Stress Response of Delta Smelt, Hypomesus transpacificus, in the Collection, Handling, Transport, and Release Phase of Fish Salvage at the John E. Skinner Delta Fish Protective Facility

By
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California Department of Fish and Wildlife
Bay Delta Region

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Abstract

The John E. Skinner Delta Fish Protective Facility (SDFPF, Skinner Fish Facility) diverts entrained fish from the State Water Project's pumping facilities in the southern Sacramento-San Joaquin Delta and transports them via tanker trucks away from the immediate influence of the export pumps. This investigation evaluated the sublethal stress associated with the terminal portion of the fish salvage process, where fish are collected, handled, transported, and released (CHTR) away from the export facilities. Physiological stress for delta smelt, *Hypomesus transpacificus*, was measured in the SDFPF. Wild and cultured adult delta smelt were inserted into the CHTR process and later sampled for cortisol, glucose, hematocrit, and lactate. Fish stress was measured by changes in the levels of these blood components. To help identify the source of overall stress response, CHTR was partitioned into 3 test phases: CH, TR, and uninterrupted CHTR. Differences in the stress response between the phases of CHTR were evaluated. Delta smelt stress in all phases of the CHTR process and in experimental handling treatments was indicated by higher plasma cortisol concentrations compared to their pre-experiment baseline levels. Wild delta smelt experienced higher levels of cortisol than cultured delta smelt. Cortisol levels did not return to pre-treatment levels within 48 hours.

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Introduction

Delta smelt, *Hypomesus transpacificus*, is a native fish found in the San Francisco Estuary, California (McAllister 1963, Aasen 1999, Moyle 2002). It once was one of the most abundant pelagic species in the upper estuary (Moyle 2002). The delta smelt population has been in decline since the mid-1980s, was listed as threatened under both the California Endangered Species and Federal Endangered Species Acts in the early 1990s, and was subsequently uplisted to endangered under the CESA. Since its listing, protection of this species has greatly influenced regional environmental restoration and water management policy. Continued decline of delta smelt in the mid-2000s triggered court decisions affecting the levels and timing of water exported from the estuary. Since the late-2000s, the federal Biological Opinion on the state and federal water operations has mandated continued pumping restrictions aimed at protecting this species.

Fish collection facilities (Figure 1) at the Central Valley Project's (CVP) Tracy Fish Collection Facility and the State Water Project's (SWP) John E. Skinner Delta Fish Protective Facility (Skinner Fish Facility) were constructed in the late 1950s and 1960s to protect mainly juvenile Chinook salmon, *Oncorhynchus tshawytscha*, and striped bass, *Morone saxatilis*. These facilities use dewatering louvers that act as behavioral barriers to direct fish away from the intakes of the SWP and CVP pumping facilities (Skinner 1974). Louvers concentrate fish and funnel them into holding tanks. Fish are later transported to release sites in the Delta within 24 hours. In the early 2000s, the Record of Decision for CALFED promoted reducing entrainment losses at SWP and CVP facilities through the construction of state-of-theart fish screens and upgraded salvage facilities (CALFED 2000a and 2000b). The implementing CALFED agencies became concerned about the feasibility to protect delta smelt and cost of the proposed salvage facilities in the southern Delta.

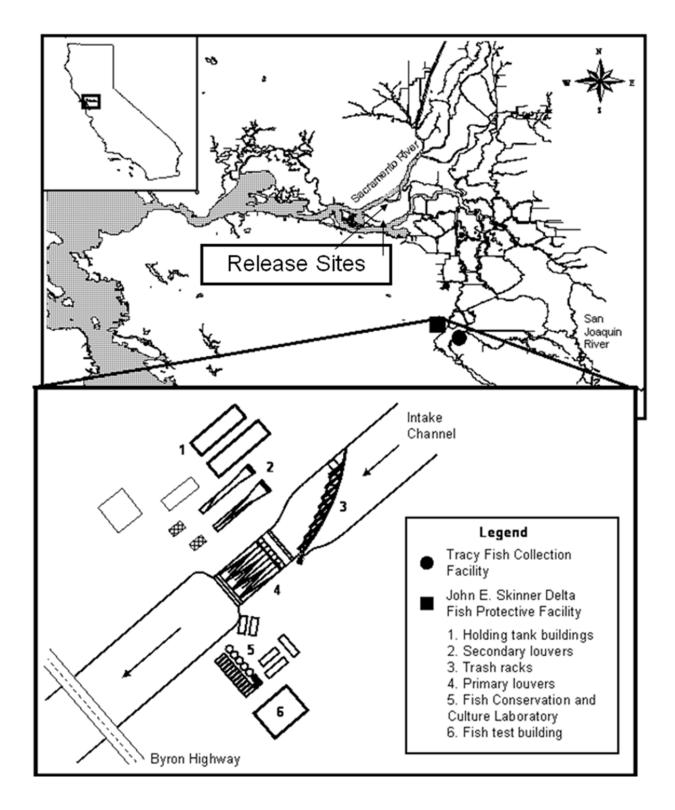


Figure 1 Map of the SWP John E. Skinner Delta Fish Protective Facility, release sites, CVP Tracy Fish Collection Facility (top) and layout of the John E. Skinner Delta Fish Protective Facility (bottom)

Although the proposed salvage facilities included design improvements intended to reduce pre-screen predation, increase collection efficiencies, improve hydraulic controls, and provide relatively fish-friendly holding conditions, the terminal portion of the fish salvage process would likely be unchanged. After salvaged fish are held and concentrated in the holding tanks, fish are further concentrated into a loading bucket then lifted and placed into a tanker vehicle for transport to release locations in the Delta (Skinner 1974). Little was known about the survival or injury of delta smelt undergoing this collection, handling, transport, and release (CHTR) portion of the salvage process, and it was thought by some that high mortality in this phase could negate the benefits of improved salvage facilities. Due to these concerns, the California Department of Fish and Wildlife (formerly the Department of Fish and Game) led a series of collaborative studies to assess the acute adverse effects of the CHTR process (or phase) on delta smelt and to recommend facility improvements and operational procedures to minimize any identified impacts.

Research on sublethal stress to delta smelt was proposed in conjunction with studies assessing acute mortality and injury, and losses due to predation. We hypothesized that delta smelt undergoing the CHTR process would experience high levels of stress due to the degree of handling that is typical of these operations. High levels of stress in fish can be immediately debilitating, decrease the ability of fish to endure subsequent challenges (Schreck 1981, Barton 2002), and result in exhaustion and death of the animal (Selye 1950). Stress at the fish facilities is likely compounded by warm water temperatures, debris, and overcrowding.

Physiological stress responses in fishes and other vertebrates are sometimes characterized as "primary," "secondary," and "tertiary," representing increasing levels of biological organization and endpoints (Wedemeyer and others 1990, Barton 2002). Physiological indicators have been employed to assess acute, chronic, and cumulative stress effects at various levels of biological organization over different time scales (Adams 1990). The primary stress response is marked by elevation of epinephrine and cortisol circulating in the blood stream after perception of a stressful stimulus by the brain (Wedemeyer and others 1990).

The primary response triggers a secondary response at the blood and tissue level. Epinephrine causes blood glucose to elevate and provide energy for a "fight-or-flight" reaction. Cortisol helps sustain elevated glucose levels through depletion of tissue glycogen. Elevation of lactate is caused by either fright or severe muscular exertion (Wedemeyer and others 1990, Wendelaar Bonga 1997). Other secondary compensatory stress responses may include elevation of hematocrit, lowering of plasma chloride, hemorrhage of the thymus, hypertrophy of the interrenal body, and immunosuppression.

Stress responses are considered adaptive and compensatory if physiological changes allow the organism to maintain homeostasis in the face of biotic or abiotic challenges. However, when stress is prolonged or severe, physiological and whole-animal responses may become detrimental to the health of the organism (Barton 2002). Stress at this magnitude can produce effects at the individual and population level (tertiary response) through reductions in growth, infectious disease resistance, reproductive success, and survival (Wendelaar Bonga 1997, Schreck and others 2001).

The purpose of our work was to investigate use of physiological assessments for quantification of stress attributable to fish-facility operation. The first objective was to evaluate a set of physiological stress indicators and their ability to measure the delta smelt stress response and recovery time within the CHTR process. The second objective was to identify the components of the CHTR process that were the most stressful to delta smelt. The results could be used along with data from the other CHTR studies to help design any new salvage facilities or make improvements to existing facilities. These results would also act as the first step toward determining likely tertiary stress responses in salvaged delta smelt.

Methods

All experiment replicates were conducted at the Skinner Fish Facility during March-May 2005 and December 2005-May 2006. The Skinner buildings have 7 large (6.1 meters deep x 6.1 meters diameter) holding tanks which temporarily collect fish diverted (salvaged) from water heading towards the SWP pumps. Prior to use, delta smelt were held in tanks with treated aqueduct water at the UCD Fish Conservation and Culture Laboratory (FCCL). Experiments took place in holding tank buildings at the Skinner Fish Facility or at the neighboring CHTR test facilities. The CHTR test building and its facilities were built in 2004 to hold test fish, conduct CHTR experiments, and provide laboratory and office space.

The CHTR phase is only a portion of a series of salvage processes that entrained fish must negotiate successfully to avoid being lost within the SWP. Fish enter the headworks of the SWP export facilities through Clifton Court Forebay. Prior to the export pumps, the Skinner Fish Facility uses a set of primary louvers to divert fish into a secondary bypass channel. Fish are then guided by louvers or conventional fish screens from the secondary channel into holding tanks. Fish generally remain in the holding tanks for a period of 8 to 24 hours. The CHTR process begins by draining the contents of each holding tank into an 1893-L loading bucket located in the bottom center of the tank (collection phase). The loading bucket is raised via a crane, and its contents are drained into a 9463- or 10599-L tanker truck (handling phase). The tanker truck is driven approximately 50 minutes to one of 2 release sites in the Delta (transport phase), where its load of salvaged fish is then released into the Delta (release phase).

Test Fish

Adult wild and cultured delta smelt were used in these experiments. Wild delta smelt were collected in late fall of 2004 and 2005 from the western Delta using a modification of methods developed by Swanson and others (1996), whereby fish were collected using a lampara net with a 0.95 cm stretch mesh landing bag. Wild fish were transported to the FCCL and held 1-3 months before use in experiments. The FCCL was expanded to accommodate the increased demands for test fish and holding facilities for CHTR studies.

Wild and cultured fish were held and cared for in a similar manner (Baskerville-Bridges, Lindberg, and Doroshov 2005; Baskerville-Bridges, Lindberg, and Cech 2006). Cultured (F₁ generation) fish were propagated from wild brood stock. Adult cultured fish were held in black circular holding tanks (550-L; 1.52 m diameter x 61 cm deep) until needed for the CHTR experiments. Fish were fed a mixture of dry pellets (Kyowa 1000-c) and Hikari plankton twice daily until the day of the experiments. Test fish were not fed immediately prior to use in the experiments.

Experimental Design

Test fish were exposed to 4 treatment types: net stress (NS), CH, TR, and CHTR. Experimental design excluded some potential stressors (e.g., other fish and debris) commonly found during routine salvage operations. Experiments were conducted during low-to-moderate seasonal temperatures.

Twenty-four randomly-selected fish were used each time a treatment was performed. Wild and cultured fish were tested separately. Four fish were randomly selected and collected using a soft-mesh brine shrimp net 30 minutes before the experiment to measure pre-treatment levels. Measurements from these fish were considered our experimental "baseline level" and are referred to in figures and tables as the "-0.5-hour interval." The 20 other fish were inserted into the experiment as treatment fish. After completion of the experiment, 4 fish were immediately sampled (0-hour post-treatment time interval) for blood collection. Another 4 fish were placed in a filled black 19-L bucket for blood collection at the 0.5-hour post-treatment time interval. In random groups of 4 fish, the remaining 12 fish were placed into 3 separate 83-L storage tubs (Rubbermaid[®]) placed in a black, 1136-L holding tank with a flow-through water supply. Three groups of 4 fish each were selected for physiological testing at 2, 24 and 48 hours posttreatment time intervals. All fish were sacrificed during bleeds. Blood from each time-interval group was used to measure 4 different stress responses: cortisol, glucose, hematocrit, and lactate. For cortisol, plasma from 2 fish was typically pooled, but sometimes — due to low volume — plasma from 4 fish was pooled. Cortisol concentration was later determined from each pooled sample. Percent hematocrit was determined using 1 or more blood samples (microhematocrit tubes) taken from each individual fish. Glucose or lactate concentration was determined using the blood from 1 fish prior to collection of the remaining blood for hematocrit and cortisol.

The NS treatment simulated the stress experienced by fish when being netted and transported, which was a procedure all fish underwent before and after each experiment. Fish were netted and placed in a filled 19-L bucket. The bucket was transported a short distance to mimic the transport of pre-treatment fish to the holding tank or transport truck. Instead of releasing fish into the tank or truck to simulate the insertion process, fish were placed in a pre-filled, black 3407-L circular tank. Fish were acclimated in a circular tank for approximately 5 minutes and then removed with nets to simulate handling during the CH, TR, and CHTR experiments. This process lasted approximately 15 minutes. These fish were held for up to 48 hours and processed the same as other treatment groups.

The CH treatment simulated the routine transfer of fish from a Skinner Fish Facility holding tank into a loading bucket and release into a 9463-L transport truck. Fish were moved between the pre-treatment holding tanks to the experiments and back to the CHTR test building. Fish were gathered using a brine shrimp net and transferred into a filled 19-L bucket. To start the experiment, the bucket was lowered by hand-line into the holding tank. Once the bottom of the bucket was just below the water surface, it was slowly inverted to release the 20 fish. The fish were allowed to acclimate in the holding tank for 5-20 minutes before the holding tank was allowed to begin draining. Once the tank drained and the fish were concentrated into the loading bucket, the bucket was raised and emptied into an 1893-L fiberglass retrieval tank. Fish were removed from the retrieval tank using a brine shrimp net over the course of about 5 minutes. Four fish were immediately sacrificed for physiological testing and the remainder held for 0.5, 2, 24, or 48 hours.

The TR treatment simulated the routine transport of fish in a tanker truck from the Skinner Fish Facility to the Delta release locations and subsequent release through a pipe into the river. Fish were loaded into

the truck using the bucket-inversion technique previously described for the CH treatment. Once all the fish were loaded, the truck drove for approximately 50 minutes. Upon return to the Skinner Fish Facility, the truck drove up a ramp to the top of a simulated release apparatus. The ramp was positioned above a 45425-L above-ground pool (8.78 m long x 4.57 m wide x 1.22 m deep). The contents of the truck were released from the back of the truck through a large pipe (25.4 cm diameter x 7.62 m long) into the previously-filled pool. Due to site limitations, the simulated release apparatus was shorter in length and lacked some features of the actual release sites. Water in the pool was drained to concentrate fish into a recessed trough (457 cm long x 46 cm wide x 30 cm deep) in the bottom of the pool. The pool water drained through a drum screen. The draining took about 15 minutes, after which 4 fish were removed and then bled.

