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Thermal Tempering Does Not Impact Rainbow Trout and Brown Trout Survival

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Abstract

Hatchery-reared fish are frequently adjusted (tempered) to the higher water temperatures present in the water bodies where they are to be stocked. This study was undertaken to determine the necessity of such tempering practices. This study used rainbow trout (Oncorhynchus mykiss) and brown trout (Salmo trutta) reared at 11.2°C. The first two trials for each species were designed to simultaneously determine the upper incipient lethal temperature and 100% lethal temperature over a 14-day period. The third trial for each species evaluated the effects of an exaggerated 12-hour tempering regime on fish survival after placement in elevated water temperatures. After transfer from a water temperature of 11.2°C, no rainbow trout survived at 26°C, and only 50% survived at 25°C. No brown trout survived at 22°C and only 50% at 20°C. Survival of rainbow trout was not improved by the 12-hour tempering regime where water temperatures were slowly increased from 11.2°C to either 25°C or 26°C. Similarly, tempering did not improve brown trout survival at either 20°C or 22°C. These results suggest that tempering is not needed when hatchery-reared trout are reared and stocked at the water temperatures within the range of those used in this study.

Keywords

Tempering, Rainbow Trout, *Oncorhynchus mykiss*, Brown Trout, *Salmo trutta*, Temperature

1. Introduction

When trout or other salmonids are transferred from cooler to warmer water, slowly tempering (adjusting) the fish to the warmer water has a long history and has been traditionally practiced by fish culturists [1-3]. This tempering process

was particularly pervasive during the stocking of hatchery-reared trout into lakes and streams until recently when the risks of disease or invasive species transmission by using thermal tempering techniques were more fully recognized [3].

Few studies have examined tempering salmonids for thermal changes. In one of the earliest experiments, Brett [4] found that very short-term (15-min) tempering had no effect on trout survival when stocked into waters at or near lethal temperatures. Smith and Hubert [3] reported no difference in the survival of rainbow trout (*Oncorhynchus mykiss*) transferred from 8°C to 24°C with or without tempering. Salmonids in general can handle a 10°C temperature change without any issues [5]. Studies involving other fish species have also found no benefits from thermal tempering of fingerling or larger fish [6, 7]. Despite these publications, thermal tempering is still widely used and recommended [1, 8, 9, 10, 11].

The South Dakota Department of Game, Fish and Parks has two publicly-owned trout and salmon hatcheries that stock 77 different water bodies within the state of South Dakota (USA) for recreational fishing [12]. With stockings occurring every month of the year, it is inevitable that hatchery fish reared at 11°C will be placed into higher temperature waters. The possible effects on the fish of stocking into such temperature gradients are unknown but given its stewardship obligations as a government agency [13], it is essential that the Department maximize the post-stocking survival of the fish. Minimizing the spread of fish pathogens and aquatic invasive species is also important [13], with increased risks associated with the use of tempering techniques such as exchanging waters between the transfer tanks and water body to be stocked [3]. Thus, the objectives of this study were two-fold, first to determine the upper thermal tolerance for the predominant trout currently stocked, and secondly to determine if thermal tempering is beneficial or needed.

2. Methods

2.1. Methods Common to Each Experiment

Six separate trials were conducted at McNenny State Fish Hatchery, Spearfish, South Dakota, USA to determine the survival of Arlee strain rainbow trout (three trials) and Saratoga strain brown trout (*Salmo trutta*) (three trials) at elevated temperatures with and without thermal tempering (**Table 1**). The first two trials determined the Upper Incipient Lethal Temperature (defined as the upper temperature where 50% of the population could theoretically survive) [14, 15] and the Upper Lethal Temperature (defined as the upper temperature where 100% of the fish die). The third trial used the results from the first two trials to determine the effect of an exaggerated simulated thermal tempering on both the Upper Incipient Lethal Temperature and the Upper Lethal Temperature.

