STRESS RESPONSE OF RAINBOW TROUT SUBJECTED TO ELEVATED AND NEAR LETHAL TEMPERATURES

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ABSTRACT

This study evaluated the short-term stress response of rainbow trout *Oncorhynchus mykiss* to rapid increases in water temperature. Trout acclimated to 11 °C water were subjected to water temperatures of 16, 22, and 25 °C, with blood glucose, hematocrit, and organosomatic indices recorded from 4 to 120 hours post-transfer. Glucose concentrations were significantly lower at 4 and 48 hours in the trout subjected to 16 °C compared to those at 22 °C and 25 °C. At 120 hours, glucose levels were not significantly different among the treatments and had returned approximately to basal levels. Hematocrit levels did not differ significantly over the course of the experiment. However, at 120 hours, hematocrit levels were significantly different between the fish subjected to 22 and 25 °C but were not significantly different than hematocrit in the fish at 16 °C. Hepatosomatic index was significantly higher in the fish in 25 °C water compared to those in water at either 16 °C or 22 °C. Viscerosomatic and splenosomatic indices were not significantly different in the fish among any of the temperatures. The temperature stress applied to the fish in this experiment was continual; water temperatures were not decreased. Thus, the return of glucose levels back to basal levels with the fish still subjected to elevated water temperatures suggests that the trout are habituating to the stress of living in elevated water temperatures, even at the near lethal temperature of 25 °C.

Keywords:
Rainbow trout, *Oncorhynchus mykiss*, temperature, stress, glucose, habituation

INTRODUCTION

Rapid increases in water temperature have been thought to be detrimental to salmonids and other fish species (Piper et al. 1982; Noga 2000; Hartman and Preston 2001; Timmons et al. 2002; Harmon 2009; Wynne and Wurts 2011). However, Wedemeyer (1996) reported that salmonids can successfully manage a

10 °C temperature change. In addition, Smith and Hubert (2003) and Huysman et al. (2020) reported no difference in survival for rainbow trout *Oncorhynchus mykiss* subjected to water temperature increases from 8 °C to 24 °C, and 11 to 22 °C respectively.

While rapid increases to water temperatures below lethal limits likely do not affect trout survival, the effects of such temperature changes on short-term stress responses are unknown. The potential effects of water temperature changes on long-term stress and possible habituation of the fish to such temperatures is also unknown. Jentoft et al. (2005) suggested that Eurasian perch *Perca fluviatilis* and rainbow trout could become habituated over time from repeated chronic handling stressors. Likewise, Basrur et al. (2010) suggested that Atlantic salmon *Salmo salar* could become habituated to weekly crowding stressors. Thus, it is possible that trout could become habituated to elevated temperatures, as indicated by a return to pre-elevated temperature stress levels.

The objective of this study was to document the short-term stress response of rainbow trout subjected to rapid and sustained increases in water temperature.

METHODS

This experiment was performed at McNenny State Fish Hatchery (Spearfish, South Dakota) using de-gassed and aerated well water (11 °C; total hardness 360 mg/L $CaCO_3$; alkalinity as $CaCO_3$, 210 mg/L; pH 7.6, total dissolved solids 390 mg/L). Twelve 190-L semi-square tanks were used, with each tank outfitted with a submersible recirculating pump (Pondmaster, Kissimmee, Florida) attached to a spray bar to maintain dissolved oxygen levels at or near saturation. Temperatures in each tank were maintained (±1 °C) using a submersible heater (Hydor, Bassano del Grappa, Italy) attached to a temperature controller (Finnex, Chicago, Illinois). The water temperature in each of the twelve tanks was 16, 22, and 25 °C, with four tanks per water temperature treatment.

Juvenile Shasta strain rainbow trout (total length 183.2 ± 2.6 mm; total weight 67 ± 3.2 g) from a common pool in water temperature of 11.2 °C were used in this study. At the start of the experiment, three trout were euthanized with 200 mg/L tricaine methanesulfonate (Tricaine-SMS-222, Syndel, Ferndale, Washington) to establish basal stress levels (Barton et al. 1980). Blood was collected by severing the caudal fin and squeezing blood from the caudal vein. Glucose was determined using an over-the-counter blood glucose monitor (Accu-Chek Aviva Plus; Roche Diabetic Care; Indianapolis, Indiana), and hematocrit was measured by collecting blood into heparinized microhematocrit capillary tubes (Fisher Scientific, Pittsburg, Pennsylvania) and centrifuging the tubes for 10 min at 11,500 rpm. In addition, liver, viscera, and spleen weights were measured to the nearest 0.0001 g.

Immediately after initial data collection, four trout were then placed into each of the twelve experimental tanks and subjected to one of the three elevated water temperature treatments. At 4, 6, 48, and 120 hours after placement in the elevated temperature tanks, one fish from each tank was euthanized. Blood samples

were again collected, with glucose and hematocrit measured. In addition, at 120 hours (end of trial), liver, viscera, and spleen weights were also recorded.