The CHTR treatment simulated the entire process of collecting, handling, transporting, and releasing salvaged fish. Fish were inserted into the holding tank and went through the same process as described for the CH experiment, except that the bucket contents were emptied directly into the transport truck rather than into a retrieval tank. The truck was then driven for 50 minutes, and its contents were emptied into the recovery pool as described for the TR treatments. Fish were collected and bled in groups of 4 individuals.

After each experiment began, fish were held in smaller tanks at the CHTR test building in minimally-treated water (sand filtered and UV treated) from the Clifton Court Forebay outlet channel. Dissolved oxygen (mg/L), water temperature ($^{\circ}$ C), and specific conductance (μ S/cm) were measured before (FCCL tanks), during (holding tanks or truck tank) and immediately after each experiment (release tank). At 2-, 24-, and 48-hour post-treatment intervals, water quality was also measured in the smaller tanks holding fish.

Physiological Measurements

Blood samples were collected and processed using an adaptation of protocols described by Young and others (2001). Four fish were netted from a bucket and wrapped in a Kimwipes® tissue before bleeding. The caudal fin of each fish was immediately severed with a scalpel just anterior to the caudal peduncle. Blood was collected from the exposed caudal vessels using standard microhematocrit tubes. The amount of blood drawn from each fish ranged from 2 - 20 μL . Plasma from individual fish was pooled to meet the minimum required volume (5 μL) for cortisol analysis. Once filled with blood, the microhematocrit tubes were sealed. The blood collections were timed using 2 timers (1 for the first 2 fish netted; 1 for the last fish netted). To avoid cortisol elevation due to this procedure, the maximum time allowed for blood collection was 2 minutes. Once bled, fish were stunned by a blow to the head with a finger flick and euthanized by sectioning the spine just posterior to the skull with a scalpel. Any fish that was mishandled or dropped during any phase of the experiment or blood collection process was excluded. Each fish was measured (fork length) to the nearest millimeter, weighed to the nearest 0.01 g, and frozen for reference purposes.

Blood samples from the microhematocrit tubes were processed in 2 ways. To separate red cells from plasma, blood was centrifuged at 9500 rpm for 5 minutes using a Vulcon Technologies Microspin 24TM centrifuge. Additionally, approximately 25% of the whole blood samples were immediately measured for either glucose or lactate concentrations. Glucose readings were obtained using an Accu-Chek[®] glucose meter and recorded to the nearest 1 mg/dL. An AccuSportTM meter was used to measure lactate concentrations to the nearest 0.1 mmol/L. Hematocrit values were obtained by measuring the centrifuged hematocrit tubes on a graduated chart for percent packed blood cells within blood samples. Hematocrit values were recorded to the nearest 1%.

Blood plasma from the centrifuged tubes was transferred into 1.5 mL microcentrifuge freezer vials. Because a minimum of 5 μ L of plasma was needed for each cortisol analysis, plasma from a minimum of 2 fish was pooled before storage at -40 °C. Frozen plasma was transported on dry ice to the UC Davis Clinical Endocrinology Laboratory for blood plasma cortisol analysis.

Cortisol concentrations from plasma were determined by Coralie Munro and Alejandro Esteller-Vico at the UCD Clinical Endocrinology Lab. The lab staff used an enzyme-linked immunosorbent assay (ELISA) method. Assays of each smelt plasma sample occurred in duplicate, flat bottom, microtiter plates (Immulon 1®) with 96 wells. Known cortisol concentrations were also added to each plate (3 wells were used for these standard concentrations on each 96-well plate) and assayed concurrent with the smelt plasma samples. After completion of the assay, plates were read in a spectrophotometer at 450 nm wavelength. Results were obtained using calibration curves developed from each assay, which returned cortisol concentration values. All results were generated electronically by an automated process employing the spectrophotometer and accompanying software. (Corbin, personal communication, see "Notes"). Cortisol readings were recorded to the nearest 0.01 ng/mL.

Data Summaries and Analyses

Treatment-time responses for each blood test (cortisol, glucose, hematocrit, and lactate) were visually examined by plotting the mean value \pm 95% confidence intervals (CI). We used standard deviation (SD) to describe the variability around mean values for each environmental measurement (electrical conductivity, dissolved oxygen, and water temperature), for fish length, and for fish weight. R© 2.14.0 (2011-10-31; R Development Core Team 2011) statistical and graphics software was used to perform the analyses and generate graphics.

The cortisol, glucose, and hematocrit data were examined for the suitability of parametric analysis. The data were inspected for normal distribution by using quantile-quantile plots and histograms (Crawley 2007). Because cortisol and glucose data were not normally distributed, cortisol data were square root transformed and glucose data were log transformed to meet assumptions of subsequent analytical tests (Sokal and Rohlf 1987; Zar 1996; Newman, personal communication, see "Notes").

Cortisol, glucose, and hematocrit data were tested for significant mean differences between treatments (NS, CH, TR, and CHTR) and between time intervals (-0.5, 0, 0.5, 2, 24, and 48 hours) using additive or factorial Type I two-way analysis of variance (ANOVA; Sokal and Rohlf 1987; Zar 1996; Newman, personal communication, see "Notes"). The additive model allowed the detection of differences between treatments and time intervals when there was no interaction between the two groups. The factorial model also allowed the detection of (1) within-treatment differences between the baseline level and all other hour intervals, (2) within-treatment differences of consecutive hour intervals, or (3) between-treatment differences within the same hour interval. Wild fish and cultured fish data were analyzed separately.

Tukey's honest significant differences (HSD) test was used to detect significant statistical differences between treatment means, time interval means, and combined treatment and time interval means. The significance level for all tests was set at P < 0.05.

A Student's t-Test (Zar 1996; Crawley 2007) was used to detect significant statistical differences between physiological parameters of wild and cultured fish at each time interval for each treatment type. The significance level was set at P < 0.05.

Quality Control for Field Measurements

The YSI 556 dissolved oxygen meter was calibrated daily according to the manufacturer's procedures. The Acculab scales were calibrated daily using a 200-g reference weight. The electrical conductivity sensor of the YSI 556 and the Accu-Chek glucose meter were checked before each study season using commercial standard solutions. The AccuSport lactate meter was calibrated using the manufacturer's calibration strips before each use. The accuracy of the other field measurements was not determined.

To evaluate precision, duplicate readings for each parameter were (generally) made at least once out of roughly every 20 measurements. An average of 8.9% of hematocrit, fork length, body weight, electrical conductivity, dissolved oxygen, and water temperature readings were repeated either by the instrument operator or 2 individuals (e.g., hematocrit and fork length). These repeat values were used to determine the percent deviation from the average of the 2 values. Percent-deviation measurements were taken on field data collected from 17 of the 80 experiments. Less than 5% deviation was the performance criterion for these readings. Precision of glucose and lactate readings was not determined, nor was the accuracy of field measurements.

Results

Number of Tests Performed

Approximately 2000 fish were sampled from 80 experiment replicates (Table 1; Appendix D). The number of physiological measurements varied among treatment groups due to the varying amounts of plasma from each fish and the need to pool plasma samples. The number of completed replicates for each experiment varied from a low of 6 TR replicates with cultured fish to a high of 13 NS replicates with wild fish.

Table 1 Number of physiological samples by analyte, fish type, and treatment type

	Time interval (h)					
	-0.5	0	0.5	2	24	48
Cortisol						
cultured						
CH	14	14	13	13	13	11
CHTR	19	19	19	22	18	18
NS	21	22	19	20	19	15
TR	11	12	12	11	11	09
wild						
СН	21	23	20	19	19	18
CHTR	15	14	16	14	13	13
NS	26	23	25	24	21	23
TR	23	21	23	16	21	23
Glucose						
cultured						
CH	07	06	07	05	06	07
CHTR	11	11	11	11	11	10
NS	11	11	11	11	11	11
TR	06	06	06	06	06	06
wild						
CH	11	11	12	12	12	10
CHTR	07	08	80	80	07	07
NS	13	13	12	13	12	12
TR	12	12	12	11	11	12
Lactate						
cultured						
СН	02	05	03	05	04	04
CHTR	09	10	80	09	08	07
NS	03	06	06	07	05	07
TR	04	04	06	05	05	03
wild						
СН	08	06	07	80	05	05
CHTR	06	06	05	06	05	03
NS	08	11	80	09	07	80
TR	07	11	09	80	09	06
Hematocrit						
cultured						
СН	22	20	20	21	22	23
CHTR	34	39	40	40	40	30
NS	38	36	32	28	31	22
TR	23	23	22	20	18	14
wild	_~		-		. •	
CH	36	32	24	30	29	23
CHTR	27	22	23	24	21	20
NS	47	38	39	37	32	30
TR	38	30	33	24	30	29

Fish Size

Cultured fish tended to be larger and heavier than wild fish used in our study. Mean fork length for wild fish was 64 ± 5.7 mm (N = 1061) and 68 ± 8.8 mm (N = 860) for cultured fish. Mean wet weight for wild fish was 2.1 ± 0.6 g (N = 1061) and 2.7 ± 1.3 g (N = 862) for cultured fish.

Mortality

Mortality for the entire study was 1.6%. A majority of mortalities were found at the 48-hour post-treatment interval. Pretest mortality at the FCCL for the period of study was 1.3%. Because mortality rates were low, we made no effort to investigate mortality rates or mechanisms.

Water Quality and Holding Conditions

Water quality and holding conditions reflected seasonal and operational conditions at the Skinner Fish Facility. Water temperatures ranged from 9 - 22°C, averaged 14 ± 3 °C (N = 481), and stayed within critical temperature maxima and minima for delta smelt (Swanson and Cech 1996). Dissolved oxygen levels varied from 6 - 15 mg/L (mean = 10 ± 1.3 mg/L; N = 481), due in part to the injection of oxygen during fish transport.

Electrical conductivity readings from experimental holding tanks and collection tanks in the Skinner Fish Facility were generally similar. Electrical conductivity for the combined NS and CH replicates ranged from 96 - 416 μ S/cm and averaged 196 \pm 71 μ S/cm (N = 206). Fish in the TR and CHTR experiments experienced higher electrical conductivity in the tanker truck and collection pool because salt (NaCl) was added to the water per standard operating procedure. Salt is added to reduce osmotic stress and increase fish survival (Swanson and others 1996). Electrical conductivity in the truck tank varied from 2277 - 9452 μ S/cm (mean = 4761 \pm 1287 μ S/cm; N = 73).

We graphically examined the effects on analyte levels of possible co-factors, namely water temperature and seasonal variation. Because of inconclusive initial findings, we did not incorporate co-factors into our statistical analyses.

Physiology

Cortisol

Plasma cortisol of wild and cultured fish elevated immediately after each treatment, remained elevated at least until the 2-hour interval, and did not return to baseline levels by the 48-hour interval (Figure 2). Cortisol levels of all 5 post-treatment intervals were significantly higher than baseline levels (Table 2). Time intervals when maximum cortisol levels occurred varied by treatment and source of fish. Cortisol reached peak levels in wild fish at the 0.5-hour interval for the CH treatment (Figure 2). Mean cortisol levels for the CHTR treatment peaked at the 2-hour interval.

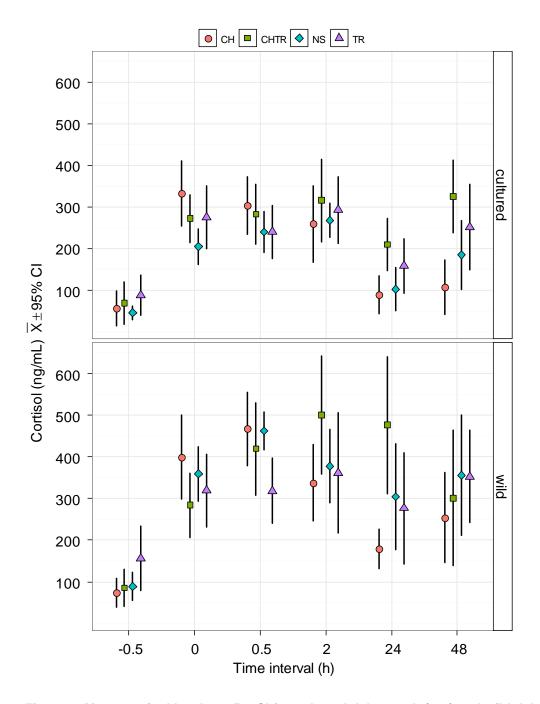


Figure 2 Mean cortisol levels \pm 95% CI for cultured delta smelt (top) and wild delta smelt (bottom) in the CH, CHTR, NS, and TR treatments over time; time interval is not to scale and -0.5 represents control condition; mean and CI are not transformed

Results from the additive-model ANOVA indicated differences in the mean cortisol levels among wild and cultured fish. Cortisol levels of wild fish were not significantly different for treatments (df = 3, F = 1.722, P = 0.16; Appendix A) but were different for intervals (df = 5, F = 35.552, P < 0.001; Appendix A). Cortisol levels for all treatments of wild fish at the 24- and 48-hour intervals were significantly lower than levels at the 0.5-hour interval, and levels at the 24-hour interval were significantly lower than levels at the 2-hour interval (Table 2).

Table 2 Type 1 ANOVA results showing *P*-values and significant (sig) differences between treatments and then hour intervals for cortisol

	Cortisol (sqrt transformed)					
Comparisons	Cı	ıltured		Wild		
·	P	<i>P</i> < 0.05	P	P < 0.05		
CHTR x CH	0.01	sig	0.29	-		
NS x CH	0.95	-	0.54	-		
TR x CH	0.20	<u>-</u>	1.00	-		
NS x CHTR	< 0.001	sig	0.93	-		
TR x CHTR	0.85	<u>-</u>	0.26	-		
TR x NS	0.04	sig	0.49	-		
0 x -0.5	< 0.001	sig	< 0.001	sig		
0.5 x -0.5	< 0.001	sig	< 0.001	sig		
2 x -0.5	< 0.001	sig	< 0.001	sig		
24 x -0.5	< 0.001	sig	< 0.001	sig		
48 x -0.5	< 0.001	sig	< 0.001	sig		
0.5 x 0	1.00	-	0.33	-		
2 x 0	0.99	-	0.97	-		
24 x 0	< 0.001	sig	0.20	-		
48 x 0	0.16	-	0.57	-		
2 x 0.5	1.00	-	0.84	-		
24 x 0.5	< 0.001	sig	< 0.001	sig		
48 x 0.5	0.14	-	< 0.01	sig		
24 x 2	< 0.001	sig	0.03	sig		
48 x 2	0.04	sig	0.17	-		
48 x 24	0.01	sig	0.99	-		

Cortisol levels of cultured fish were significantly different for treatments (df = 3, F = 6.843, P < 0.001; Appendix A) and intervals (df = 5, F = 42.596, P < 0.001; Appendix A). Cortisol levels of cultured fish in CHTR and TR treatments were significantly higher than levels of NS (Table 2). Also, cortisol levels at the 24-hour interval were significantly lower than levels at 0-, 0.5-, and 2-hour intervals (Figure 2; Table 2). Levels at the 48-hour interval were significantly lower than levels at the 2-hour interval but significantly higher than levels at the 24-hour interval (Figure 2; Table 2).