Twelve 190-L semi-square tanks were used for each trial. Each tank was outfitted with a submersible recirculating pump (Pondmaster, Kissimmee, Florida, USA) attached to a spray bar that maintained dissolved oxygen levels at or near

Table 1. Mean (±SE) total length and weight of rainbow and brown trout for six temperature-related trials.

Species	Trial	Length (mm)	Weight (g)
Rainbow trout	1	155 ± 3	36.2 ± 2.5
	2	172 ± 3	51.5 ± 3.4
	3	175 ± 6	56.7 ± 5.0
Brown trout	1	183 ± 3	77.2 ± 5.6
	2	174 ± 4	61.4 ± 5.0
	3	186 ± 8	79.0 ± 5.4

saturation. A submersible heater (Hydor, Bassano del Grappa, Italy) with temperature controller (Finnex, Chicago, Illinois, USA) was used to maintain tank temperatures ($\pm 1^{\circ}$ C) over the course of each trial. Prior to the start of each trial, tanks were cleaned and filled with well water (11.2°C; total hardness 360 mg/L CaCo₃; alkalinity as CaCO₃ 210 mg/L; pH 8.3; total dissolved solids 390 mg/L) and allowed to temperature stabilize to the experimental temperatures.

Tanks were monitored daily for total ammonia levels using a water total ammonia test kit (LaMotte, Chestertown, Maryland, USA). Ammonia levels were maintained at levels < 0.50 mg/L (free ammonia) with the addition of AmQuel Plus Ammonia Detoxifier (Kordon, Hayward, California; USA). Each tank was stocked with one fish netted from a common tank receiving the same well water that was used in the experiments. Fish were not fed the day before the trial or over the course of each 14-day trial. The trial duration was selected because 70% of the trout stocked in South Dakota are assumed to be caught within 14 days post-stocking [16]. Fish were weighed (g) and measured (total length, mm) either at the end of the trial or when mortality occurred.

Following the experiments, a chi-square analysis (SPSS 24.0; IBM, Armonk, New York, USA) was performed on each separate experiment to determine if there were any differences in survival between treatments. In addition, a one-way Analysis of Variance (ANOVA) was conducted on the time of mortality for each trial, with all fish assumed to have died by the end of the trial (day 14). Tu-key's means comparison procedure was performed if the ANOVA indicated significant differences. Significance was pre-determined at p < 0.05.

2.2. Rainbow Trout

The first rainbow trout trial used three different temperatures (n = 4): 19°C, 22°C, and 25°C. The trout were directly placed in each tank from the common pool tank containing 11.2°C water. The upper temperature of 25°C was selected based on the assumption that it was the Upper Lethal Temperature for rainbow trout [14, 17, 18], and the other temperatures have been observed in South Dakota trout stocking waters. Based on the results from Trial 1, temperatures of 18°C, 22°C, and 26°C were used in Trial 2. The second trial was identical to the first, except for the difference in some of the temperatures used.

The third trial used an exaggerated tempering protocol. All of the tanks used in this trial initially contained 11.2° C water. After the rainbow trout were transferred from the common pool, also at 11.2° C, the tank heaters were engaged, and water temperatures in the tanks slowly began to increase to three predetermined temperatures: 16° C, 25° C, and 26° C. Four tanks were used for each temperature (n = 4). The overall tempering times and thermal rate of increase for each temperature treatment were: 16° C - 12° C hours (0.41°C/hour); 25° C - 11 hours (1.27°C/hour); and 26° C - 12 hours (1.25°C/hour).

