma cortisol levels in the control group, but not in the treatment group, increased between 6 and 24 h. This increase could be the result of an unknown disturbance (e.g., Sharp et al. 1998), or it could be a natural secondary increase in cortisol in response to a stressor that did not appear to be related to a secondary disturbance (e.g., Barton et al. 1987; Schreck et al. 1989). For example, McCormick and Smith (2004) measured plasma cortisol levels in PIT-tagged and untagged groups of the marine damselfish Ambon damsel *Pomacentrus amboinensis* 63 d after tag insertion and reported that plasma cortisol levels were "considerably lower" in the control group but not significantly so at $\alpha = 0.05$. McCormick and Smith (2004) reported standard error values for nontransformed plasma cortisol data of 10.33 and 11.93 for the PIT-tagged ($N = 12$) and control groups ($N = 12$), respectively. For comparison, the standard errors for nontransformed plasma cortisol in the present study ranged from 1.6 to 7.2.

We did not find any change in the expression levels of hepatic hsp70 during the course of this experiment. Furthermore, the primary antibody used to measure hsp70 in this study in liver tissue detected only one hsp70 band. For this reason, we did not attempt to differentiate between the constitutive and inducible isoforms of hsp70. According to the manufacturer, the polyclonal rabbit anti-hsp70/hsc70 antibody detects proteins of approximately 70 and 73 kDa, corresponding to the apparent molecular mass of hsp70 (hsp72) and hsc70 (hsp73), respectively (Stressgen technical specifications, SPA-758). Given the lack of change in overall hsp70 levels through time and no differences between treatment groups, we concluded that PIT tagging had no effect on overall hsp70 levels.

Unlike cortisol, which is a fast-acting hormone that can exhibit dramatic changes within minutes of a stressor, heat shock proteins exhibit a different time course in response to physiological stressors. When cellular hsp70 protein levels increase, these levels can remain elevated for extended periods (>24 h). For example, in brook trout *Salvelinus fontinalis*, peak hsp70 proteins in both red blood cells and white muscle tissue reached peak levels 12 h after an acute temperature stress and remained elevated for over 48 h (Lund et al. 2003). With juvenile Chinook salmon, a single exposure to 26°C for 10–15 min was sufficient to produce significantly elevated hepatic hsp70 levels for 14 d (Mesa et al. 2002). Therefore, we focused our analysis of hsp70 protein levels at 24 and 120 h posttreatment. If PIT tagging caused an increase in hsp70 levels, we would expect to detect this response in both the 24-h and 120-h sampling periods. The finding that PIT tagging did not alter hepatic hsp70 levels supports other research showing that handling

stress does not alter hsp70 levels (Vijayan et al. 1997; Washburn et al. 2002).

We saw no evidence in this study that PIT tagging and subsequent changes in plasma cortisol levels altered hepatic hsp70 levels. This finding is important because there is some evidence that cortisol plays a role in mediating hsp70 levels in fish tissue after physiological stress (Basu et al. 2001), and chronically stressed fish may have decreased ability to produce hepatic hsp70 (Basu et al. 2002). Subsequently, if plasma cortisol levels were increased in a chronic fashion, it could decrease a fish's ability to mount a heat shock response to a stressor (e.g., temperature, disease, and toxins). If increases in cortisol levels associated with PIT tagging were to impair the heat shock response, this would indicate that PIT-tagging fish has unforeseen physiological consequences that could compromise the fitness of PIT-tagged fish.

These results support a growing literature documenting the suitability of PIT tags as a low-impact tagging procedure for juvenile salmonids. However, because this experiment was completed in a controlled setting where we were able to control factors such as predation, density, feeding, and water temperature; in an uncontrolled setting, differences, particularly those in the plasma cortisol response, may emerge between fish that are handled versus those that are handled and tagged. Additionally, physiological responses to tagging may differ by fish species (McCormick and Smith 2004) or developmental stage. For example, Baras et al. (1999) reported low survival (10–50%) in PIT-tagged Nile tilapia that was size dependent. Variation in the stress response between species and the effects of stress on development and immunocapacity are discussed by Schreck et al. (2001).

In conclusion, changes in plasma cortisol levels indicate that for fish in our experiment, PIT tagging was moderately more stressful, as indicated by higher plasma cortisol levels, in the PIT-tagged group 6 h after sampling. Hepatic hsp70 levels, however, were not altered by PIT tagging or handling. Because PIT tagging does not alter hepatic hsp70 levels in juvenile rainbow trout, PIT tags have the potential to be used as individual fish identifiers during laboratory and field manipulations when one is examining changes in hsp70 levels.