The factorial ANOVA results among the treatment- and time-interval combinations showed a greater number of statistically significant differences for cultured fish (N = 22; Table 3) than for wild fish (N = 13; Table 3). Except for wild fish in the TR treatments, cortisol was significantly elevated from baseline levels by the 0- or 0.5-hour interval and remained significantly elevated at least to the 2-hour interval or up to the 48-hour interval for NS (wild fish) and CHTR (cultured fish) treatments (Table 3). Cultured fish were more likely than wild fish to experience significant decreases in cortisol levels by the 24- and 48-hour intervals, as well as significant increases during the TR treatment. At the 48-hour interval, cortisol levels for cultured fish were significantly elevated for the CHTR treatment compared to the CH treatment (Table 3; Figure 2).

Table 3 Factorial Type 1 ANOVA results showing only *P*-values for significant differences between comparable time intervals within treatment and between treatments

Cortisol (wild, sqrt transformed)						
Comparisons	diff	lwr	upr	P		
CH:0 x CH:-0.5	11.591	4.852	18.331	< 0.001		
CH:0.5 x CH:-0.5	13.539	6.562	20.516	< 0.001		
CH:2 x CH:-0.5	9.916	2.846	16.986	< 0.001		
CHTR:0.5 x CHTR:-0.5	11.786	3.760	19.811	< 0.001		
CHTR:2 x CHTR:-0.5	13.690	5.391	21.988	< 0.001		
CHTR:24 x CHTR:-0.5	12.739	4.277	21.200	< 0.001		
NS:0 x NS:-0.5	10.525	4.133	16.917	< 0.001		
NS:0.5 x NS:-0.5	13.285	7.031	19.540	< 0.001		
NS:2 x NS:-0.5	10.456	4.135	16.777	< 0.001		
NS:24 x NS:-0.5	8.040	1.489	14.592	< 0.01		
NS:48 x NS:-0.5	8.954	2.562	15.346	< 0.001		
CH:24 x CH:0.5	-8.436	-15.589	-1.282	< 0.01		
CHTR:24 x CH:24	8.043	0.006	16.081	< 0.05		
Cortisol (cultured, sqrt transf	ormed)					
Comparisons	diff	lwr	upr	P		
CH:0 x CH:-0.5	11.329	5.068	17.590	< 0.001		
CH:0.5 x CH:-0.5	10.519	4.139	16.899	< 0.001		
CH:2 x CH:-0.5	8.987	2.607	15.368	< 0.001		
CHTR:0 x CHTR:-0.5	8.812	3.438	14.187	< 0.001		
CHTR:0.5 x CHTR:-0.5	9.178	3.803	14.552	< 0.001		
CHTR:2 x CHTR:-0.5	9.448	4.260	14.636	< 0.001		
CHTR:24 x CHTR:-0.5	6.354	0.905	11.802	< 0.01		
CHTR:48 x CHTR:-0.5	10.122	4.674	15.571	< 0.001		
NS:0 x NS:-0.5	7.584	2.531	12.638	< 0.001		
NS:0.5 x NS:-0.5	8.748	3.503	13.993	< 0.001		
NS:2 x NS:-0.5	9.789	4.613	14.965	< 0.001		
NS:48 x NS:-0.5	6.030	0.430	11.630	< 0.05		
TR:0 x TR:-0.5	7.679	0.764	14.593	< 0.05		
TR:2 x TR:-0.5	8.242	1.179	15.305	< 0.01		
CH:24 x CH:0	-9.218	-15.598	-2.838	< 0.001		
CH:48 x CH:0	-8.573	-15.247	-1.899	< 0.001		
CH:24 x CH:0.5	-8.408	-14.905	-1.911	< 0.001		
CH:48 x CH:0.5	-7.763	-14.549	-0.977	< 0.01		
NS:24 x NS:0.5	-6.106	-11.481	-0.732	< 0.01		
CH:24 x CH:2	-6.877	-13.374	-0.379	< 0.05		
NS:24 x NS:2	-7.147	-12.454	-1.841	< 0.001		
CHTR:48 x CH:48	8.101	1.761	14.440	< 0.001		
Hematocrit (wild)						
Comparisons	diff	lwr	upr	Р		
TR:0 x CH:0	-8.410	-13.798	-3.023	< 0.001		
NS:0 x CHTR:0	5.702	0.023	11.382	< 0.05		

TR:0 x NS:0	-9.632	-14.810	-4.455	< 0.001
NS:48 x NS:0	-5.632	-10.810	-0.455	< 0.05
Hematocrit (cultured)				
Comparisons	diff	lwr	upr	P
TR:0 x CH:0	-5.580	-10.991	-0.170	< 0.05
NS:0 x CHTR:0	5.448	1.358	9.538	< 0.001
TR:0 x NS:0	-6.705	-11.429	-1.980	< 0.001

In general, wild fish had higher cortisol levels than cultured fish for all treatments (Figure 3). Cortisol levels for wild fish were significantly higher than levels observed for cultured fish at the 0.5-hour and 24-hour intervals for all treatments except TR (Figure 3; Appendix B). Significantly higher cortisol levels for wild fish were also observed at the 0-hour interval for NS, the 2-hour interval for CHTR, and the 48-hour interval for CH treatments (Figure 3; Appendix B). No significant differences were observed between wild and cultured fish for the TR treatment.

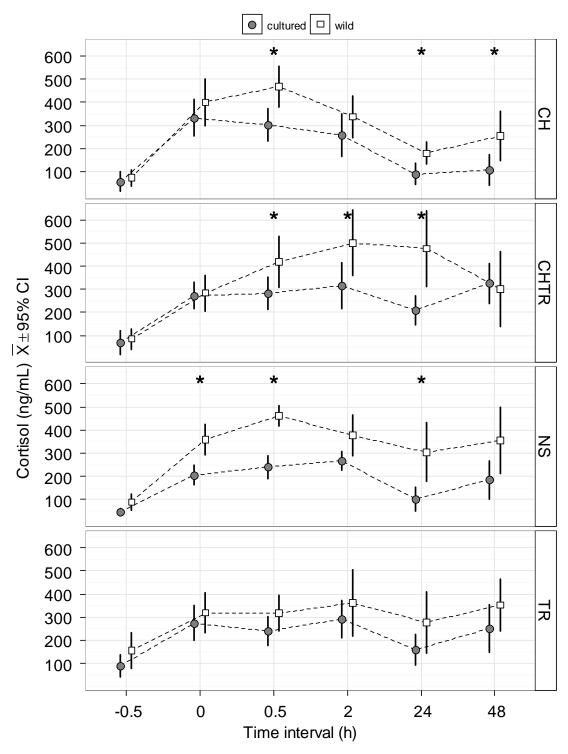


Figure 3 Mean cortisol levels \pm 95% CI for delta smelt in the CH, CHTR, NS, and TR treatments comparing wild and cultured over time; asterisk indicates a significant difference (P < 0.05) between wild and cultured delta smelt at that interval; time interval is not to scale and -0.5 represents control condition; mean and CI are not transformed

Glucose

Glucose levels of wild fish rose gradually to the 24-hour interval (Figure 4). Glucose concentration of the CH and NS treatments nearly returned to baseline levels at the 48-hour interval. In contrast, cultured-fish glucose remained elevated for TR, CHTR, and NS treatments (Figure 4).

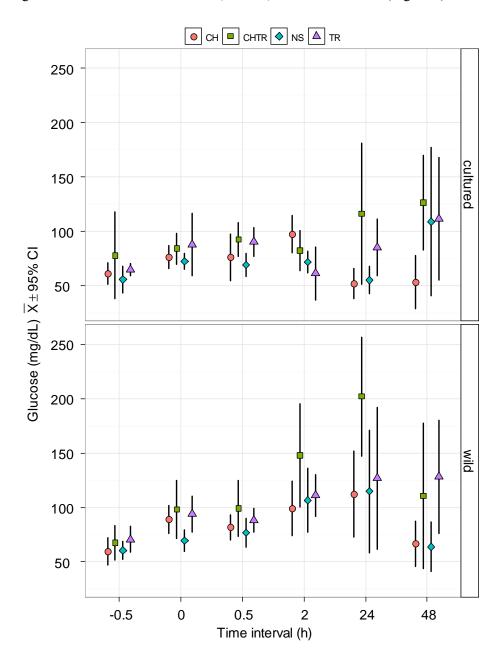


Figure 4 Mean glucose levels ± 95% CI for cultured delta smelt (top) and wild delta smelt (bottom) in the CH, CHTR, NS, and TR treatments over time; time interval is not to scale and -0.5 represents control condition; mean and CI are not transformed

Results from the additive-model ANOVA indicated that wild and cultured fish responded differently in terms of glucose concentrations. Glucose levels for wild fish were significantly different for treatments (df = 3, F = 6.681, P < 0.001; Appendix A) and time intervals (df = 5, F = 7.279, P < 0.001; Appendix A). Glucose levels of the CHTR treatment were significantly higher than levels of the CH and NS treatments (Table 4). Glucose levels of the TR treatment were also significantly higher than levels of the NS treatment (Table 4). Glucose concentrations at the 2- and 24-hour intervals were significantly higher than values for the baseline levels and at 48-hour interval (Figure 4; Table 4).

Table 4 Type 1 ANOVA results showing *P*-values and significant (sig) differences between treatments and then hour intervals for glucose

	Glucose (log 10 transformed)				
Comparisons	Cu	ltured	W	ild	
	P	<i>P</i> < 0.05	P	<i>P</i> < 0.05	
CHTR x CH	0.03	sig	0.01	sig	
NS x CH	1.00	<u>-</u>	0.81	-	
TR x CH	0.38	-	0.23	-	
NS x CHTR	0.01	sig	< 0.001	sig	
TR x CHTR	0.78	-	0.42	-	
TR x NS	0.38	-	0.02	sig	
0 x -0.5	0.20	-	0.07	-	
0.5 x -0.5	0.15	-	0.08	-	
2 x -0.5	0.58	-	< 0.001	sig	
24 x -0.5	0.98	-	< 0.001	sig	
48 x -0.5	0.06	-	0.68	-	
0.5 x 0	1.00	-	1.00	-	
2 x 0	0.99	-	0.35	-	
24 x 0	0.62	-	0.17	-	
48 x 0	1.00	-	0.83	-	
2 x 0.5	0.97	-	0.32	-	
24 x 0.5	0.53	-	0.16	-	
48 x 0.5	1.00	-	0.85	-	
24 x 2	0.94	-	1.00	-	
48 x 2	0.86	-	0.02	sig	
48 x 24	0.30	<u>.</u>	0.01	sig	

Glucose levels for cultured fish were generally below 100 mg/dL (Figure 4) and were significantly different for treatments (df = 3, F = 4.145, P < 0.01; Appendix A) and intervals (df = 5, F = 2.379, P = 0.04; Appendix A). Cultured fish had fewer significant differences in treatment and interval comparisons of mean glucose levels (N = 2) compared to wild fish (N = 7; Table 4). Glucose levels of the CHTR treatment were significantly higher than levels of the CH and NS treatments (Table 4). Tukey post-hoc testing showed no significant difference between intervals (Figure 4; Table 4).

Glucose levels of wild fish were significantly elevated above levels of cultured fish at the 2-hour interval for the TR and CHTR treatments and at the 24-hour interval for the CH and CHTR (Figure 5; Appendix B). No significant differences were observed between wild and cultured fish for the NS treatment.

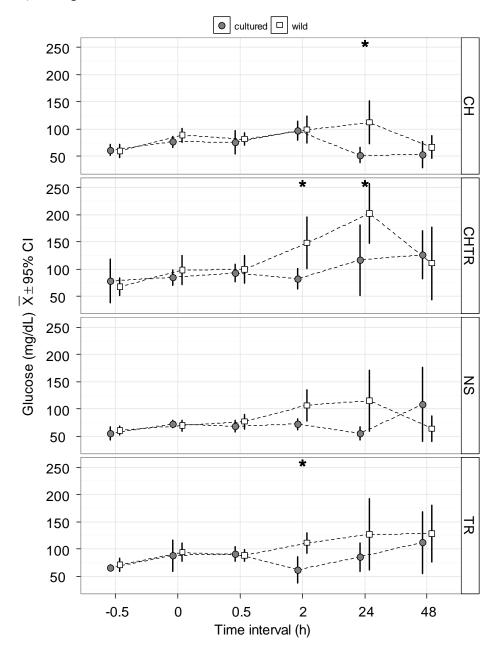


Figure 5 Mean glucose levels ± 95% CI for delta smelt in the CH, CHTR, NS, and TR treatments comparing wild and cultured over time; asterisk indicates a significant difference (*P* < 0.05) between wild and cultured delta smelt at that interval; time interval is not to scale and -0.5 represents control condition; mean and CI are not transformed

Hematocrit

Changes in hematocrit levels for wild and cultured fish were short-term and less dramatic compared to the other physiological indicators. Hematocrit levels increased slightly for CH and NS or decreased slightly for TR immediately after these treatments. Hematocrit concentrations returned to levels similar to baseline levels by the 2-hour interval (Figure 6).

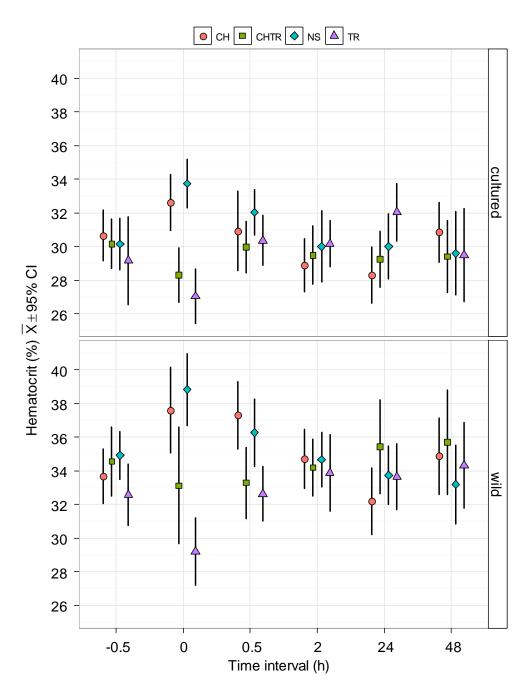


Figure 6 Mean hematocrit levels \pm 95% CI for cultured delta smelt (top) and wild delta smelt (bottom) in the CH, CHTR, NS, and TR treatments over time; time interval is not to scale and -0.5 represents control condition

Results from the factorial model ANOVA indicated significant differences in mean hematocrit levels for both wild and cultured fish among treatments but not for time intervals (Appendix A). Hematocrit levels of wild fish from TR were significantly lower than levels for CH, CHTR, and NS (Table 5; Figure 6). Hematocrit levels of cultured fish from CHTR were significantly lower than levels of NS (Table 5; Figure 6).