2.3. Brown Trout

The experimental designs used for all three brown trout trials were similar to those described previously for rainbow trout. The first trial used temperatures of 13°C, 22°C, and 25°C, and the brown trout were originally held in 11.2°C water. The Upper Lethal Temperature for brown trout has been reported from 22°C to 25°C [14, 17, 19]. Based on the results from Trial 1, temperatures of 13°C, 20°C and 22°C were used in the second trial. In the third trial, brown trout were directly placed into their respective tanks containing 11.2°C water and slowly tempered to 13°C, 20°C and 22°C. The overall tempering times and thermal rate of increase for each temperature treatment were: 13°C - 9 hours (0.22°C/hour); 20°C - 9 hours (1.00°C/hour); and 22°C - 10 hours (1.10°C/hour).

3. Results

3.1. Rainbow Trout

In trial 1, all of the fish in the 19°C tanks survived, compared to 75% and 50% of the trout in the 22°C and 25°C tanks (**Table 2**). However, there was no significant difference among these means (x = 2.67, p = 0.26). All mortality occurred

Table 2. Mean number of days (\pm SE) and percent survival (%) of rainbow trout from three separate 14-day trials involving placement of fish into tanks at different water temperatures without tempering (Trial 1 and 2) and with an exaggerated tempering routine (Trial 3). Means for each trial in a column followed by different superscript letters are significantly different (p = 0.05).

Trial	Temp (°C)	Days Alive	Survival (%)
1—Untempered	19	14.0 ± 0.0	100
	22	13.0 ± 1.0	75
	25	10.8 ± 1.9	50
2—Untempered	18	$14.0\pm0.0^{\rm z}$	100 ^z
	22	14.0 ± 0.0^{z}	100 ^z
	26	$0.75 \pm 0.3^{\text{y}}$	0_{λ}
3—Tempered	16	14.0 ± 0.0^{z}	100 ^z
	25	10.3 ± 1.9^{z}	25 ^y
	26	$5.3 \pm 0.9^{\text{y}}$	0_{a}

between seven and ten days after the start of the experiment. In the second trial, all fish survived in the 18° C and 22° C tanks, which was significantly different than the 100% mortality observed in the 26° C tanks (x = 12.0, p = 0.002). The mortality at 26° C occurred within 24 hours after placement of the trout in the tanks. Thus, 25° C was the rainbow trout Upper Incipient Lethal Temperature and 26° C was the Upper Lethal Temperature. Mean survival was significantly different (x = 8.91, p = 0.01) among the tempering treatments in third trial, with 100% survival at 16° C, 50% at 25° C, and 0% at 26° C. These results were consistent with the other two trials. Compared to the rapid mortality at 26° C in Trial 2 however, the timing of mortality was delayed by several days in the 26° C trout that were tempered in Trial 3.

3.2. Brown Trout

Brown trout survival was significantly different (x = 8.90, p = 0.01) among the treatments in the first trial, with 100% survival at 13°C, 25% at 22°C, and 0% at 25°C (**Table 3**). All the brown trout placed into 25°C water died within the first day. Results were similar in the second trial, with significantly greater survival at 13°C (100%), compared to 50% at 20°C, and 0% at 22°C (x = 8.0, p = 0.02), with most of the mortality occurring within the first week of the trial. Thus 20°C was the brown trout Upper Incipient Lethal Temperature and 22°C the Upper Lethal Temperature. Thermal tempering in the third trial did not change brown trout survival, with the results similar to the second trial. Survival was significantly greater at 13°C where all of the fish survived, compared to 25% at 20°C, and 0% at 22°C (x = 8.9, p = 0.01). Tempering also did not influence the timing of mortality.

Table 3. Mean number of days (\pm SE) and percent survival (%) of brown trout from three separate 14-day trials involving placement of fish into tanks at different water temperatures without tempering (Trial 1 and 2) and with an exaggerated tempering routine (Trial 3). Means for each trial in a column followed by different superscript letters are significantly different (p = 0.05).