The following formulas were used:

Hepatosomatic index (HSI) = [liver weight (g) /total weight (g)] * 100
Viscerosomatic Index (VSI) = [viscera weight (g) / total weight (g)] * 100
Splenosomatic Index (SSI) = [spleen weight (g) / total weight (g)] * 100

All data were analyzed using the SPSS statistical analysis computer program (Version 24.0; IBM; Chicago, Illinois, USA) with significance pre-determined at $P < 0.05$. Glucose and hematocrit data over the course of the trial were analyzed using Repeated Levels Analysis of Variance along with a Bonferroni post-hoc test. Glucose and hematocrit data at each sampling point, and HSI, VSI, and SSI data, were analyzed using one-way Analysis of Variance and Tukey post-hoc means tests.

RESULTS

No trout died from experimental conditions over the course of the trial. Glucose concentrations were significantly lower ($F_{2, 6} = 31.51$, $P = 0.001$) at 4 and 48 hours in the 16 °C treatment compared to the 22 °C and 25 °C treatments (Figure 1). At 120 hours, glucose levels were not significantly different among the treatments and had returned approximately to basal levels.

Hematocrit levels did not differ significantly over the course of the experiment ($F_{2,6} = 4.29$, $P = 0.07$). However, at 120 hours, hematocrit levels were significantly different between the 22 and 25 °C treatments ($P = 0.022$), but neither were significantly different than hematocrit in the 16 °C treatment (Figure 2). The hepatosomatic index was significantly higher in the 25 °C treatment compared to both the 16 °C and 22 °C treatments ($P = 0.001$; Table 1). There were no significant differences in the viscerosomatic or splenosomatic indices among the treatments.

DISCUSSION

The elevated glucose response in trout subjected to the largest sudden temperature increase was not unexpected. Glucose levels, as secondary stress indicators (Sopinka et al. 2016), should increase in response to the stress of highly elevated water temperatures (Elliott 1981; Thompson et al. 2008; López-Luna et al. 2016; Kim et al. 2019). The glucose response to elevated temperature stress observed in this study is similar to that reported in response to different stress events such as crowding (Biron and Benfey 1994; Wagner and Driscoll 1994; Iwama et al. 1995; Benfey and Biron 2000), handling (Barton et al. 1980; Barton et al. 1986; Vijayan and Moon 1992; Wells and Pankhurst 1999; Jentoft et al. 2005; Fazio et al. 2015), chasing (Barton et al. 1987; Ball and Weber 2017), transporting (Barton 2000), or starving (López-Luna et al. 2016).

The increase in glucose at four hours observed in this study, along with the gradual decrease thereafter, supports the observations of Mazaeud et al. (1977) and Pickering et al. (1982) that the secondary glucose response peaks from three to ten hours after a stressful event. The relatively small rise in glucose at four hours and the quick return to basal levels in the 16 °C treatment suggests the stress was a result of handling the fish and may not have been due to the slight increase in water temperature. The return of glucose in the 25 °C treatment to near basal levels at 120 hours was unexpected, given that Huysman et al. (2020) documented that 26 °C was lethal for the same strain of rainbow trout.

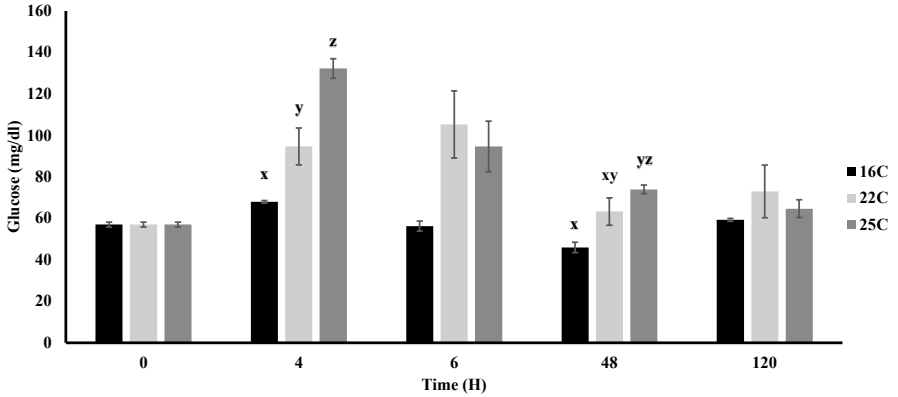

Figure 1. Mean (± se) blood glucose (mg/dl) levels at the start of trial (0 hour) and at 4, 6, 48, and 120 hours for rainbow trout directly transferred from a water temperature of 11 °C to 16, 22, and 25 °C. Letters represent significant differences (P = 0.05) among treatments at individual timepoints.