Table 5 Type 1 ANOVA results showing *P*-values and significant (sig) differences between treatments and then hour intervals for hematocrit

	Hematocrit				
Comparisons	Cul	tured	W	/ild	
•	P	<i>P</i> < 0.05	P	P < 0.05	
CHTR x CH	0.30	-	0.78	-	
NS x CH	0.58		0.91	-	
TR x CH	0.63		< 0.001	sig	
NS x CHTR	< 0.01	sig	0.37	-	
TR x CHTR	0.98	- -	0.049	sig	
TR x NS	0.06		< 0.001	sig	
0 x -0.5	0.98	- -	0.60	-	
0.5 x -0.5	0.80	- -	0.73	-	
2 x -0.5	1.00	- -	1.00	-	
24 x -0.5	1.00	- -	1.00	-	
48 x -0.5	1.00	- -	0.98	-	
0.5 x 0	0.99	- -	1.00	-	
2 x 0	0.85	- -	0.91	-	
24 x 0	0.89	-	0.43	-	
48 x 0	0.96	- -	0.97	-	
2 x 0.5	0.50	- -	0.96	-	
24 x 0.5	0.56	-	0.56	-	
48 x 0.5	0.73	-	0.99	-	
24 x 2	1.00	-	0.96	-	
48 x 2	1.00	-	1.00	-	
48 x 24	1.00	-	0.92	-	

Results from the factorial ANOVA showed that significant changes in hematocrit levels occurred mostly between treatments at the 0-hour interval (Table 3). Hematocrit levels for wild and cultured fish at the 0-hour interval were significantly higher for the NS and CH treatments compared to the TR. NS treatment levels for both groups were also significantly higher than their associated CHTR levels at the 0-hour interval. Within the NS treatment for wild fish, the 48-hour hematocrit levels were significantly lower than levels at the 0-hour interval (Table 3).

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Comparisons of the hematocrit levels for wild and cultured fish suggest pre-existing differences. With some exceptions, hematocrit levels of wild fish were significantly higher than hematocrit levels of cultured fish for all treatments at each interval (Figure 7; Appendix B); the exceptions were for the TR treatment at 0-, 0.5-, and 24-hour intervals.

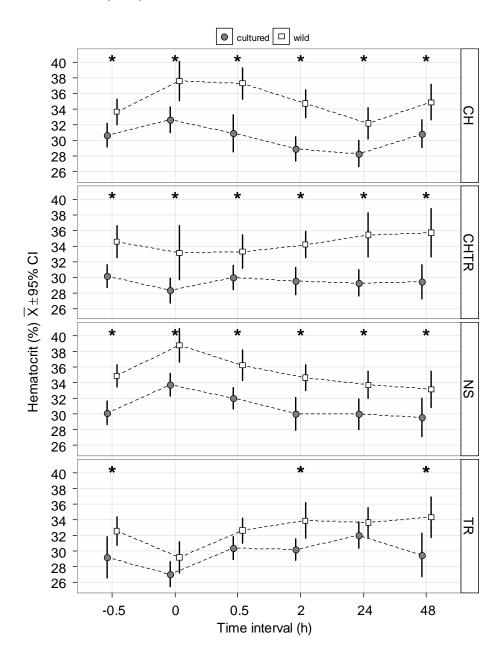


Figure 7 Mean hematocrit levels ± 95% CI for delta smelt in the CH, CHTR, NS, and TR treatments comparing wild and cultured over time; asterisk indicates a significant difference (*P* < 0.05) between wild and cultured delta smelt at that interval; time interval is not to scale and -0.5 represents control condition

Lactate

Average lactate baseline levels in wild and cultured fish were approximately 2.5 mmol/L (Figure 8). Lactate levels for both groups of fish increased to the 2-hour interval but returned to baseline levels by the 24-hour interval (Figure 8). The greatest differences in lactate levels were observed between wild fish and cultured fish in the TR and CHTR treatments at the 0-hour interval (Figure 8). No statistical comparisons were attempted because of small sample sizes for some treatments (Table 1) due to a high number of non-numeric readings (e.g., readings of "high" or "low").

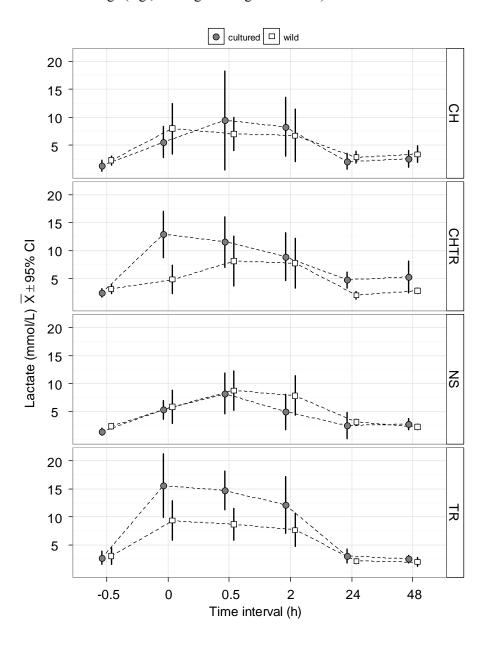


Figure 8 Mean lactate levels \pm 95% CI for wild and cultured delta smelt in the CH, CHTR, NS, and TR treatments over time; means were not statistically compared between wild and cultured delta smelt at each time interval; time interval is not to scale and -0.5 represents control condition

Quality Control

Field Measurements

Percent deviations of replicate fork length and weight measurements were within acceptable limits. Mean percent deviation for length measurements was $0.71 \pm 2.01\%$, and all but 2 values were under 5% deviation (Appendix C). Weight measurements had a mean percent deviation of $0.41 \pm 0.42\%$ and all values were within acceptable levels.

Hematocrit and water quality parameter measurements showed notable measurement variability. Hematocrit quality-control readings had a mean percent deviation of $1.50 \pm 2.26\%$. Nine out of 154 hematocrit measurements exceeded 5% deviation (Appendix C). The mean percent deviations for electrical conductivity, dissolved oxygen (mg/L), and water temperature were 2.87, 3.87, and 1.80% respectively.

Some of the large differences in replicate field measurements for electrical conductivity and dissolved oxygen were attributable to changing conditions in the quality of water held in the tanker truck. The measurement variability likely reflected the slow dissolution of salt and the continuous injection of oxygen.

Cortisol

Coefficients of variation (CVs) were calculated for each duplicate sample using the concentration values. These were given as percent for each duplicate set. CVs from the standard cortisol concentrations (called "controls" by the Endocrinology lab) were used for the following cortisol ELISA quality control results. The individual CV values were averaged for each plate and each assay to determine the reliability of the ELISA. The whole-assay CV was less than 23% for the low control, less than 11% for the medium control, and less than 6% for the high control. The CV for each plate was less than 43% for the low control, less than 18% for the medium control, and less than 10% for the high control.

Data Entry

Five percent of the data entry was randomly checked for accuracy, and corrective action was taken because an error rate of approximately 25% was discovered. The corrective action entailed a second line-by-line edit of 100% of the data entered into the database. This editing resulted in a near-0% data entry error rate.

Discussion

Plasma cortisol showed patterns of rapid elevation similar to those reported in fishes including juvenile Chinook salmon (Barton, Schreck, and Sigismondi and others 1986; Portz, Woodley, and Cech 2006), rainbow trout (Woodward and Strange 1987), olive flounder (Hur, Park, and Chang 2007), lingcod (Milston and others 2006), and wild delta smelt (Swanson and others 2001). Elevated levels of blood glucose of wild fish at the 2- and 24-hour intervals were consistent with the observed stress responses in other fishes (Hattingh 1977; Barton, Schreck, and Fowler 1988).

The CHTR treatment resulted in the greatest elevation (experienced by wild fish) of cortisol and the least amount of recovery (experienced by cultured fish) during the post-experimental period. We attribute these responses to the cumulative exposure to stressful events — including necessary handling. Swanson and others (2001) found that peak cortisol elevation was additive with increasing flow regimes.

The NS treatments showed response trends slightly less or comparable to the other treatments. The similarities in cortisol response suggest that experimental handling stress may limit our ability to interpret the impacts of CHTR — in whole or in part — on sublethal stress as measured by cortisol. Swanson and others (2001) found that wild delta smelt experienced significantly higher cortisol concentrations 0.5 hour after experimental release of test fish into a testing apparatus and 0.5 hour after test-fish removal from the apparatus. Weber and Borthwick (2000) concluded that most of the elevated cortisol response seen in juvenile Chinook salmon passed through "fish-friendly" pumps was due to handling and transport of the test fish prior to insertion into the pumps.

The results also suggest that after sequential stressors, cortisol levels failed to return to baseline within 24 hours. Similar studies have found prolonged elevation of stress hormones in other fishes (Milston and others 2006; Hur, Park, and Chang 2007). Swanson and others (2001) found that cortisol levels peaked at 0.5 hour and remained elevated up to 48 hours in adult wild delta smelt exercised for 2 hours at all sweeping and approach flow velocities.

Elevated cortisol levels at the 48-hour interval suggest that post-experimental stress inhibited recovery or that recovery may take longer than 48 hours. We suspect the former hypothesis, in part because Swanson and others (2001) reported slightly-elevated cortisol levels in wild delta smelt held for 48 hours in separate 5-L containers following exercise in a fish treadmill.

Significant elevation of hematocrit levels at the 0-hour interval of CH and NS treatments relative to levels at the 0-hour interval of TR treatments was consistent with past studies. Swanson and others (2001) reported that significantly-elevated hematocrit readings of wild delta smelt immediately after release into a test apparatus were due to erythrocytic swelling and/or erythrocyte addition from splenic contraction due to pre-experimental handling of fish. The lower hematocrit levels for the CHTR and TR treatments at the 0- and 0.5-hour intervals may also be attributable to the addition of NaCl to water in the tanker truck. Similar changes of hematocrit and blood volume resulting from exposure to NaCl were observed in salmon (Redding and Schreck 1983). The overall higher hematocrit levels for wild fish compared to cultured fish suggest pre-existing physiological differences between those groups.

Differences in the stress responses between wild and cultured delta smelt suggests that cultured fish exhibited muted stress responses and are more tolerant to anthropogenic stressors. Congleton and others (2000) reported such differences between hatchery and wild Chinook salmon responding to stress from human impacts. Woodward and Strange (1987) suggested that fish reared in hatchery situations are subjected to an unnatural selection pressure that favors individuals with muted stress responses. Because our test fish were the first generation cultured artificially, these observed differences are noteworthy. The relative differences in stress indicators of wild and cultured delta smelt should be considered before using only cultured delta smelt for research.

Recommendations

Although we were able to collect the physiological data, the experimental-handling stress responses made it difficult to discern responses to the CHTR process in whole or in part. Developing methods to reduce experimental handling or post-experimental holding stress appear to be a major requirement for future stress evaluation of delta smelt using the parameters in our study. Adding 6- and 96-h sampling intervals would have been helpful in observing the recovery responses in greater detail. Increasing the sample size

would likely have improved our ability to make statistical inferences.

References

- Aasen, GA. 1999. Juvenile delta smelt use of shallow-water and channel habitats in California's Sacramento San Joaquin Estuary. California Fish and Game 85(4): 161-169.
- Adams, SM. Editor. 1990. Biological indicators of stress in fish. American Fisheries Society, Symposium 8, Bethesda, Maryland. 191 p.
- Barton, BA. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology 42:517-525.
- Barton, BA, CB Schreck, and LG 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.
- Barton, BA, CB Schreck, and LA Sigismondi. 1986. Multiple acute disturbances evoke cumulative physiological stress responses in juvenile Chinook salmon. Transactions of the American Fisheries Society 115:245-251.
- Baskerville-Bridges, B, JC Lindberg, and JJ Cech Jr. 2006. Delta smelt culture, production, and facility expansion, 2003-2005. Department of Water Resources. Project title: Delta smelt culture and swimming performance. Calfed Bay-Delta agreement number 4600002963.
- Baskerville-Bridges, B, JC Lindberg, and SI Doroshov. 2005. Manual for the intensive culture of Delta smelt (*Hypomesus transpacificus*). Calfed Bay-Delta agreement number 4600002881.
- CALFED Bay-Delta Program. 2000a. Programmatic Record of Decision. Volume 1 Record of Decision and Attachments 1 through 4. August 28, 2000. Department of Water Resources. 84 p.
- CALFED Bay-Delta Program. 2000b. Implementation Plan. Final Programmatic EIS/EIR Technical Appendix. July 2000. CD-ROM version. 139 p.
- Congleton, JL, WJ LaVoie, CB Schreck, and LE Davis. 2000. Stress indices in migrating juvenile Chinook salmon and steelhead of wild and hatchery origin before and after barge transportation. Transactions of the American Fisheries Society 129: 946-961.
- Crawley, MJ. 2007. The R Book. West Sussex, England: John Wiley & Sons Ltd. 942 p.
- Hattingh, J. 1977. Blood sugar as an indicator of stress in the freshwater fish, *Labeo capensis* (Smith). Journal of Fish Biology 10(2): 191–195.
- Hur, JW, I-S Park, and YJ Chang. 2007. Physiological responses of the olive flounder, *Paralichthys olivaceus*, to a series stress during the transportation process. Ichthyological Research 54(1): 32-37.

- McAllister, DE. 1963. A revision of the smelt family, Osmeridae. National Museum of Canada Bulletin 191. 53 p.
- Milston, RH, MW Davis, SJ Parker, BL Olla, S Clements, and CB Schreck. 2006. Characterization of the physiological stress response in lingcod. Transactions of the American Fisheries Society 135:1165-1174.
- Moyle, PB. 2002. Inland Fishes of California. Revised and expanded. University of California Press, Berkeley. 517 p.
- Portz, DE, CM Woodley and JJ Cech. 2006. Stress-associated impacts of short-term holding on fishes. Reviews in Fish Biology and Fisheries 16 (2): 125-170.
- R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3 900051-07-0, URL http://www.R-project.org/.
- Redding, JM and CB Schreck. 1983. Influence of ambient salinity on osmoregulation and cortisol concentration in yearling coho salmon during stress. Transactions of the American Fisheries Society 112(6): 800–807.
- Schreck, CB. 1981. Stress and compensation in teleostean fishes: response to social and physical factors. Pages 295-321. Stress in fish. AD Pickering, editor. Academic Press, London. 367 p.
- Schreck, CB, W Contreras-Sanchez, and MS Fitzpatrick. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture 197:3-24.
- Selye, H. 1950. Stress and the general adaptation syndrome. British Medical Journal 1:1383-1392.
- Skinner, JE. 1974. A functional evaluation of a large louver screen installation and fish facilities research on California water diversion projects. Pages 225-249. LD Jensen, editor. Proceedings of the second entrainment and intake-screening workshop. Johns Hopkins University, Cooling Water Research Project. Report number 15. Baltimore, Maryland.
- Sokal, RR and JF Rohlf. 1987. Introduction to Biostatistics. 2nd ed. Stony Brook, NY: W.H. Freeman and Company. 363 p.
- Swanson, C and JJ Cech. 1996. Environmental Tolerances and Habitat Requirements of Delta Smelt. Final report to California Department of Water Resources. 77 p.
- Swanson, C, RC Mager, SI Doroshov, and JJ Cech Jr. 1996. Use of salts, anesthetics, and polymers to minimize handling and transport mortality in delta smelt. Transactions of the American Fisheries Society 125:326-329.
- Swanson, C, PS Young, S Chun, T Chen, T MacColl, L Kanemoto, and JJ Cech Jr. 2001. Part 2. Biological studies. *In* Fish treadmill-developed fish screen criteria for native Sacramento-San

Joaquin watershed fishes. Final report. Prepared by JJ Cech, PS Young, C Swanson, (Department of Wildlife, Fish, and Conservation Biology) ML Kavvas, ZQ Chen, and H Bandeh (Department of Civil and Environmental Engineering), University of California, Davis. November 2001; revised April 2002. Department of Wildlife, Fish and Conservation Biology, University of California, Davis, CA. 98 p.

