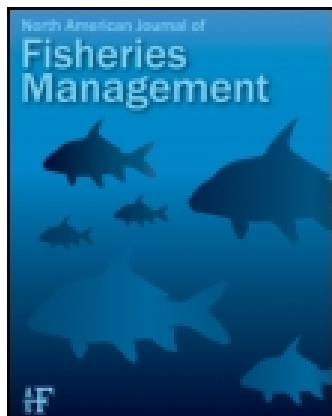


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### Absence of Handling-Induced Saprolegnia Infection in Juvenile Rainbow Trout with Implications for Catch-and-Release Angling

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## MANAGEMENT BRIEF

# Absence of Handling-Induced *Saprolegnia* Infection in Juvenile Rainbow Trout with Implications for Catch-and-Release Angling

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### Abstract

Catch-and-release angling is common in recreational fisheries. During handling and dehooking, fish are subjected to stress and dermal injuries, which may result in infections by pathogens after the fish is released. The objective of this study was to evaluate the consequences of common handling practices used by anglers on the postrelease behavior and fate, particularly the susceptibility to disease, of undersized Rainbow Trout *Oncorhynchus mykiss*. Behavior immediately following capture and subsequent release of fish was examined in a 40-L container, and long-term fate was studied for 2 weeks in tanks incubated with *Saprolegnia parasitica* zoospores. Trout were behaviorally impaired as indicated by the ease of being netted following the simulated fight associated with catch and release, but there were no further behavioral impacts due to subsequent handling. None of the Rainbow Trout developed fungal infections nor was any significant mortality observed after 2 weeks; only 1 out of 137 fish died. Our data indicate that juvenile hatchery-reared Rainbow Trout have a high resilience to *Saprolegnia* infection handling-induced stress.

Globally, as many as two-thirds of angled fish are released (Cooke and Cowx 2004) because of legal requirements or for voluntary reasons (Arlinghaus et al. 2007). Any catch-and-release event involves handling fish during the landing and dehooking processes. Handling may take place using bare hands, towels, or other equipment used by the angler to constrain the fish (Danylchuk et al. 2008). Prior to dehooking, fish may also be exposed to a landing net, which may remove some of the mucus from fish (Barthel et al. 2003; De Lestang et al. 2008). Rough handling may affect survival postrelease due to the physical damage to the epidermis resulting in infections, predation, or other forms of stress-induced mortality (Barthel et al. 2003; Colotelo and Cooke 2011). Relatively few studies have examined how fish behave after release (reviewed by Donaldson et al. 2008), and aside from the duration of air exposure (Danylchuk et al. 2007; Arlinghaus et al. 2009)

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no research known to the authors has evaluated the impact of common handling practices by anglers on fish behavior postrelease. It is pertinent to study how fish behavior and probability of infection with pathogens vary with common handling techniques used by anglers during dehooking.

One ubiquitous pathogen that may infect freshwater fish is *Saprolegnia parasitica* (Noga 1993; van West 2006). *Saprolegnia parasitica* is able to parasitize all species of freshwater fish, especially salmonids (Noga 1993). Infections are visible as white to grey mold on the fish's skin and often lead to death of the fish (Howe and Stehly 1998; van West 2006). In salmonids, predisposing factors leading to an infection include epidermal wounds (Harai and Hoshiai 1993) and physiological stress (e.g., Noga 1993). As handling by anglers after capture might interact with initial physical exhaustion during the fight, it is possible that some released fish become infected with *S. parasitica* on damaged epithelial layers.

Particularly under conditions of high predation threat postrelease (Cooke and Philipp 2004), rapid resumption of normal behavior after catch and release is important to safeguard survival. Exercise alone already impairs the swimming of fish after release (Schreer et al. 2005; Arlinghaus et al. 2009), and any handling during dehooking may aggravate the situation. Due to a lack of studies available it is currently unknown whether this actually is the case.

The objective of this study was to evaluate whether handling practices used by anglers during the dehooking event influence behavior and mortality of juvenile Rainbow Trout. We hypothesized that harsh handling practices would result in *Saprolegnia* infections in juvenile Rainbow Trout. The impact of handling on behavior postrelease was studied as well to better understand how handling may affect fish behavior after their release.