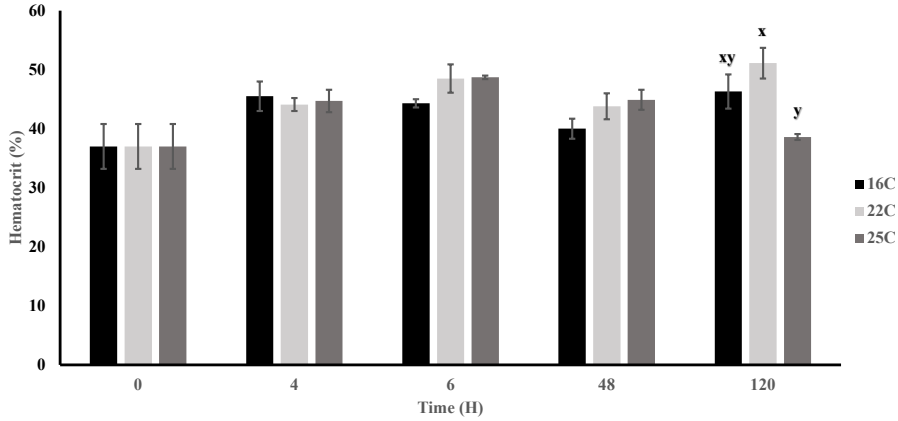

Figure 2. Mean (± se) hematocrit (%) levels at the start of (0 hour) and at 4, 6, 48, and 120 hours for rainbow trout directly transferred from a water temperature of 11 °C to 16, 22, and 25 °C. Letters represent significant differences (P = 0.05) among treatments at individual timepoints.

The temperature stress applied to the fish in this experiment was continual; water temperatures were not decreased. Thus, the return of glucose levels back to basal levels with the fish still subjected to elevated water temperatures suggests that the trout are habituating to the stress of living in elevated water temperatures, even at the near lethal temperature of 25 °C (Martínez-Porchas et al. 2009). These results support the suggestions of Jentoft et al. (2005) and Basrur et al. (2010) that salmonids could become habituated to repeated handling and crowding stressors respectively.

The blood glucose values obtained in this study were within the range previously reported (Iwama et al. 1995; Wells and Pankhurst 1999; Beecham et al. 2006; Stoot et al. 2014; Ball and Weber 2017). However, these values should not be considered as definitive. Portable meters are a validated technique to obtain relative data but may not accurately record absolute glucose values (Iwama et al. 1995; Wells and Pankhurst 1999; Beecham et al. 2006; Stoot et al. 2014; Ball and Weber 2017).

Although hematocrit levels in this study did not change over 120 hours, hematocrit levels typically increase following a significant stress event (Biron and Benfey 1994; Benfey and Biron 2000; Sopinka et al. 2016). Hematocrit may increase or decrease in response to stressors, making changes in hematocrit difficult to interpret (Sopinka et al. 2016). It is possible that the decrease in hematocrit in the 25 °C treatment at 120 hours could be due to an elevated immune response from the stress of being at or near lethal temperatures. It is also possible that the changes in hematocrit among the treatments at 120 hours, while statistically significant, may not actually represent any biological differences.

Values lower or higher than normal for the hepatosomatic and viscerosomatic indices indicate that energy allocation to these areas has been affected by the stressor (Kebus et al. 1992). Although the hepatosomatic index was significantly elevated in the 25 °C treatment, this value was still below that reported previously for rainbow trout (Parker and Barnes 2015; López-Luna et al. 2016). In hindsight, the collection of organosomatic data was likely not needed in this study. Organosomatic indices are typically used for longer term stress studies (Barton et al. 2002) and require a very large effect to detect differences (Sopinka et al. 2016). In addition, hepatosomatic and viscerosomatic indices are influenced by food consumption (Daniels and Robinson 1986; Kim and Kaushik 1992; Sakamota and Yone 1978), and the trout were not fed during this study. It should also be noted that food-deprived rainbow trout may be more sensitive to stress events (Vijayan and Moon 1992).

This study lasted only five days, but the results should be applicable longer-term. Huysman et al. (2020) showed that rainbow trout transferred from 11 °C water temperatures could survive up to 14 days at 25 °C. However, neither this study, nor the others examining trout survival at elevated water temperatures (Wedemeyer 1996; Smith and Hubert 2003; Huysman et al. 2020) examined behavioral changes at higher water temperatures. The trout were not required to expend any energy foraging, avoiding predation, or finding suitable habitat.

In conclusion, this study documents the initial stress response and likely habituation of rainbow trout subjected to immediate and sustained elevated water

temperatures. Additional research is needed to determine the long and short-term effects of elevated temperatures on trout survival, growth, behavior, and reproductive performance.

Table 1. Mean (± SE) hepatosomatic index (HSI)[a], viscerosomatic index (VSI)[b], and splenosomatic index (SSI)[c] values for rainbow trout directly transferred from a water temperature of 11 °C to 16, 22, and 25 °C at the start and end of trial. Means in a row followed by different letters are significantly different (P = 0.05).

		Temperature (°C)		
	Start (11 °C)	**16**	**22**	**25**
HSI	1.05 ± 0.05x	0.85 ± 0.03x	0.97 ± 0.07x	1.28 ± 0.02y
VSI	9.50 ± 0.42x	6.06 ± 0.32y	5.97 ± 0.10y	6.42 ± 0.30y
SSI	0.10 ± 0.03	0.18 ± 0.06	0.12 ± 0.02	0.19 ± 0.01

[a] HSI = [liver weight (mg) /total weight (g)] * 100
[b] VSI = [viscera weight (mg) / total weight (g)] * 100
[c] SSI = [spleen weight (mg) / total weight (g)] * 100

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