- Weber, ED and SM Borthwick. 2000. Plasma cortisol levels and behavioral stress responses of juvenile Chinook salmon passed through Archimedes lifts and an internal helical pump at Red Bluff Research Pumping Plant, Sacramento River, California. Red Bluff Research Pumping Plant Report Series: Volume 8. U.S. Bureau of Reclamation. Red Bluff Fish Passage Program. Red Bluff, California. 31 p.
- Wedemeyer, GA, BA Barton, and DJ McLeay. 1990. Stress and acclimation. Pages 451-477. Methods for Fish Biology. CB Schreck and PB Moyle, editors. American Fisheries Society, Bethesda, Maryland. 684 p.
- Wendelaar Bonga, SE 1997. The stress response in fish. Physiological Reviews 77(3): 591-625.
- Woodward, CC and RJ Strange. 1987. Physiological responses in wild and hatchery-reared rainbow trout. Transactions of the American Fisheries Society 116: 574-579.
- Young, PS, C Swanson, S Chun, T Chen, T MacColl, and JJ Cech Jr. 2001. Responses and recovery in delta smelt exposed to handling stress during fish treadmill experiments at winter temperature. Interagency Ecological Program for the Sacramento-San Joaquin Estuary. IEP Newsletter 14(3):38-40.
- Zar, JH. 1996. Biostatistical Analysis. 3rd ed. Upper Saddle River, NJ: Prentice-Hall. 662 p.

Notes

Jo Corbin, UCD Endocrinology Lab, personal communication with Virginia Afentoulis as communicated to Marty Gingras through e-mail on 02 October 2012.

Ken Newman (Mathematical Statistician, US Fish and Wildlife Service), in-person conversations, 06 October 2011, 16 November 2011, and 15 December 2011.

Appendixes

Appendix A ANOVA results for each analyte

Cortisol (wild, sqrt transfor	med)				
	df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	3	192	64.1	1.722	0.16168
Time interval	5	6616	1323.2	35.552	< 0.001
Treatment:Time interval	15	1172	78.2	2.100	0.00911
Residuals	450	16748	37.2		
Cortisol (cultured, sqrt tran	sforme	ed)			
	df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	3	419	139.7	6.843	0.000171
Time interval	5	4347	869.3	42.596	< 0.001
Treatment:Time interval	15	630	42.0	2.057	0.011524
Residuals	351	7164	20.4		
Glucose (wild, log transfor	med)				
	df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	3	0.897	0.2991	6.681	0.000235
Time interval	5	1.629	0.3259	7.279	< 0.001
Residuals	249	11.148	0.0448		
Glucose (cultured, log tran	sforme	ed)			
	df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	3	0.491	0.16351	4.145	0.00709
Time interval	5	0.469	0.09383	2.379	0.04017
Residuals	196	7.732	0.03945		
Hematocrit (wild, not trans	formed)			
	df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	3	829	276.5	8.205	< 0.001
Time interval	5	183	36.5	1.083	0.368
Treatment:Time interval	15	1829	121.9	3.618	< 0.001
Residuals	694	23385	33.7		
Hematocrit (cultured, not t	ransfor	,		_	
	df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	3	312	103.94	4.430	0.004297
Time interval	5	112	22.41	0.955	0.44483
Treatment:Time interval	15	937	62.49	2.663	0.000611
Residuals	634	14875	23.46		

Appendix B Student's t-Test results for each analyte; statistical significance where P < 0.05 is indicated by sig

_	Time	Time Cortisol (sqrt transformed)					Glucose (log 10	transfor	med)	Hematocrit			
Treatment	Interval	df	statistic	P	P < 0.05	df	statistic	P	P < 0.05	df	statistic	P	P < 0.05
	-0.5	33	-0.8693201	0.39	-	16	0.42671056	0.68	-	56	-2.447001	0.02	sig
	0	35	-0.8297168	0.41	-	15	-1.2217693	0.24	-	50	-2.808549	0.01	sig
011	0.5	31	-2.6619859	0.01	sig	17	-0.7237575	0.48	-	42	-4.088251	< 0.001	sig
СН	2	30	-1.1071828	0.28	-	15	0.35726435	0.73	-	49	-4.582239	< 0.001	sig
	24	30	-2.7137665	0.01	sig	16	-2.2149434	0.04	sig	49	-2.817721	0.01	sig
	48	27	-2.0667282	0.049	sig	15	-1.0857823	0.29	-	44	-2.726034	0.01	sig
	-0.5	32	-0.5617444	0.58	-	16	0.02297116	0.98	-	59	-3.520721	< 0.001	sig
	0	31	-0.1850262	0.85	-	17	-0.5505763	0.59	-	59	-2.802617	0.01	sig
CUTD	0.5	33	-2.1522605	0.04	sig	17	-0.2525824	0.80	-	61	-2.548966	0.01	sig
CHTR	2	34	-2.412416	0.02	sig	17	-2.4762862	0.02	sig	62	-3.561689	< 0.001	sig
	24	29	-3.2528814	< 0.01	sig	16	-2.5256064	0.02	sig	59	-3.948038	< 0.001	sig
	48	29	0.5995286	0.55	-	15	0.69664424	0.50	-	48	-3.383652	< 0.01	sig
	-0.5	45	-1.4286554	0.16	-	22	-0.9054927	0.38	-	83	-4.508636	< 0.001	sig
	0	43	-4.1812313	< 0.001	sig	22	0.65110459	0.52	-	72	-3.79568	< 0.001	sig
NC	0.5	42	-6.4021208	< 0.001	sig	21	-0.6612074	0.52	-	69	-3.28811	< 0.01	sig
NS	2	42	-1.5999553	0.12	-	22	-1.5099807	0.15	-	63	-3.491814	< 0.001	sig
	24	38	-3.7260576	< 0.001	sig	21	-1.9988723	0.06	-	61	-2.809083	0.01	sig
	48	36	-1.8686022	0.07	-	21	1.3909362	0.18	-	50	-2.045956	0.046	sig
	-0.5	32	-0.9480616	0.35	-	16	-0.2270707	0.82	-	59	-2.144728	0.04	sig
	0	31	-0.17326	0.86	-	16	-0.555721	0.59	-	51	-1.565001	0.12	-
TD	0.5	33	-1.0043103	0.32	-	16	0.2856821	0.78	-	53	-1.913445	0.06	-
TR	2	25	-0.333657	0.74	-	15	-2.9944718	0.01	sig	42	-2.608529	0.01	sig
	24	30	-0.9373913	0.36	-	15	-0.6745072	0.51	-	46	-1.122511	0.27	-
	48	30	-0.7397629	0.47	-	16	-0.1037734	0.92	-	41	-2.280585	0.03	sig

Appendix C Percent deviation results for biological and water quality field measurements

Measurement	N	Mean % deviation	Range	Standard deviation	Number exceed 5%
Fork length* (mm)	138	0.71	0.00 - 16.79	2.01	2
Wet weight (g)	138	0.41	0.00 - 2.10	0.42	0
Hematocrit* (%)	154	1.5	0.00 - 12.66	2.26	9
Electrical conductivity (µS/cm)	41	2.87	0.00 - 59.04	9.41	3
Dissolved oxygen (mg/L)	41	3.87	0.00 - 27.45	6.04	8
Water temperature (°C)	41	1.8	0.00 - 11.72	2.98	8

^{*}Deviations based on the difference between two observers

Appendix D Delta smelt cortisol, glucose, hematocrit, and lactate results by experiment, smelt type, and post-experiment interval (h). Experiments are identified by treatment acronym-test number-date (e.g., collection and handling experiment number 1 on 23-March-2005 = CH-1-3/23/2005); Note: NA or NaN means result is not a number (e.g., high or low), and <NA> means no sample taken or sample taken but not enough blood volume to test for analyte

Experiment	Fish Type	Hour interval	Cortisol (ng/mL)	Glucose (mg/dL)	Hematocrit (%)	Lactate (mmol/L)
CH-1-3/23/2005	wild	-0.5	NA,289.07	60	NaN,28,33,38	2.3
CH-1-3/23/2005	wild	0	1223.37	90	NaN,39,34,26	17.5
CH-1-3/23/2005	wild	0.5	931.4,788.62	46	<na></na>	10.1
CH-1-3/23/2005	wild	2	586.14,125.58	111	NaN,31,29,29	8.8
CH-1-3/23/2005	wild	24	284.82	29	37,NaN	2.5
CH-2-3/28/2005	wild	-0.5	116.33,30.29	<na></na>	31,38,35,NaN	2
CH-2-3/28/2005	wild	0	574.84,415.01	84	36,38,NaN,44	9.4
CH-2-3/28/2005	wild	0.5	567.41,761.72	92	NaN,NaN,41,40	6.2
CH-2-3/28/2005	wild	2	282.23,161.56	77	NaN,38,38,35	14.7
CH-2-3/28/2005	wild	24	396.84,233.86	86	NaN,34,39,33	3.9
CH-2-3/28/2005	wild	48	73.86,42.78	64	40,35,38,40	3.4
NS-2-4/4/2005	wild	-0.5	253.45,98.46	54	29,33,36	2.2
NS-2-4/4/2005	wild	0	346.64,327.62	61	36,NaN,35	16.6

Experiment	Fish Type	Hour interval	Cortisol (ng/mL)	Glucose (mg/dL)	Hematocrit (%)	Lactate (mmol/L)
NS-2-4/4/2005	wild	0.5	387.84,495.46	100	14,26,37,40	15.3
NS-2-4/4/2005	wild	2	727.66	116	NaN,36,35,34	14.5
NS-2-4/4/2005	wild	24	222.27,70.82	94	NaN,30,34,35	3.3
NS-2-4/4/2005	wild	48	340.9,NA,100.88	39	NaN,33,47,NaN	1.8
TR-1-4/6/2005	wild	-0.5	29.56,50.52	25	42,40,NaN	4.5
TR-1-4/6/2005	wild	0	<na></na>	81	<na></na>	19.6
TR-1-4/6/2005	wild	0.5	307.15,581.24	86	NaN,38,33,32	7.7
TR-1-4/6/2005	wild	2	304.44,NA	71	28,42,NaN,47	7.8
TR-1-4/6/2005	wild	24	17.46,58.28	52	NaN,29,43	1.8
TR-1-4/6/2005	wild	48	168.98	46	NaN,44	2.5
CHTR-1-4/11/2005	cultured	-0.5	95.42,18.33	42	NaN,NaN,33,35	NA
CHTR-1-4/11/2005	cultured	0	NA,419.24	46	34,38,NaN,32	21.6
CHTR-1-4/11/2005	cultured	0.5	819.72,311.18	37	28,36,24,NaN	19.3
CHTR-1-4/11/2005	cultured	2	616.93,218.92,NA	48	37,NaN,30,40	NA
CHTR-1-4/11/2005	cultured	24	180.46,330.01	40	35,26,34,32	4.5
CHTR-1-4/11/2005	cultured	48	239.54,571.16,379.53	47	41,27,28,38	11.9
NS-3-4/12/2005	wild	-0.5	308.39,193.42	46	36,41,43,39	NA
NS-3-4/12/2005	wild	0	276.87,197.78	49	NaN,37,39,47	1.4
NS-3-4/12/2005	wild	0.5	337.24,NA,478.18	67	38,37,40,50	NA
NS-3-4/12/2005	wild	2	279.45,296.71	46	35,39,36,40	18.4
NS-3-4/12/2005	wild	24	274.5,225.62	40	41,36,39,NaN	4.3
NS-3-4/12/2005	wild	48	227.77,252.33	33	36,49,38,35	1.4
CH-3-4/18/2005	wild	-0.5	33.59,56.77	64	36,43,42,37	NA
CH-3-4/18/2005	wild	0	223.08,514.85	91	NaN,43,NaN,39	10.3
CH-3-4/18/2005	wild	0.5	268.87,619.74,593.41	77	39,42,42,38	NA
CH-3-4/18/2005	wild	2	218.63,207.33,289.75	100	41,27,33,30	19.1
CH-3-4/18/2005	wild	24	63.68,80.48	55	27,39,30,37	1.3
CH-3-4/18/2005	wild	48	432.42,214.72	46	NaN,NaN,37,40	4.7
TR-2-4/19/2005	wild	-0.5	179.5,117.89	62	31,30,33,22	1.4
TR-2-4/19/2005	wild	0	177.34,168.98	71	27,NaN,28,33	5.8
TR-2-4/19/2005	wild	0.5	69.86,59.23,62.34	76	34,29,28,34	4.1
TR-2-4/19/2005	wild	2	68.01,189.01	84	34,33,NaN	6.2
TR-2-4/19/2005	wild	24	147.05,39.91	<na></na>	41,36,37	1.7
TR-2-4/19/2005	wild	48	260.95,387.19	53	41,NaN,44,49	1
CHTR-2-4/25/2005	cultured	-0.5	32.33,NA	59	28,30,35,34	3.2
CHTR-2-4/25/2005	cultured	0	396.32,437.69	71	29,26,37,28	20.4
CHTR-2-4/25/2005	cultured	0.5	197.8,261.2	104	26,28,33,34	14.4
CHTR-2-4/25/2005	cultured	2	676.17,43.19,58.53	65	31,30,42,28	6.1
CHTR-2-4/25/2005	cultured	24	176.38,395.88	39	24,31,30,25	5.9
CHTR-2-4/25/2005	cultured	48	25.92,162.2,123.5	149	30,27,34,24	1.7
NS-4-4/26/2005	wild	-0.5	165.1,49.95	78	42,46,38,43	<na></na>
NS-4-4/26/2005	wild	0	305.59,360.04	69	43,48,43,32	13.8
NS-4-4/26/2005	wild	0.5	477.4,222.45	54	36,29,36,34	16.2
NS-4-4/26/2005	wild	2	334.4,661.02	103	32,31,28	5.8