## METHODS

Experimental fish were undersized juvenile Rainbow Trout (mean TL =  $19.4 \pm 1.49$  cm [mean  $\pm$  SD],  $n = 137$ ) obtained from a commercial hatchery (Forellenzucht Werdermühle, Niemege, Germany). Juvenile fish were chosen because they have to be released in most jurisdictions and may thus suffer particularly from angling-induced handling. Fish were acclimated to experimental conditions over a period of 2 weeks in an 800-L ( $1.1 \times 1.1 \times 1.0$  m), aerated tank with a water inflow rate of  $\sim 8$  L/h. To ensure identification of each individual, fish were tagged with  $1.2 \times 0.2$ -cm PIT tags (TROVAN, model ID-100B; EURO I.D., Weilerswist, Germany), inserted into the dorsal musculature left of the dorsal fin 1 week prior to the experiment. Before experimentation, treatments were assigned at random to PIT tag numbers (see below). Behavior or mortality of Rainbow Trout is not significantly influenced by PIT tags (Prentice and Park 1984).

Water quality during both acclimation and the subsequent experiment was measured daily and was within the optimal

range for Rainbow Trout (water temperature =  $18.7 \pm 0.5^\circ\text{C}$  [mean  $\pm$  SD], dissolved oxygen =  $6.6 \pm 0.7$  mg/L; Schmidt 1998). Fish were fed a maintenance diet of commercial trout pellets (i.e., 1.5% of body weight per day; Kaushik and Gomes 1988).

During the experiments, fish were individually netted with a knotless landing net (Barthel et al. 2003) from the holding tank (any excess fish captured were released immediately back into the tank), identified using a PIT tag reader (TROVAN), subsequently placed in a bucket filled with 40 L of water, and chased by tail pinching for 30 s to simulate a fight on the rod as described in other simulated catch-and-release studies (e.g., Kieffer 2000; Arlinghaus et al. 2009). Subsequently, fish were exposed to one of three randomly allocated handling treatments or one of two controls. Control groups included (1) netting only ( $N = 28$ ) and (2) netting and chasing ( $N = 27$ ). Treatments included netting, chasing, and handling with (3) dry hand ( $N = 28$ ), (4) wet hand ( $N = 27$ ), and (5) dry towel ( $N = 27$ ). Each handling event was 5 s in duration (similar to experimental data in Danylchuk et al. 2007) that mimicked rapid dehooking of an undersized fish without actually hooking the fish; this controlled for any confounding effects of hooking injury to directly link the results to handling. Fish in the unhandled groups were individually caught with a landing net from the holding tank and either not chased at all or exposed to physical exhaustion by tail pinching only.

To measure postrelease behavior, a behavioral impairment score immediately after the simulated release event was recorded following Gingerich et al. (2007). Impairment was defined as a state that would render the fish an easy prey to predators, as indicated by impairment of swimming or escape. All fish were individually released into a white container (40 L; upper and lower diameters, 60 and 49 cm, respectively; height, 38 cm), where the behavior of each fish postrelease was observed for a total period of 300 s. At each of five intervals (0, 30, 60, 180, and 300 s), the fish's behavioral state was categorized and assigned a value as follows:

- (0) fish appeared normal with normal swimming behavior,
- (1) fish appeared normal with visible signs of fatigue,
- (2) fish swam rapidly and randomly, and somewhat erratically,
- (3) fish rested on the bottom of the tank,
- (4) fish displayed partial lateral equilibrium loss,
- (5) fish displayed complete equilibrium loss (either floating on its side or upside down).

The five observed values were subsequently summed to yield a release behavioral condition score after 300 s. Afterwards, as a second measure of behavioral impairment, the ease of netting the fish from the bucket was scored as either difficult (1) or easy (0). Easy was scored when the fish was netted with the first attempt. The difficult category meant that the fish evaded the netting event actively, such that more than one netting attempt was needed to capture the fish. This second behavioral

metric was used to simulate predator evasion ability postrelease. It was assumed that an easily netted fish would probably fall victim to a natural predator.