Acknowledgments

Funding for this research was obtained through grants from the U.S. Department of the Interior and the National Oceanic and Atmospheric Administration (NOAA). We thank Chris Jordan (NOAA fisheries) and Michael Newsom (U.S. Bureau of Reclamation) for their efforts to help secure funding. We thank Lisa Madsen of the Oregon State University's Department of Statistics for statistical consultation. We thank Carriska Anthony, Rob Chitwood, Jodi Feldhaus, Grant Feist, Francisco Madriñan, Tracey Momoda, Morgan Packard, Ian Tattam, Jeremiah Osborne-Gowey, and many others for laboratory assistance, and scientific discussion. The use of trade, product, or firm names in this publication is for descriptive purposes only and does not imply endorsement by the U.S. Government.

References

Baras, E., L. Westerloppe, C. Merlard, J. C. Philippart, and V. Benech. 1999. Evaluation of implantation procedures for PIT-tagging juvenile Nile tilapia. North American Journal of Aquaculture 61:246–251.

Barton, B. A., C. B. Schreck, and L. D. Barton. 1987. Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress response in juvenile rainbow trout. Diseases of Aquatic Organisms 2:173–185.

Barton, B. A., C. B. Schreck, and L. G. Fowler. 1988. Fasting and diet content affect stress-induced changes in plasma glucose and cortisol in juvenile Chinook salmon. Progressive Fish-Culturist 50:16–22.

Basu, N., T. Nakano, E. G. Grau, and G. K. Iwama. 2001. The effects of cortisol on heat shock protein 70 levels in two fish species. General and Comparative Endocrinology 124:97–105.

Basu, N., A. E. Todgham, P. A. Ackerman, M. R. Bibeau, K. Nakano, P. M. Schulte, and G. K. Iwama. 2002. Heat shock protein genes and their functional significance in fish. Gene 295:173–183.

Cucherousset, J., J. M. Roussel, R. Keeler, R. A. Cunjak, and R. Stump. 2005. The use of two new portable 12-mm PIT-tag detectors to track small fish in shallow streams. North American Journal of Fisheries Management 25:270–274.

Feder, M. E., and G. E. Hofmann. 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annual Review of Physiology 61:243–282.

Foster, L. B., and R. T. Dunn. 1974. Single-antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. Clinical Chemistry 20:365–368.

Hazon, N., and R. J. Balment. 1998. Endocrinology. Pages 441–463 *in* D. H. Evans, editor. The physiology of fishes, 2nd edition. CRC Press, Boca Raton, Florida.

Hochachka, P. W., and G. N. Somero. 2002. Biochemical adaptation: mechanism and process in physiological evolution. Oxford University Press, New York.

Hockersmith, E. E., W. D. Muir, S. G. Smith, B. P. Sandford, R. W. Perry, N. S. Adams, and D. W. Rondorf. 2003. Comparison of migration rate and survival between radio-tagged and PIT-tagged migrant yearling Chinook salmon in the Snake and Columbia rivers. North American Journal of Fisheries Management 23:404–413.

Iwama, G. K., P. T. Thomas, R. H. B. Forsyth, and M. M. Vijayan. 1998. Heat shock protein expression in fish. Reviews in Fish Biology and Fisheries 8:35–56.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227:680–685.

Lund, S. G., M. E. A. Lund, and B. L. Tufts. 2003. Red blood cell hsp70 mRNA and protein as bioindicators of temperature stress in the brook trout (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences 60:460–470.

McCormick, M. I., and S. Smith. 2004. Efficacy of passive integrated transponder tags to determine spawning-site visitations by a tropical fish. Coral Reefs 23:570–577.

Mesa, M. G., and C. B. Schreck. 1989. Electrofishing mark–recapture and depletion methodologies evoke behavioral and physiological changes in cutthroat trout. Transactions of the American Fisheries Society 118:644–658.

Mesa, M. G., L. K. Weiland, and P. Wagner. 2002. Effects of acute thermal stress on the survival, predator avoidance, and physiology of juvenile fall Chinook salmon. Northwest Science 76:118–128.

Muir, W. D., S. G. Smith, J. G. Williams, E. E. Hockersmith, and J. R. Skalski. 2001. Survival estimates for migrant yearling Chinook salmon and steelhead tagged with passive integrated transponders in the lower Snake and lower Columbia rivers, 1993–1998. North American Journal of Fisheries Management 21:269–282.