Experiment	Fish Type	Hour interval	Cortisol (ng/mL)	Glucose (mg/dL)	Hematocrit (%)	Lactate (mmol/L)
NS-4-4/26/2005	wild	24	133.74,225.4	30	34,34,47,39	2.4
NS-4-4/26/2005	wild	48	481.36,232.19	36	NaN,34,33,39	2.5
TR-3-4/26/2005	wild	-0.5	569.5,325.6	54	33,40,35,38	6.4
TR-3-4/26/2005	wild	0	84.83,106.23	48	34,40,33,NaN	5
TR-3-4/26/2005	wild	0.5	296.38,325.99	69	43,32,34,NaN	3.2
TR-3-4/26/2005	wild	2	92.48,86.75	69	41,42,29,25	4.8
TR-3-4/26/2005	wild	24	104.22,34.48	52	36,32,35,39	1.9
TR-3-4/26/2005	wild	48	486.66,335.63,114.85	34	40,30,43,45	2.2
CH-4-5/9/2005	wild	-0.5	63.45,88.46	52	38,39,30,40	1.6
CH-4-5/9/2005	wild	0	118.6,669.85	87	58,41,NaN	2.9
CH-4-5/9/2005	wild	0.5	535.52,278.99,193.91	131	52,35,36,33	14.4
CH-4-5/9/2005	wild	2	219.28,108.14	145	32,41,40	2.4
CH-4-5/9/2005	wild	24	25.04,228.89	191	30,31,44	NA
CH-4-5/9/2005	wild	48	64.23,76.75	63	40,32,30,33	5.3
NS-5-5/9/2005	wild	-0.5	164.48,39.54	53	36,31,40,40	2.2
NS-5-5/9/2005	wild	0	277.95,300.99	61	35,30,48,40	6.7
NS-5-5/9/2005	wild	0.5	406.61,515.52	75	38,50,42,40	5.9
NS-5-5/9/2005	wild	2	93.56,694.54	220	36,NaN,32,42	4.1
NS-5-5/9/2005	wild	24	290.83,174.36	57	NaN,33,30,33	3.8
NS-5-5/9/2005	wild	48	328.43,67.66	47	33,NaN,34,37	3.8
CH-5-5/16/2005	wild	-0.5	108.6,53.69	77	34,30,38,40	4.9
CH-5-5/16/2005	wild	0	180.6,252.64,581.34	127	47,48,51,43	NA
CH-5-5/16/2005	wild	0.5	494.56,316.88	82	42,NaN,44,NaN	2.9
CH-5-5/16/2005	wild	2	89.72,101.45	173	45,40,44	2.2
CH-5-5/16/2005	wild	24	NA,76.76	92	44,33,42,NaN	4.3
CH-5-5/16/2005	wild	48	709.08,450.68,500.86	79	40,47,NaN,NaN	2.4
CHTR-3-5/17/2005	wild	-0.5	132.6,47.7	58	36,34,42,36	4.3
CHTR-3-5/17/2005	wild	0	224.04,47.43	132	42,33,NaN,41	3.8
CHTR-3-5/17/2005	wild	0.5	230.64,139.24	121	NaN,39,40,NaN	13.2
CHTR-3-5/17/2005	wild	2	236.79,409.34	175	37,32,38,27	12.7
CHTR-3-5/17/2005	wild	24	259.55,68.49	116	NaN,44,40,NaN	1.3
CHTR-3-5/17/2005	wild	48	75.32,119.42	137	36,39,40,44	3
NS-6-5/23/2005	wild	-0.5	6.83,81.53	67	36,37,39,41	1.5
NS-6-5/23/2005	wild	0	542.53,219.11	81	52,48,49,49	5.7
NS-6-5/23/2005	wild	0.5	583.38,455.63	109	45,35,33,NaN	11.7
NS-6-5/23/2005	wild	2	653.01,208.89,287.9	130	44,43,45,27	7.2
NS-6-5/23/2005	wild	24	256.64,205.16	237	30,23,39,32	2.9
NS-6-5/23/2005	wild	48	949.93,57.36	53	14,38,39,33	1.9
CHTR-4-5/24/2005	wild	-0.5	182.62,174.76	83	33,42,33,40	<na></na>
CHTR-4-5/24/2005	wild	0	68.25,195.59	139	42,45,NaN,57	4.3
CHTR-4-5/24/2005	wild	0.5	282.34,183.81	150	NaN,30,31,34	4.5
CHTR-4-5/24/2005	wild	2	451.45,290.67	172	36,36,32,NaN	1.5
CHTR-4-5/24/2005	wild	24	485.79,NA	161	30,35,41,NaN	2.6
CHTR-4-5/24/2005	wild	48	112.28,516.23	109	45,28,29,42	2.3
5 G/2 //2000	******	.5			.5,25,25,72	2.0

Fyneriment	Fish Type	Hour interval	Cortisol (ng/mL)	Glucose (mg/dL)	Hematocrit (%)	Lactate (mmol/L)
NS-1-12/20/2005 cu	Iltured	-0.5	5.94,36.99	34	NaN,28,NaN,31	1.6
NS-1-12/20/2005 cu	ıltured	0	81.86,152.77	59	36,36,NaN,35	<na></na>
NS-1-12/20/2005 cu	ıltured	0.5	127.68,72.17	54	30,NaN,28,NaN	8.1
NS-1-12/20/2005 cu	ıltured	2	212.69,89.36	66	NaN,21,27,33	2
NS-1-12/20/2005 cu	ıltured	24	22.72,45.69	83	33,32,NaN,47	NA
NS-1-12/20/2005 cu	ıltured	48	228.63,72.62	49	25,34,NaN,32	<na></na>
CH-1-12/28/2005	wild	-0.5	18.26,NA	69	NaN,NaN,NaN,NaN	1.3
CH-1-12/28/2005	wild	0	555.1	102	NaN,NaN,NaN	<na></na>
CH-1-12/28/2005	wild	0.5	NA	102	NaN,NaN,NaN	NA
CH-1-12/28/2005	wild	2	NA	117	NaN,NaN,30,NaN	NA
CH-1-12/28/2005	wild	24	222.02	251	NaN,NaN	<na></na>
NS-2-1/4/2006 cu	ıltured	-0.5	33.14,13.16	58	34,32,31,31	1.8
NS-2-1/4/2006 cu	ıltured	0	84.89,128.12	67	36,34,27,NaN	8.1
NS-2-1/4/2006 cu	ıltured	0.5	260.59,224.02	108	31,NaN,NaN,33	1.2
NS-2-1/4/2006 cu	ıltured	2	271.54	74	NaN,NaN,NaN	2.6
NS-2-1/4/2006 cu	ıltured	24	NA	36	<na></na>	7.2
NS-2-1/4/2006 cu	ıltured	48	13.46,35.39	38	44,NaN,39,30	1.1
CH-2-1/9/2006 cu	ıltured	-0.5	3.79,25.63	78	30,31,NaN,34	NA,NA,NA,NA
CH-2-1/9/2006 cu	ıltured	0	65.62,155.6	85	NaN,35,32,38	NA,NA,NA,NA
CH-2-1/9/2006 cu	ıltured	0.5	301.51,397.47	84	33,31,NaN,NaN	NA,NA,NA,NA
CH-2-1/9/2006 cu	ıltured	2	312.17,66.37	<na></na>	28,24,NaN,28	NA,NA,NA,NA
CH-2-1/9/2006 cu	ıltured	24	36.32,7.01	53	33,27,27,32	NA,NA,NA,NA
CH-2-1/9/2006 cu	ıltured	48	10.53,25.02	44	23,NaN,33,32	NA,NA,NA,NA
NS-3-1/11/2006 cu	ıltured	-0.5	87.06,61.01	67	29,34,26,29	NA,NA,NA,NA
NS-3-1/11/2006 cu	ıltured	0	218.83,151.88	83	30,34,36,34	NA,NA,NA,NA
NS-3-1/11/2006 cu	ıltured	0.5	371.8,148.54	40	26,31,34,29	NA,NA,NA,NA
NS-3-1/11/2006 cu	ıltured	2	317.17,146.4	56	33,30,24,34	NA,NA,NA,NA
NS-3-1/11/2006 cu	ıltured	24	67.59,44.97	32	29,38,25,26	NA,NA,NA,NA
NS-3-1/11/2006 cu	ıltured	48	NA,70.9	67	NaN,NaN,17,35	NA,NA,NA,NA
CH-4-1/17/2006	wild	-0.5	4.82,22.43	106	26,30,25,NaN	NA,NA,NA,NA
CH-4-1/17/2006	wild	0	460.14,247.82	124	NaN,NaN,27,NaN	NA,NA,NA,NA
CH-4-1/17/2006	wild	0.5	NA,488.2	81	NaN,NaN,NaN,NaN	NA,NA,NA,NA
CH-4-1/17/2006	wild	2	604.35,392.12	130	30,NaN,NaN,31	NA,NA,NA,NA
CH-4-1/17/2006	wild	24	NA,214.16	71	NaN,NaN,NaN,31	NA,NA,NA,NA
CH-4-1/17/2006	wild	48	53.37,51.75	62	NaN,NaN,NaN,23	NA,NA,NA,NA
NS-4-1/18/2006	wild	-0.5	16.63,4.25	93	NaN,25,NaN,NaN	NA,NA,NA,NA
NS-4-1/18/2006	wild	0	243.75,NA	79	NaN,27,23,NaN	NA,NA,NA,NA
NS-4-1/18/2006	wild	0.5	NA,567.85	80	NaN,NaN,NaN,34	NA,NA,NA,NA
NS-4-1/18/2006	wild	2	82.82,NA	99	NaN,NaN,NaN,NaN	NA,NA,NA,NA
NS-4-1/18/2006	wild	24	315.82	88	31,NaN,NaN,NaN	NA,NA,NA,NA
NS-4-1/18/2006	wild	48	307.94	93	NaN,NaN,NaN,27	NA,NA,NA,NA
NS-5-1/24/2006 cu	ıltured	-0.5	50.7,63.76	69	24,27,26,30	NA,NA,NA,NA
NS-5-1/24/2006 cu	ıltured	0	134.14,79.12	57	32,38,34,38	NA,NA,NA,NA
NS-5-1/24/2006 cu	ıltured	0.5	357.99	71	36,37,27	NA,NA,NA,NA

Experiment	Fish Type	Hour interval	Cortisol (ng/mL)	Glucose (mg/dL)	Hematocrit (%)	Lactate (mmol/L)
NS-5-1/24/2006	cultured	2	335.2,302.91	69	27,36,23,NaN	NA,NA,NA,NA
NS-5-1/24/2006	cultured	24	15.89,13.31	44	30,26,28,NaN	1.2
NS-5-1/24/2006	cultured	48	156.78,354.63	56	30,NaN,25,NaN	2.4
TR-1-1/25/2006	cultured	-0.5	26.41,15.04	55	28,25,25,23	1.6
TR-1-1/25/2006	cultured	0	216.81,232.89	44	25,31,26,26	<na></na>
TR-1-1/25/2006	cultured	0.5	297.08,327.17	87	30,27,32	12.5
TR-1-1/25/2006	cultured	2	448.15,598.52	43	NaN,29,39,27	14.7
TR-1-1/25/2006	cultured	24	96.25,35.43	56	34,30,NaN,31	3.9
TR-1-1/25/2006	cultured	48	143.64,236.86	102	30,32,NaN,25	NA
TR-2-1/25/2006	wild	-0.5	7.97,NA	105	NaN,NaN,NaN,27	NA
TR-2-1/25/2006	wild	0	2.6,277.62	157	22,NaN,22,NaN	14.7
TR-2-1/25/2006	wild	0.5	533.61,NA	89	NaN,29,NaN,NaN	15
TR-2-1/25/2006	wild	2	845.8	121	25,NaN	<na></na>
TR-2-1/25/2006	wild	24	95.24	93	NaN,NaN,NaN	NA
TR-2-1/25/2006	wild	48	67.35,78.88	60	NaN,NaN,NaN,NaN	3.4
TR-3-1/30/2006	wild	-0.5	6.02,26.22	69	28,27,NaN,NaN	3.6
TR-3-1/30/2006	wild	0	2.25,501.97	135	30,23,NaN,26	13.6
TR-3-1/30/2006	wild	0.5	261.07,107.9	105	NaN,NaN,NaN,NaN	14.7
TR-3-1/30/2006	wild	2	357.32	129	NaN,NaN,NaN	10.1
TR-3-1/30/2006	wild	24	443.75,NA	158	31,NaN,28,32	1
TR-3-1/30/2006	wild	48	670.36,696.41	163	NaN,26,26,29	<na></na>
TR-4-1/31/2006	cultured	-0.5	49.34,41.57	67	32,31,27,31	2.7
TR-4-1/31/2006	cultured	0	440.78,225.44	97	31,25,32,26	NA
TR-4-1/31/2006	cultured	0.5	245.44,502.77	78	29,29,30,38	14.8
TR-4-1/31/2006	cultured	2	NA,272.55	74	30,32,29,NaN	8.7
TR-4-1/31/2006	cultured	24	244.95,184.26	64	NaN,30,28	3.2
TR-4-1/31/2006	cultured	48	29.37	47	33,NaN,35	<na></na>
NS-6-2/1/2006	wild	-0.5	33.63,126.32	67	30,NaN,28,35	3.8
NS-6-2/1/2006	wild	0	323.77,NA	98	NaN,NaN,NaN,30	5.6
NS-6-2/1/2006	wild	0.5	430.09,405.36	113	31,NaN,33,32	6
NS-6-2/1/2006	wild	2	583.44	157	30,31,NaN,30	<na></na>
NS-6-2/1/2006	wild	24	166.59,98.85	130	30,NaN,34,NaN	2.3
NS-6-2/1/2006	wild	48	158.7	48	30,NaN,NaN,NaN	2.6
TR-5-2/1/2006	wild	-0.5	121.3,692.07	99	NaN,26,NaN,25	NA
TR-5-2/1/2006	wild	0	463.79	91	NaN,NaN,NaN,NaN	4.6
TR-5-2/1/2006	wild	0.5	624.86,NA	98	NaN,29,31,NaN	<na></na>
TR-5-2/1/2006	wild	2	481.09,NA	140	29,NaN,30,NaN	16.8
TR-5-2/1/2006	wild	24	88.72	74	NaN,NaN,NaN,NaN	2
TR-5-2/1/2006	wild	48	36.72,22.91	30	NaN,NaN,NaN,26	NA
CHTR-1-2/6/2006	cultured	-0.5	22.32,8.7	36	30,29,30,32	2.6
CHTR-1-2/6/2006	cultured	0	206.98,217.94	65	32,29,30,28	16.1
CHTR-1-2/6/2006	cultured	0.5	423.42,373.33	120	38,28,26,26	16.7
CHTR-1-2/6/2006	cultured	2	301.13,276.57	76	NaN,31,32,27	10.6
CHTR-1-2/6/2006	cultured	24	160.46,288.99	63	30,31,32,22	5.8