To assess infections with *S. parasitica* and survival, after the 300-s observation period all fish from each treatments were evenly allocated into three replicate tanks so that in each tank all treatments were jointly represented to control for tank effects. Each tank had a capacity of 400 L ( $1.1 \times 1.1 \times 0.5$  m; tap water inflow,  $\sim 8$  L/h; temperature,  $18.7 \pm 0.5^\circ\text{C}$ ; dissolved oxygen,  $6.6 \pm 0.7$  mg/L) and was stocked with trout at a density of  $3.58$  kg/m<sup>3</sup> (tank 1, 46 individuals; tank 2, 46 individuals; tank 3, 45 individuals). To standardize the density of *S. parasitica* spores across tanks, each tank was inoculated with *S. parasitica* at a concentration of 4 zoospores/mL, which was slightly lower than the concentration targeted by Howe and Stehly (1998) in their study on Rainbow Trout. The strain of *S. parasitica* originated from the American Type Culture Collection (ATCC 22284) and was recultivated at least 20 times. According to ATCC this strain of *S. parasitica* is highly pathogenic, and Gieseke et al. (2006) were able to infect Rainbow Trout using this strain.

Seeds of hemp together with pieces from the pure culture of *S. parasitica* were put into glucose–yeast extract (GY) agar culture media (Howe and Stehly 1998; Meinelt et al. 2007). After 3 d, the seeds were moved into a liquid culture media (50 ppm carbon), and after another 2 d, 1.2 L of a liquid culture media was produced and equally distributed to the three tanks. Before the beginning of the experiment, the number of spores was determined by streaking culture samples onto the GY agar and counting the number of spores. A 2-week observation period was chosen to capture delayed mortalities as a result of handling and possibly infection by the fungus. After 2 weeks, each surviving fish was visually assessed for mortality or infection by *S. parasitica* as either absent or present. Two weeks should be sufficient for *S. parasitica* to infect the fish (Willoughby and Pickering 1977). In other experimental infection studies, trout developed visible infections after only 2 to 5 d (Howe and Stehly 1998; Fregeneda Grandes et al. 2001). Barnes et al. (2004) showed that for water hardness (calcium) of about 50–150 mg/L, 70–80 h were needed to observe microscopic growth of *S. diclina*. Hardness of the water used in the present study was similar: calcium, 94–147 mg/L; magnesium, 8.4–18.8 mg/L (data retrieved from local drinking water industry). Therefore, an observation period of 2 weeks seemed valid.

Statistical comparisons of the behavioral impairment score upon release among treatments were conducted in SPSS (version 9.0.1) by a one factorial ANOVA at  $P < 0.05$  that assumed the ordinal behavioral scale has equidistant qualities similar to a rating scale in survey research conducted with human subjects. Tests on homogeneity of variances were conducted using Levené tests, and normality was tested using a Shapiro–Wilk test. Subsequently, a Tukey honestly significantly different (HSD) post hoc test was used for

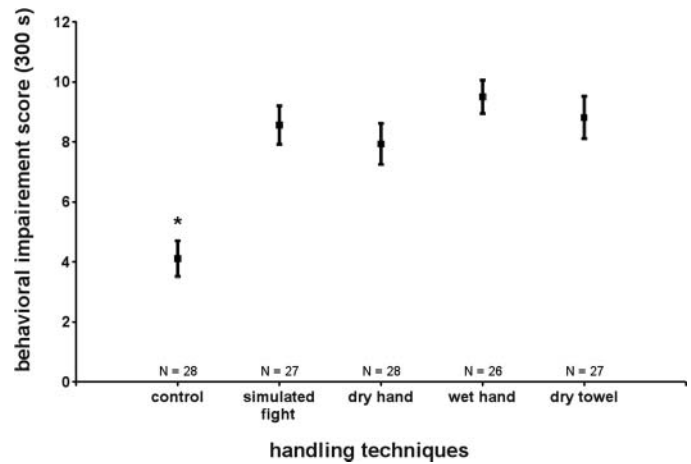


FIGURE 1. Index of behavioral impairment summarized over 300 s postrelease in juvenile Rainbow Trout displayed as mean score ( $\pm$ SE). State of behavior was categorized as: (0) fish appeared normal with normal swimming behavior, (1) fish appeared normal with visible signs of fatigue, (2) fish swam rapidly and randomly in the tank, (3) fish rested on the bottom of the tank, (4) fish displayed partial lateral equilibrium loss, (5) fish displayed complete equilibrium loss. Note that similar patterns existed at all time periods, but to save space results are only shown for the 300-s integrated measure. Significant differences in mean score are marked with an asterisk (\*) (ANOVA and Tukey HSD post hoc test:  $F = 11.25$ ,  $df = 4$ ,  $P < 0.05$ ).

homogeneous variances. The ease of netting was evaluated using a chi-square test using a standard contingency table that compared expected and revealed frequencies.