Trial	Temp (°C)	Days Alive	Survival (%)
1—Untempered	13	14.0 ± 0.0^{z}	100 ^z
	22	7.3 ± 2.3^{y}	25 ^y
	25	1.0 ± 0.0^{x}	$0_{\mathrm{\lambda}}$
2—Untempered	13	$14.0\pm0.0^{\rm z}$	100^{z}
	20	11.3 ± 1.7^{z}	50 ^y
	22	6.0 ± 0.8^{y}	0_x
3—Tempered	13	$14.0\pm0.0^{\rm z}$	100 ^z
	20	13.8 ± 0.3^{z}	75 ^z
	22	7.3 ± 0.6^{y}	0_{a}

4. Discussion

The results of this experiment strongly suggest that adjusting trout to increased water temperatures during stocking activities is not needed. Similarly, Smith and Hubert [3] did not observe any positive effects from tempering during the stocking for juvenile rainbow trout from 8°C to 24°C water. Brett [4] also found no benefit from short term thermal tempering with brook trout (*Salvelinus fontinalis*). These results also support the statement that salmonids in general can handle a 10°C temperature change without any issues [5]. In other species, thermal tempering has also been shown to be ineffective. Mather and Wahl [6] found little to no effect of tempering three different esocids to near lethal water temperatures pre-stocking. In addition, Clapp *et al.* [7] found no benefits to tempering in juvenile percids. Although the results of this study apply to only two salmonid species at relatively large sizes, the blanket recommendations for thermal tempering proposed by Piper *et al.* [1], Noga [8], Timmons *et al.* [9], Harmon [10] and Wynne and Wurts [11] may need to be reconsidered.

Although thermal tempering may not be needed, tempering of fish in relation to water chemistry has been shown to be beneficial [20-22]. Rapidly changing water chemistry, such as hardness, pH, and salinity, is a major stressor for fish and can even cause mortality [23]. Water chemistry was controlled for in the present study; only temperature was changed among the treatments.

Eliminating the need for thermal tempering reduces the risk of fish pathogen transmission or the spread of aquatic invasive species [3]. Traditional tempering practices involved slowly exchanging the water in the transfer (stocking) tanks with water from the lake or stream to be stocked [2], thereby allowing for the introduction of foreign organisms into the transfer tank and possibly beyond. By ceasing this water exchange, a possible contamination vector is eliminated, as well as the need for subsequent tank disinfection or anti-invasive species chemical treatments [24-26].

While the exaggerated thermal tempering regime used in this study did slightly delay mortality, the same percentage of fish died in the tempered and untempered treatments. Thus, the two-week post-tempering duration in this study provided results that may not have been observed if the study only lasted for the shorter time frames used by Smith and Hubert [3] or Clapp *et al.* [7]. Of course, additional mortality could have possibly occurred after the 14-day observation period of this study. While short-term experiments hold value, longer term experiments may more accurately express the potential survivability of the fish in the receiving waters. Wehrly *et al.* [27] suggested that a 7-day Upper Incipient Lethal Temperature may not be long enough to ascertain when 50% of a population may die, and Selong *et al.* [28] used a 60-day Acclimated Chronic Exposure method to determine thermal tolerance in bull trout (*Salvelinus confluentus*).

The Upper Incipient Lethal Temperature of 25°C and Upper Lethal Temperature of 26°C for rainbow trout observed in this study are similar to what has been previously reported. An Upper Lethal Temperature of 25°C was also ob-

served by Cherry *et al.* [17] and Hokanson *et al.* [18], as reviewed by Jobling [14]. In contrast, Bidgood and Berst [29] and Alabaster and Welcomme [30] found the Upper Lethal Temperature of rainbow trout was 26°C and 26.5°C respectively. The brown trout Upper Lethal Temperature of 22°C in the present study is close to the 23°C value reported by both Bishai [19] and Cherry *et al.* [17].

The results from this set of experiments strongly suggest that as long as water chemistry is similar between hatchery water and waters to be stocked, using an exaggerated tempering regime to adjust trout to elevated temperatures will not positively affect survival. Future studies should examine the possible interactions between changes in both temperature and water chemistry, as well as evaluate thermal tempering in other fish species.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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