Experiment	Fish Type	Hour interval	Cortisol (ng/mL)	Glucose (mg/dL)	Hematocrit (%)	Lactate (mmol/L)
CHTR-1-2/6/2006	cultured	48	205.73,307.92	122	36,30,31	9.1
CHTR-2-2/7/2006	wild	-0.5	4.08,24.32	64	29,28,NaN,NaN	2.6
CHTR-2-2/7/2006	wild	0	292.97	96	NaN,NaN,27,25	NA
CHTR-2-2/7/2006	wild	0.5	392.68,313.89	140	NaN,NaN,27,25	1
CHTR-2-2/7/2006	wild	2	435.37	165	NaN,NaN,NaN	2.4
CHTR-2-2/7/2006	wild	24	NA	<na></na>	NaN,NaN,NaN	<na></na>
CHTR-2-2/7/2006	wild	48	97.99	54	NaN,NaN,30	<na></na>
NS-7-2/7/2006	cultured	-0.5	35.17,3.93	94	38,38,38,32	NA
NS-7-2/7/2006	cultured	0	71.11,198.41	75	NaN,27,NaN,30	3.6
NS-7-2/7/2006	cultured	0.5	211.35,343.7	68	NaN,44,34,NaN	4.6
NS-7-2/7/2006	cultured	2	356.36,342.57	64	35,34,NaN,32	3.1
NS-7-2/7/2006	cultured	24	26.01,12.15	49	35,35,32,33	NA
NS-7-2/7/2006	cultured	48	188.71,472.12	154	NaN,30,29,27	1.8
TR-6-2/8/2006	wild	-0.5	5.36,12.91	82	30,30,26,22	1.4
TR-6-2/8/2006	wild	0	394.52,339.07	98	NaN,28,20,29	11.5
TR-6-2/8/2006	wild	0.5	362.1,424.89	101	29,NaN,NaN,37	10
TR-6-2/8/2006	wild	2	171.18	150	NaN,NaN,NaN	5.7
TR-6-2/8/2006	wild	24	173.98,164.41	63	27,NaN,32,28	3.4
TR-6-2/8/2006	wild	48	51.11,52.78	48	NaN,34,NaN,32	NA
NS-8-2/14/2006	cultured	-0.5	9.61	77	27,NaN,NaN,NaN	0.8
NS-8-2/14/2006	cultured	0	364.45,152.99	77	33,32,NaN,33	3
NS-8-2/14/2006	cultured	0.5	379.26,175.18	65	28,NaN,31,31,27 NaN,NaN,NaN,28,2	NA
NS-8-2/14/2006	cultured	2	144.53,378.4,NA	78	7	5
NS-8-2/14/2006	cultured	24	126.82,63.19	52	26,NaN,24,32,28	NA
			,,		NaN,NaN,NaN,NaN,	
NS-8-2/14/2006	cultured	48	NA	50	NaN	4.5
TR-8-2/14/2006	cultured	-0.5	31.84,NA	74	36,47,27,38	NA
TR-8-2/14/2006	cultured	0	409.48,196.64	72	32,30,30,28	16.3
TR-8-2/14/2006	cultured	0.5	131.35,187.84	70	NaN,36,32,35	16.7
TR-8-2/14/2006	cultured	2	173.6,216.98	67	32,28,32,28	15.7
TR-8-2/14/2006	cultured	24	NA,59.34	89	34,26,27,31	4.6
TR-8-2/14/2006	cultured	48	NA,149.57	33	27,NaN,28,NaN	1.9
CHTR-3-2/15/2006	cultured	-0.5	40.87,NA	41	24,31,30,31	2.5
CHTR-3-2/15/2006	cultured	0	138.19,25.28	56	26,28,22,28	6
CHTR-3-2/15/2006	cultured	0.5	NA,140.27	97	26,28,NaN,30	4.1
CHTR-3-2/15/2006	cultured	2	85.02,94.51	36	35,26,29,27	13.1
CHTR-3-2/15/2006	cultured	24	12.64,67.02	36	25,33,31,26	NA
CHTR-3-2/15/2006	cultured	48	NA,414.45	129	NaN,NaN,26,NaN	NA
CH-5-2/21/2006	wild	-0.5	27.96,28.76	63	24,27,33,NaN	1.9
CH-5-2/21/2006	wild	0	172.55,223.12	64	37,34,32,NaN	<na></na>
CH-5-2/21/2006	wild	0.5	327.85,431.84	79	36,NaN,36,33	4.1
CH-5-2/21/2006	wild	2	707.38,520.6	86	31,32,32,NaN	2
CH-5-2/21/2006	wild	24	250.77,220.81	50	NaN,28,31,NaN,32	NA

Experiment	Fish Type	Hour interval	Cortisol (ng/mL)	Glucose (mg/dL)	Hematocrit (%)	Lactate (mmol/L)
CH-5-2/21/2006	wild	48	NA,670.73	73	38,NaN,NaN,NaN	NA
CH-6-2/21/2006	cultured	-0.5	50.34,28.01	65	34,28,32,32	1.8
CH-6-2/21/2006	cultured	0	321.83,418.97	78	31,35,33,40	6.1
CH-6-2/21/2006	cultured	0.5	392.63,436.17	88	32,40,NaN,37	8.3
CH-6-2/21/2006	cultured	2	209.97,104.57	100	29,35,33,30,32	7.3
CH-6-2/21/2006	cultured	24	8.46,53.84	55	NaN,30,NaN,22,29	3.6
CH-6-2/21/2006	cultured	48	124.38,139.61	38	30,29,NaN,27	4.5
CH-7-2/22/2006	cultured	-0.5	1.24,80.32	48	31,30,NaN,27	8.0
CH-7-2/22/2006	cultured	0	459.41,301.79	84	NaN,31,30,35	10.5
CH-7-2/22/2006	cultured	0.5	406.99,265.15	55	33,30,38,29	<na></na>
CH-7-2/22/2006	cultured	2	128.05,188.37	96	30,24,31,29	13.7
CH-7-2/22/2006	cultured	24	142.29,77.99	31	30,NaN,NaN,33	0.9
CH-7-2/22/2006	cultured	48	38.38,44.8	24	28,32,33,33	1.2
NS-9-2/22/2006	wild	-0.5	20.06,6.23	63	32,32,28,24	3.2
NS-9-2/22/2006	wild	0	184.15,166.28	64	38,38,30,36	2.4
NS-9-2/22/2006	wild	0.5	364.63,750.13	67	NaN,35,NaN,35	3
NS-9-2/22/2006	wild	2	515.3,271.45	124	34,30,28,NaN	3.9
NS-9-2/22/2006	wild	48	438.74,136.5	54	36,NaN,NaN,30	NA
CHTR-4-2/27/2006	wild	-0.5	11.47,NA	<na></na>	NaN,29,33,31	2.6
CHTR-4-2/27/2006	wild	0	557.71	31	28,NaN,30,28	1.2
CHTR-4-2/27/2006	wild	0.5	667.9,600.68	40	35,NaN,33,NaN	<na></na>
CHTR-4-2/27/2006	wild	2	776.83	52	35,NaN,NaN	<na></na>
CHTR-4-2/27/2006	wild	24	NA,724.73	195	31,36,NaN	<na></na>
NS-10-3/6/2006	cultured	-0.5	174.09,23.86	54	36,34,22,36	NA
NS-10-3/6/2006	cultured	0	415.05,192.6	84	39,34,32,39	6.7
NS-10-3/6/2006	cultured	0.5	236.56,105.4	87	33,32,34,33	12.2
NS-10-3/6/2006	cultured	2	211.69,246.97	89	31,38,NaN,41	14.2
NS-10-3/6/2006	cultured	24	130.97,43.21	47	25,27,NaN,NaN	1.8
NS-10-3/6/2006	cultured	48	NA,67.68	34	31,20,31,NaN	4
CH-8-3/7/2006	wild	-0.5	20.2,NA	43	NaN,34,NaN,30	NA
CH-8-3/7/2006	wild	0	424.21,571.2	65	38,28,34,37	NA
CH-8-3/7/2006	wild	0.5	NA,NA	78	37,34,NaN,NaN	NA
					34,NaN,NaN,NaN,N	
CH-8-3/7/2006	wild	2	641.84	50	aN	<na></na>
CH-8-3/7/2006	wild	24	160.6,137.46	190	23,NaN,NaN,29	NA
CH-8-3/7/2006	wild	48	81.27,130.32	30	30,30,33,37	<na></na>
CHTR-5-3/7/2006	cultured	-0.5	21.04,44.68	61	NaN,30,NaN,31	2.4
CHTR-5-3/7/2006	cultured	0	484.98,400.84	114	NaN,NaN,22,29	NA
CHTR-5-3/7/2006	cultured	0.5	253.26,143.36	62	26,22,30,32	3.3
CHTR-5-3/7/2006	cultured	2	96.21,201.83	59	23,23,28,27	5
CHTR-5-3/7/2006	cultured	24	44.03,13.91	68	24,24,26,NaN	NA
NS-11-3/8/2006	wild	-0.5	2.26,15.26	46	38,34,35,36	1.6
NS-11-3/8/2006	wild	0	NA,232.2	49	NaN,NaN,NaN,36	NA
NS-11-3/8/2006	wild	0.5	523.72,319.08	<na></na>	NaN,NaN,35,36	<na></na>

Experiment	Fish Type	Hour interval	Cortisol (ng/mL)	Glucose (mg/dL)	Hematocrit (%)	Lactate (mmol/L)
NS-11-3/8/2006	wild	2	641.85,224.29	51	34,NaN,30,30	<na></na>
NS-11-3/8/2006	wild	24	181.83,67.54	169	NaN,NaN,33,NaN	NA
NS-11-3/8/2006	wild	48	160.54,40.4	33	NaN,NaN,NaN,33	NA
CH-9-3/13/2006	cultured	-0.5	14.51,42.57	72	25,25,NaN,24	NA
CH-9-3/13/2006	cultured	0	276,252.12	86	27,29,29,32	5.1
CH-9-3/13/2006	cultured	0.5	255.93,220.64	87	26,NaN,27,28	<na></na>
CH-9-3/13/2006	cultured	2	306.4,221.94	121	NaN,31,21,NaN	1.8
CH-9-3/13/2006	cultured	24	238.63,131.84	44	NaN,23,25,25,24	3.1
					27,NaN,NaN,NaN,3	
CH-9-3/13/2006	cultured	48	389.35,131.6	125	2	<na></na>
NS-12-3/14/2006	wild	-0.5	2.97,3.63	35	35,36,35,34	<na></na>
NS-12-3/14/2006	wild	0	453.03,245.43	51	37,42,34,36	3
NS-12-3/14/2006	wild	0.5	459.86,497.94	31	43,NaN,39,NaN	NA
NS-12-3/14/2006	wild	2	170.98,61.07	29	NaN,34,36,NaN	8.6
NS-12-3/14/2006	wild	24	451.48	46	NaN,32,NaN	NA
NS-12-3/14/2006	wild	48	369.43,251.27	43	25,30,30	NA
CH-10-3/15/2006	wild	-0.5	283.69,20.57	32	32,29,40,NaN	2.9
CH-10-3/15/2006	wild	0	190.74,146.1	67	28,38,32,32	3.9
CH-10-3/15/2006	wild	0.5	206.29,431.86	77	36,37,NaN,27	<na></na>
CH-10-3/15/2006	wild	2	483.99	54	37,38	<na></na>
CH-10-3/15/2006	wild	24	112,100.40	58	28,NaN,28,27	<na></na>
CH-10-3/15/2006	wild	48	105.43	63	NaN,34,NaN	1.2
CHTR-6-3/20/2006	cultured	-0.5	23.69,29.63,29.54	61	25,19,NaN,NaN	0.9
CHTR-6-3/20/2006	cultured	0	151.91,113.07	76	20,NaN,22,24	5
CHTR-6-3/20/2006	cultured	0.5	165,282.93	129	24,23,24,25	NA
CHTR-6-3/20/2006	cultured	2	159.74,181.76	68	18,32,28,NaN	3.9
CHTR-6-3/20/2006	cultured	24	NA,152.7	43	27,NaN,28,29	NA
CHTR-6-3/20/2006	cultured	48	436.86,171.73	53	NaN,NaN,36,28	3.2
NS-13-3/21/2006	cultured	-0.5	17.37,68.07	51	26,30,23,21	<na></na>
NS-13-3/21/2006	cultured	0	126.61,298.8	85	27,30,27,27	6.6
NS-13-3/21/2006	cultured	0.5	144.02,342.23	69	31,40,28,32	12.2
NS-13-3/21/2006	cultured	2	436.86,145	94	NaN,24,23,27	2.7
NS-13-3/21/2006	cultured	24	75.91,270.33	104	24,25,28,24	<na></na>
NS-13-3/21/2006	cultured	48	77.55,90.91	79	32,30,NaN,30	NA
CHTR-7-3/22/2006	wild	-0.5	8.26,2.85	32	41,NaN,34,37	<na></na>
CHTR-7-3/22/2006	wild	0	361.62,551.61	71	NaN,NaN,29,29	NA
CHTR-7-3/22/2006	wild	0.5	927.37,754.5	75	34,NaN,32,27	NA,NA,NA,N
CHTR-7-3/22/2006	wild	2	766.96,1210.84	77	NaN,37,33,NaN	<na></na>
CHTR-7-3/22/2006	wild	24	313.85,258.2	143	NaN,29,NaN,41	1.3
CHTR-7-3/22/2006	wild	48	117.88,180.94	50	NaN,40,29	<na></na>
CHTR-8-3/27/2006	cultured	-0.5	NA,NA	276	26,34,NaN,NaN	1.4
CHTR-8-3/27/2006	cultured	0	NA,NA	79	20,21,20,17	10.5
CHTR-8-3/27/2006	cultured	0.5	NA,NA	78	30,29,27,NaN	17.5
CHTR-8-3/27/2006	cultured	2	NA,NA	94	28,25,19,25,36	20.7