## RESULTS

There were significant differences in postrelease behavioral impairment scores among treatments (ANOVA and Tukey HSD post hoc test:  $F = 11.25$ ,  $df = 4$ ,  $P < 0.05$ ; Figure 1). Only the control group captured by landing net showed less behavioral impairment compared with all other groups (Figure 1). A similar pattern was apparent based on the ease of netting: the control group not chased was significantly more difficult to net than all other treatment groups ( $\chi^2 = 32.92$ ,  $df = 4$ ,  $P < 0.05$ ; Figure 2).

During the 2-week observation period after release, we observed only one mortality that occurred in the chased-only control group. At the termination of the experiment, no fish had visible signs of skin damage or *S. parasitica* infection. Therefore, the mortality rate as a function of handling treatment by dry hand, wet hand, or dry towel was 0%.

## DISCUSSION

The results of this study suggest that handling does not affect the behavior of juvenile hatchery Rainbow Trout following a catch-and-release event; rather, exercise fatigue appeared to be responsible for the statistical differences among treatments in terms of their postrelease behavior. These findings agree with a number of other studies that show fish are

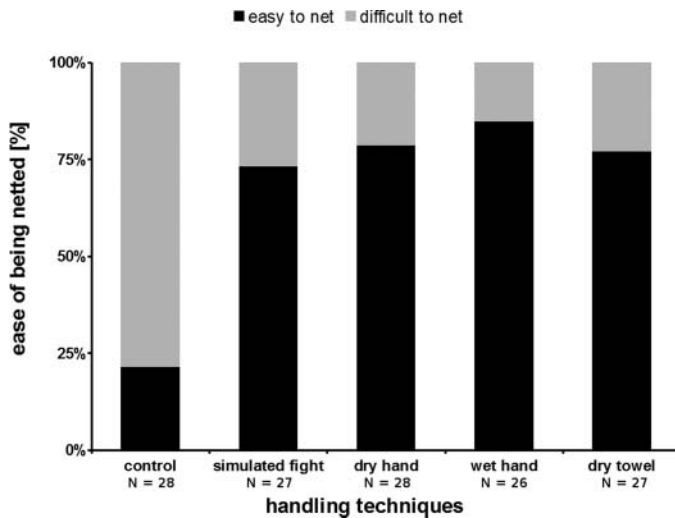


FIGURE 2. The ease of netting juvenile Rainbow Trout after 300 s of chasing. The distributional differences were significant ( $\chi^2 = 32.92$ ,  $df = 4$ ,  $P < 0.05$ ).

behaviorally impaired immediately after release when they have been physically exhausted during the fight (e.g., Thompson et al. 2008; Arlinghaus et al. 2009). This study adds to this literature by reporting that rapid handling with limited air exposure of 5 s does not aggravate the behavioral status of Rainbow Trout postrelease relative to exhaustion alone.

The experiments were conducted at mild environmental conditions within the thermal optimum range for Rainbow Trout (although at the upper part of the range), avoiding rapid changes in water temperature or extended air exposure. Gingerich et al. (2007) showed that air exposure and water temperature may interact to cause behavioral changes and potentially large, delayed mortality in centrarchid fish. Increased handling time can also increase recovery times (White et al. 2008; Arlinghaus et al. 2009; Rapp et al. 2012) and result in air exposure thresholds after which postrelease swimming performance declines (Schreer et al. 2005). It appeared that our handling protocols did not exceed physiological thresholds for juvenile Rainbow Trout. Therefore, despite the lack of effects of rapid handling on behavior and mortality in our study, it is possible that negative effects of handling may be evident under actual angling conditions. For example, if handling coincides with longer air exposure than tested in the present study or when release events occur during unfavorable (e.g., too warm or too cold) temperature conditions (Arlinghaus et al. 2007), an effect may be evident. Moreover, in systems where juvenile trout are released in the presence of cannibalistic larger trout or other predators, mortality postrelease might be more elevated than what was revealed in the present tank experiments because the physical impairment after exhaustion can limit escape abilities and result in predation postrelease (Danylchuk et al. 2007; reviewed by Raby et al. 2014). However, in light of the result

of this study, such effects would likely be unrelated to common handling practices in Rainbow Trout during dehooking and rather be caused by physical exhaustion or air exposure.