Ombredane, D., J. L. Bagliniere, and F. Marchand. 1998. The effects of passive integrated transponder tags on survival and growth of juvenile brown trout (*Salmo trutta* L.) and their use for studying movement in a small river. Hydrobiologia 372:99–106.

Parsell, D. A., and S. Lindquist. 1993. The function of heat shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Annual Review of Genetics 27:437–496.

Peterson, N. P., E. F. Prentice, and T. P. Quinn. 1994. Comparison of sequential coded wire and passive integrated transponder tags for assessing overwinter growth and survival of juvenile coho salmon. North American Journal of Fisheries Management 14:870–873.

Prentice, E. F., T. A. Flagg, and C. S. McCutcheon. 1990a. Feasibility of using implantable passive integrated transponder (PIT) tags in salmonids. Pages 317–322 *in* N. C. Parker, A. E. Giorgi, R. C. Heidinger, D. B. Jester, Jr., E. D. Prince, and G. A. Winans, editors. Fish-marking techniques. American Fisheries Society, Symposium 7, Bethesda, Maryland.

Prentice, E. F., T. A. Flagg, C. S. McCutcheon, and D. F. Brastow. 1990b. PIT-tag monitoring systems for hydroelectric dams and fish hatcheries. Pages 323–334 *in* N. C. Parker, A. E. Giorgi, R. C. Heidinger, D. B. Jester, Jr., E. D. Prince, and G. A. Winans, editors. Fish-marking techniques. American Fisheries Society, Symposium 7, Bethesda, Maryland.

Redding, J. M., C. B. Schreck, E. K. Birks, and R. D. Ewing. 1984. Cortisol and its effects on plasma thyroid hormone and electrolyte concentrations in freshwater and during seawater acclimation in yearling coho salmon, *Oncorhynchus kisutch*. General and Comparative Endocrinology 56:146–155.

Roussel, J. M., R. A. Cunjak, R. Newbury, D. Caissie, and A. Haro. 2004. Movements and habitat use by PIT-tagged Atlantic salmon parr in early winter: the influence of anchor ice. Freshwater Biology 49:1026–1035.

Roussel, J. M., A. Haro, and R. A. Cunjak. 2000. Field test of a new method for tracking small fishes in shallow rivers using passive integrated transponder (PIT) technology. Canadian Journal of Fisheries and Aquatic Sciences 57:1326–1329.

Ruetz, C. R., III, B. M. Earl, and S. L. Kohler. 2006. Evaluating passive integrated transponder tags for marking mottled sculpins: effects on growth and mortality. Transactions of the American Fisheries Society 135:1456–1461.

Sanders, B. M. 1993. Stress proteins in aquatic organisms: an environmental perspective. Critical Reviews in Toxicology 23:49–75.

SAS Institute. 2003. SAS OnlineDoc, version 9. 1. SAS Institute, Cary, North Carolina.

Schreck, C. B. 2000. Accumulation and long-term effects of stress in fish. Pages 147–158 *in* G. P. Moberg and J. A. Mench, editors. The biology of animal stress: basic principles and implications for animal welfare. CABI Publishing, Wallingford, UK.

Schreck, C. B., W. Contreras-Sanchez, and M. S. Fitzpatrick. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture 197:3–24.

Schreck, C. B., M. F. Solazzi, S. L. Johnson, and T. E. Nickelson. 1989. Transportation stress affects performance of coho salmon, *Oncorhynchus kisutch*. Aquaculture 82:15–20.

Sharpe, C. S., D. A. Thompson, H. L. Blankenship, and C. B. Schreck. 1998. Effects of routine handling and tagging procedures on physiological stress responses in juvenile Chinook salmon. Progressive Fish-Culturist 60:81–87.

Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk. 1985. Measurement of protein using bicinchoninic acid. Analytical Biochemistry 150:76–85.

Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proceedings of the National Academy of Sciences of the USA 16:4340–4354.

Vijayan, M. M., C. Pereira, R. B. Forsyth, C. J. Kennedy, and G. K. Iwama. 1997. Handling stress does not affect the expression of hepatic heat shock protein 70 and conjugation enzymes in rainbow trout treated with beta-naphthoflavone. Life Sciences 61:117–127.

Washburn, B. S., J. J. Moreland, A. M. Slaughter, I. Werner, D. E. Hinton, and B. M. Sanders. 2002. Effects of handling on heat shock protein expression in rainbow trout (*Oncorhynchus mykiss*). Environmental Toxicology and Chemistry 21:557–560.

Zugel, U., and S. H. E. Kaufmann. 1999. Role of heat shock proteins in protection from and pathogenesis of infectious diseases. Clinical Microbiology Reviews 12:19–39.