Experiment	Fish Type	Hour interval	Cortisol (ng/mL)	Glucose (mg/dL)	Hematocrit (%)	Lactate (mmol/L)
CHTR-8-3/27/2006	cultured	24	NA,NA	250	32,27,24,24,36	1.8
CHTR-8-3/27/2006	cultured	48	NA,NA	257	24,22,33,NaN	NA
NS-14-3/27/2006	wild	-0.5	170.18,8.37	69	32,34,31,30	<na></na>
NS-14-3/27/2006	wild	0	459.14,521.59	55	40,44,39,39	4
NS-14-3/27/2006	wild	0.5	560.55,588.1	60	NaN,NaN,34,42	<na></na>
NS-14-3/27/2006	wild	2	626.11,563.4	57	29,38,NaN,NaN	NA
NS-14-3/27/2006	wild	24	47.85	79	24,24,NaN	<na></na>
NS-14-3/27/2006	wild	48	81.53,52.01	<na></na>	NaN,27,NaN,32	1.6
TR-9-3/27/2006	wild	-0.5	75.55,82.26	60	32,32,NaN,32	<na></na>
TR-9-3/27/2006	wild	0	752.66,649.48	75	32,NaN,NaN,26	8.2
TR-9-3/27/2006	wild	0.5	594.46,559.13	48	30,NaN,25,24	<na></na>
TR-9-3/27/2006	wild	2	757.16	74	37	<na></na>
TR-9-3/27/2006	wild	24	43.24,94.84	57	NaN,34,33,34,NaN	1.5
TR-9-3/27/2006	wild	48	282.9,339.32	196	31,NaN,30,28,30	NA
CH-11-3/28/2006	cultured	-0.5	26.25,21.52	43	32,26,NaN,28	NA
CH-11-3/28/2006	cultured	0	468.68,552.03	51	36,27,NaN,NaN	1.9
CH-11-3/28/2006	cultured	0.5	180.74,492.19	44	22,NaN,34,NaN	2.3
CH-11-3/28/2006	cultured	2	315.26	<na></na>	NaN,NaN,NaN	<na></na>
CH-11-3/28/2006	cultured	24	NA,269.8	<na></na>	NaN,21,NaN,32	NA
CH-11-3/28/2006	cultured	48	NA,158.15	52	33,30,30,NaN	<na></na>
NS-15-4/03/2006	cultured	-0.5	75.26,50.26	24	NaN,26,25,26	<na></na>
NS-15-4/03/2006	cultured	0	297.08,197.44	54	30,37,NaN,33	<na></na>
NS-15-4/03/2006	cultured	0.5	NA,NA	60	NaN,33,NaN,NaN	NA
NS-15-4/03/2006	cultured	2	270.62,232.05	44	24,NaN,23,NaN	5.2
NS-15-4/03/2006	cultured	24	NA,55.51	47	NaN,29,43,33	<na></na>
NS-15-4/03/2006	cultured	48	381.02	121	25,NaN,NaN,NaN	NA
CH-12-4/04/2006	wild	-0.5	143.37,10.37	35	29,39,33,34	<na></na>
CH-12-4/04/2006	wild	0	437.13,468.68	<na></na>	33,42,29,NaN	3.9
CH-12-4/04/2006	wild	0.5	NA,484.18	72	NaN,NaN,34,34	5.2
CH-12-4/04/2006	wild	2	NA,215.15	16	NaN,NaN,33,39,31	2.3
CH-12-4/04/2006	wild	24	23.13,208.61	113	34,30,NaN,29,30	2.3
CH-12-4/04/2006	wild	48	59.41	35	25,NaN,NaN	<na></na>
CH-13-4/05/2006	cultured	-0.5	18,91.79	53	36,33,35,NaN	<na></na>
CH-13-4/05/2006	cultured	0	202.33,574.15	<na></na>	NaN,NaN,28,NaN	<na></na>
CH-13-4/05/2006	cultured	0.5	362.81	126	27,NaN,NaN,25	NA
CH-13-4/05/2006	cultured	2	600.12,174.84	102	NaN,33,27,31	15.2
CH-13-4/05/2006	cultured	24	35.47,27.98	45	28,32,NaN,NaN,36	NA
CH-13-4/05/2006	cultured	48	NA,115.38	46	NaN,28,19,NaN,34	1.7
CH-14-4/10/2006	wild	-0.5	38.51,78.32	54	34,28,NaN,35	1.6
CH-14-4/10/2006	wild	0	220.56,314.58	77	43,40,33,NaN	NA
CH-14-4/10/2006	wild	0.5	303.61,318.62	64	35,NaN,NaN,32	6.2
CH-14-4/10/2006	wild	2	450.51	130	41,30,NaN	2.4
CH-14-4/10/2006	wild	24	362.85	160	25,NaN,28	<na></na>
	wild	48	336.31,522.78	151	36,NaN,28,36	NA

Experiment	Fish Type	Hour interval	Cortisol (ng/mL)	Glucose (mg/dL)	Hematocrit (%)	Lactate (mmol/L)
NS-16-4/10/2006	cultured	-0.5	49.29,40.23	42	25,30,30,34	<na></na>
NS-16-4/10/2006	cultured	0	206.95,308.43	69	39,28,NaN,33	4
NS-16-4/10/2006	cultured	0.5	92.09,198.4	80	31,37,30,NaN	<na></na>
NS-16-4/10/2006	cultured	2	221.2,NA	98	NaN,24,NaN,NaN	<na></na>
NS-16-4/10/2006	cultured	24	210.73,53.38	58	NaN,NaN,30,NaN	8.0
NS-16-4/10/2006	cultured	48	67.15	112	NaN,23,NaN,NaN	1.7
CH-15-4/11/2006	cultured	-0.5	68.25,317.76	71	33,35,35	NA
CH-15-4/11/2006	cultured	0	371.68,230.85	75	37,NaN,32,34	4.2
CH-15-4/11/2006	cultured	0.5	179.94,41.39	49	33,33,38,21	17.7
CH-15-4/11/2006	cultured	2	595.67,145.67	67	28,NaN,23,32,28	3.4
CH-15-4/11/2006	cultured	24	63.73,68.33	83	NaN,25,30,28,30	0.8
CH-15-4/11/2006	cultured	48	3.98,NA	43	35,31,34,37,39	3
ΓR-10-4/12/2006	cultured	-0.5	15.07,142.13	67	21,NaN,31,36	NA
TR-10-4/12/2006	cultured	0	123.13,204.56	65	NaN,26,24,22	18.7
TR-10-4/12/2006	cultured	0.5	249.78,72.17	86	30,28,27,23	8.2
TR-10-4/12/2006	cultured	2	359.53,340.08	15	33,32,28,27	3.7
ΓR-10-4/12/2006	cultured	24	142.46,306.59	120	NaN,NaN,27,35	<na></na>
ΓR-10-4/12/2006	cultured	48	270.78,279.2	218	NaN,NaN,29,NaN	NA
CHTR-9-4/24/2006	wild	-0.5	50.23,152.65	64	26,30,22,NaN	2.6
CHTR-9-4/24/2006	wild	0	271.48,231.98	83	30,33,23,37	10.8
CHTR-9-4/24/2006	wild	0.5	379.95,314.36	87	28,30,37	10.5
CHTR-9-4/24/2006	wild	2	217.29,238.87	89	NaN,24,32,32,38	6.1
CHTR-9-4/24/2006	wild	24	258.58,206.86,349.1	247	NaN,33,NaN,39,42	3.1
CHTR-9-4/24/2006	wild	48	170.69,218.8	71	40,42,NaN,38,NaN	NA
ΓR-11-4/25/2006	wild	-0.5	50.13,55.52	60	33,32,33,30	NA
TR-11-4/25/2006	wild	0	323.56,185.26	94	22,24,34,NaN	2.2
ΓR-11-4/25/2006	wild	0.5	153.2,130.07	98	38,34,33,34	5.9
ΓR-11-4/25/2006	wild	24	89.85,270.41	58	23,28,29,29	NA
ΓR-11-4/25/2006	wild	48	968.01,189.26	276	32,33,41,NaN	1.2
TR-12-4/25/2006	cultured	-0.5	126.41,64.43	67	38,24,22,27	4.3
ΓR-12-4/25/2006	cultured	0	186.78,170.76	146	28,25,21,20	20.2
ΓR-12-4/25/2006	cultured	0.5	182.94,278.9	107	30,29,28,34	21.3
TR-12-4/25/2006	cultured	2	156.2,191.54	105	30,31,NaN,NaN	NA
ΓR-12-4/25/2006	cultured	24	174.11,41.41	128	38,35,32,38	1.8
TR-12-4/25/2006	cultured	48	516.76,165.24	102	43,30,26,28	2.3
NS-17-4/26/2006	wild	-0.5	101.47,74.37	58	35,40,37,33	2.3
NS-17-4/26/2006	wild	0.5	434.23,485.8	108	33,NaN,NaN,38	0.9
NS-17-4/26/2006	wild	0.5	235.48,480.92	80	30,39,33,32	8.5
NS-17-4/26/2006	wild	2	393.43,117.25	94	29,40,40,29	6.5
NS-17-4/26/2006 NS-17-4/26/2006	wild	24	327.7,375.24	43	39,31,32,40	NA
NS-17-4/26/2006 NS-17-4/26/2006	wild	48	1400.67,710.29	43 146	39,31,32,40 31,NaN,32,25	2.5
CHTR-10-5/1/2006	cultured	-0.5	61.52,522.77	89		2.5 4.8
			•		25,32,26,33	
CHTR-10-5/1/2006	cultured	0	220.77,119.85	100	NaN,32,30,31	6.1
CHTR-10-5/1/2006	cultured	0.5	288.34,382.06	89	29,24,28,34	NA

Experiment	Fish Type	Hour interval	Cortisol (ng/mL)	Glucose (mg/dL)	Hematocrit (%)	Lactate (mmol/L)
	-		-		NaN,23,NaN,NaN,3	
CHTR-10-5/1/2006	cultured	2	850.83,705.59	118	4	1.3
CHTR-10-5/1/2006	cultured	24	344.34	70	14,NaN,34,33,36	6.6
CHTR-10-5/1/2006	cultured	48	543.34,795.73	87	26,28,14	<na></na>
CHTR-11-5/1/2006	wild	-0.5	64.31,33.23	101	33,44,42,35	4.4
CHTR-11-5/1/2006	wild	0	297.8,316.98	143	NaN,25,39,NaN	4.5
CHTR-11-5/1/2006	wild	0.5	395.69,182.6	76	42,28,33,42	NA
CHTR-11-5/1/2006	wild	2	382.17,496.25	250	29,34,35,33,41	15.4
CHTR-11-5/1/2006	wild	24	559.39,718.68	216	25,45,36,42,42	NA
CHTR-11-5/1/2006	wild	48	463.31,178.58	53	NaN,27,46,24,37	NA
CHTR-12-5/2/2006	cultured	-0.5	46.23,37.91	81	28,33,31,27	1.8
CHTR-12-5/2/2006	cultured	0	262.46,345.93	97	37,30,33,NaN	9.3
CHTR-12-5/2/2006	cultured	0.5	149.1,328.32	113	NaN,NaN,40,30	NA
CHTR-12-5/2/2006	cultured	2	390.05,222.87	115	32,30,39,27	3.5
CHTR-12-5/2/2006	cultured	24	417.64,308.3	326	26,37,NaN,26	3.2
CHTR-12-5/2/2006	cultured	48	211.32,474.08	78	28,32,34,36	2.1
TR-13-5/2/2006	wild	-0.5	89.44,142.18	74	30,34,43,40	1.4
TR-13-5/2/2006	wild	0	211.64,389.04	76	27,NaN,33,26	2.1
TR-13-5/2/2006	wild	0.5	423.66,163.91	71	31,43,34,34	10.8
TR-13-5/2/2006	wild	2	212.54,867.13	117	30,36,30,NaN	3
TR-13-5/2/2006	wild	24	131.08,693.1	412	33,NaN,NaN,39	2.8
TR-13-5/2/2006	wild	48	661.83,693.75	201	28,37,30,45	NA
NS-18-5/3/2006	cultured	-0.5	23.79,44.14	42	36,31,30,39	<na></na>
NS-18-5/3/2006	cultured	0	365.98,282.25	86	NaN,38,44,43	NA
NS-18-5/3/2006	cultured	0.5	364.97,396.06	57	31,36,28	11.2
NS-18-5/3/2006	cultured	2	290.42,399.47	58	34,40,35,33	<na></na>
NS-18-5/3/2006	cultured	24	191.18,472.74	54	24,NaN,27,32	1.6
NS-18-5/3/2006	cultured	48	492.01	437	NaN,32,NaN	3.9
TR-14-5/3/2006	cultured	-0.5	229.68,227.21	59	25,24,26,26	1.9
TR-14-5/3/2006	cultured	0	315.96,571.69	103	22,36,30,26	7.2
TR-14-5/3/2006	cultured	0.5	136.4,268.68	113	27,30,28,36	14.7
TR-14-5/3/2006	cultured	2	191.98,270.64	63	28,25,34,29	17.8
TR-14-5/3/2006	cultured	24	372.9,84.89	54	30,35,NaN,36	1.2
TR-14-5/3/2006	cultured	48	467.31	167	26,21	3.1
CHTR-13-5/8/2006	wild	-0.5	91.89,300.47	71	32,38,33,38	2.2
CHTR-13-5/8/2006	wild	0	346.33,211.1	89	31,NaN,31,24	4.3
CHTR-13-5/8/2006	wild	0.5	589.02,351.11	104	29,40,39,31	11.2
CHTR-13-5/8/2006	wild	2	480.03,613.33	203	39,35,32,42,31	8.2
CHTR-13-5/8/2006	wild	24	1063.36,926.04	336	29,33,28,23	1.7
CHTR-13-5/8/2006	wild	48	1138.55,522.59	301	NaN,34,NaN,24	3
TR-15-5/8/2006	wild	-0.5	58.02,155.74	78	30,46,33,28	NA
TR-15-5/8/2006	wild	0	242.53,467.18	102	23,33,28,28	NA
TR-15-5/8/2006	wild	0.5	242.53,555.62	112	28,36,35,NaN	NA
TR-15-5/8/2006	wild	2	108.7,134.92	156	28,39,33,38	NA

Experiment	Fish Type	Hour interval	Cortisol (ng/mL)	Glucose (mg/dL)	Hematocrit (%)	Lactate (mmol/L)
TR-15-5/8/2006	wild	24	1072.44,432.11	151	47,33,43,34	NA
TR-15-5/8/2006	wild	48	541.36	251	36,NaN	<na></na>
CHTR-14-5/9/2006	cultured	-0.5	122.2,40.12	55	NaN,32,36,NaN	1.7
CHTR-14-5/9/2006	cultured	0	281.76,273.84	104	29,30,36,29,24	21.6
CHTR-14-5/9/2006	cultured	0.5	277.41,282.49	89	30,38,31,32,33	4.5
CHTR-14-5/9/2006	cultured	2	173.8,507.03,252.31	92	30,30,21,38,22	NA
CHTR-14-5/9/2006	cultured	24	395.06,108.36	274	29,41,21	7.5
CHTR-14-5/9/2006	cultured	48	283.66,200.77	109	34,34,25,30	4.5
CHTR-15-5/9/2006	cultured	-0.5	9.11,110.17	55	20,36,40,29	<na></na>
CHTR-15-5/9/2006	cultured	0	373.2,300.06	117	27,33,30,32	12
CHTR-15-5/9/2006	cultured	0.5	84.34,209.35	100	40,30,40,36	12.2
CHTR-15-5/9/2006	cultured	2	605.05,228.22	134	33,31,33	15.7
CHTR-15-5/9/2006	cultured	24	256.07,118.44	70	35,37,33	2.4
CHTR-15-5/9/2006	cultured	48	308.61	233	32,15	4.1
NS-19-5/10/2006	wild	-0.5	202.09,164.84	57	30,34,24,36	2.2
NS-19-5/10/2006	wild	0	873.4,478.76	78	39,NaN,50,36	4.5
NS-19-5/10/2006	wild	0.5	553.81,450.43	85	33,42,43,34	3.4
NS-19-5/10/2006	wild	2	147.52,425.79	159	43,33,NaN,40	1.6
NS-19-5/10/2006	wild	24	1082.3,1196.28	364	37,35,36,32	2.7
NS-19-5/10/2006	wild	48	1031.55	140	35	NA
TR-7-5/15/2006	wild	-0.5	249.23,477.64	78	40,33,31,40	2.1
TR-7-5/15/2006	wild	0	555.45,404.94	100	32,34,35,44	15.2
TR-7-5/15/2006	wild	0.5	122.85,357.76	106	31,41,30,24	6.2
TR-7-5/15/2006	wild	2	796.9,315.89	111	34,35,33,34	6.6
TR-7-5/15/2006	wild	24	887.41,727.62	227	36,NaN,29	2.4
TR-7-5/15/2006	wild	48	361.86,646.06	179	27,26,33	1.1