Mucus loss and damage of epithelia is to be expected during handling in any fish, e.g., handling with towels during dehooking or with dry hands. Indeed, Murchie et al. (2009) observed a maximum loss of slime in Bonefish *Albula vulpes* during handling by bare hands or gloved hands. Thus, the handling we exposed the juvenile Rainbow Trout to should also have caused some mucus abrasion. Abrasion promotes infection with *S. parasitica* in Rainbow Trout (Fregeneda Grandes et al. 2001). Because our handling did not result in *Saprolegnia* infection within a 2-week observation period, it appears that handling-induced abrasion, common in recreational angling, may not be severe enough to cause adverse effects in Rainbow Trout under reasonably benign environmental conditions.

Our results can be explained by the fact that hatchery fish often show a reduced stress response (e.g., Weil et al. 2001) and overall are more resistant to handling (Rapp et al. 2012) than wild fish. Alternative explanations include the existence of environmental conditions within an optimal range (Schmidt 1998) that prevented a further deterioration of the fish's condition, use of generally well-conditioned fish (e.g., good nutritional status from artificial feed), and short air exposure of only 5 s during handling. It is also conceivable that our experimental design failed to establish a large enough and long-lasting enough zoospore load because we incubated the tanks just once and not repeatedly over the entire 2-week observation period as was conducted, for example, by Howe and Stehly (1998). Howe and Stehly (1998) also used a much harsher abrasion treatment involving daily dewatering of experimental tanks and letting Rainbow Trout flop for 1 min over an abrasive paper. Yet, despite this harsh treatment only 25% of those fish became infected with *S. parasitica* within 14 d at a continuous concentration of  $>5$  zoospores/mL (Howe and Stehly 1998). In their study, it was only when abrasion of the intensity just described was matched with temperature stress (simulated by rapidly dropping water temperatures) that the mortalities rose to 75% over 14 d. Similarly, Fregeneda Grandes et al. (2001) exposed batches of 15 Rainbow Trout to shaking for 2 min in a fan-shaped landing net made of a 6-mm knotless mesh at zoospore concentrations of 200–300 zoospores/mL over 3 d with a subsequent 12-d observation period. Yet, despite this harsh treatment, infection rates and mortalities varied between 0% and 100% depending on the *Saprolegnia* morphotype and isolate. In light of these data, the lack of infections in response to a single, relatively mild, handling event in our study does not appear unreasonable. Possibly, our treatment was simply not harsh enough and led to only superficial mucus loss that was insufficient to enable infection by *Saprolegnia*. Similarly, Pope et al. (2007) reported a very high survival rate of, on average, 96% in mouth-hooked Rainbow Trout under experimental laboratory conditions and found no

relationship between mortality and number of hooking events (up to four). Hence, under similar environmental conditions as those in the present study, juvenile Rainbow Trout of hatchery origin are likely to tolerate moderate injury caused by handling and by hooking (Pope et al. 2007) without suffering infections with *S. parasitica* and without experiencing significant mortality.

The present study joins a number of recent examples that have reported very low or even zero postrelease mortality in various freshwater fishes (e.g., Pope and Wilde 2004; Rapp et al. 2012), including Rainbow Trout (Pope et al. 2007). Negligible hooking mortality should generally be expected when environmental conditions are benign and the injury induced by the capture event is mild (Arlinghaus et al. 2007; Pope et al. 2007). However, based on the present work alone one should not conclude that handling is generally not problematic for fish. There is still merit for anglers to not artificially prolong the fight to minimize behavioral impairment and to use wet hands or dehook fish entirely under water because this will minimize the stress response of the fish and maintain the fish in a better condition upon release (FAO 2012). If these actions are followed and deep hooking is avoided, the survival of undersized Rainbow Trout of hatchery origin can approach 100%.

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