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Effects of recreational angling and air exposure on the physiological status and reflex impairment of European grayling (*Thymallus thymallus*)

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European grayling (*Thymallus thymallus*) is a popular recreational fish that may be lifted out of the water to facilitate hook removal or for admiration. To evaluate the effects of air exposure and angling-induced exhaustive exercise on released grayling condition, we assessed blood physiology and reflexes of grayling after angling and air exposure in the subarctic River Lakselva (Norway) at midsummer temperatures (i.e., 17–18 °C). Blood samples were drawn 30 min after angling and analyzed for lactate anions, glucose, sodium ions, and pH. Reflex impairment was determined with orientation and tail grab reflex action assessments immediately after landing, after air exposure, and after 30 min holding. Blood physiology did not indicate an exacerbating effect of air exposure relative to just angling-induced exercise, but significant and prolonged reflex impairment was associated with the 120 s air exposure interval. Anglers must take care to minimize air exposure to adhere to best handling practices.

Introduction

Recreational fisheries are components of local economies and natural resource conservation efforts in regions throughout the world (Cowx 2002, Arlinghaus and Cooke 2008, Cooke *et al*. 2015). Whereas recreational fishers may choose to harvest fish, many anglers practice catch-and-release. Catch-and-release is a minimally consumptive form of recreational angling that is practiced either voluntarily or via legal regulation (Arlinghaus *et al*. 2007). The operational assumption of catch-and-release is that fish are released in good condition and will return to the common population with negligible effects on lifetime fitness (Barnhart 1989, Cooke and Schramm 2007). However, many studies have demonstrated that catch-and-release can have both lethal and sublethal consequences for fish that are captured (reviewed in Muoneke and

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Childress 1994, Bartholomew and Bohnsack 2005, Arlinghaus *et al*. 2007). This has inspired research into the components of recreational fisheries that contribute to mortality of caught and released fish in an effort to identify best angling and handling practices. That body of work has revealed that mortalities can be attributed to physical damage from hooking injuries or to physiological alterations associated with stress (Arlinghaus *et al*. 2007, Cooke and Schramm 2007).

The magnitude of the physiological alteration associated with angling can affect the recovery from exercise, with longer recovery times decreasing the likelihood of survival. Stress associated with angling is exacerbated by factors such as water temperature, playing time, fish size, or air exposure duration (Ferguson and Tufts 1992, Meka and McCormick 2005, Gingerich *et al*. 2007, Arlinghaus *et al*. 2007). Inducing hypoxia by lifting fish out of the water for hook removal or admiration (i.e., photography, weighing) is stressful for fish (Ferguson and Tufts 1992, Schreer *et al*. 2005, Gingerich *et al*. 2007). Anaerobic cell respiration during air exposure produces lactic acid in white muscle, which is broken down to lactate ion [lac$^-$] and a free metabolic proton [H$^+$], the latter contributing to intracellular acidosis, which is a primary factor influencing mortality of exercised fish (Wood *et al*. 1983). In addition, the oxidation of lactate to restore glycogen reserves is costly and time-consuming (Wood 1991, Jain and Farrell 2003), meaning that high [lac$^-$] associated with extended anaerobiosis from exercise or air exposure can indicate the degree of departure from homeostasis and can thus be inversely related to the likelihood of recovery. The primary stress response initiated by both exercise and air exposure involves the release of catecholamines epinephrine and norepinephrine as well as cortisol, the primary glucocorticoid in fish (McCormick and Macleod 1925, Mazeaud *et al*. 1977, Mazeaud and Mazeaud 1981, McDonald and Milligan 1997, Barton 2002). During stress or exercise, freshwater fish lose sodium [Na$^+$] across the gill lamellae because of increased gill permeability caused by catecholamine action (Mazeaud *et al*. 1977, Mazeaud and Mazeaud 1981, McDonald and Milligan 1997), although this may take several hours to manifest (Carey and McCormick 1998). Immediately after exercise, [Na$^+$] is expected to increase in the blood, moving out of muscle cells when [lac$^-$] accumulates (Wood *et al*. 1983). As part of the secondary stress response, glucose [glu] is produced from hepatic glycogen (Barton 2002, Mazeaud and Mazeaud 1977, McDonald and Milligan 1992, Mommsen *et al*. 1999), increasing in the blood between five minutes (Wydoski *et al*. 1976) and one hour (Carey and McCormick 1998, Ristori and Laurent 1985) after induction of stress. In spite of the documented responses by fish to air exposure, some anglers lift fish out of the water after recreational angling events, even when they intend to practice catch-and-release. Yet, recreational fishing guidelines rarely provide science-based air exposure recommendations that anglers can adopt, making it relevant to identify and test air exposure intervals that could be useful from a management perspective (Pelletier *et al*. 2007, Cook *et al*. 2015).

European grayling (*Thymallus thymallus*) is native throughout Europe and is popular among recreational anglers (Swatidpong *et al*. 2010). Grayling populations in many countries are declining due to habitat alterations, eutrophication, climate change, pollution, and overfishing (Northcote 1995, Persat 1996, Uiblein *et al*. 2001, Gum *et al*. 2003, 2005, Duftner *et al*. 2005). Anglers that release grayling often do so after handling and photographing them in air with the characteristic dorsal fin extended, meaning that many of these fish undergo air exposure prior to release. We measured the effects of air exposure on European grayling after exhaustive exercise using blood-based physiological metrics and reflex impairment observations. Blood physiology metrics of interest were blood pH as a measurement of intracellular acidosis (Wood *et al*. 1983), lactate anion concentration as a measurement of white muscle exhaustion and anaerobiosis (Dobson and Hochachka 1987, Wood 1991, Kieffer 2000), glucose concentration as an index of the secondary stress response (Mommsen *et al*. 1999, Barton 2002), and sodium ion concentration as a measure of osmoregulatory disruption (Wood and Randall 1973). Reflex impairment indices are useful for assessing post-capture condition of fish (e.g., Campbell *et al*. 2010) and have been validated for other salmoniformes (e.g., Raby *et* *al*. 2012) as a whole-organism metric for assessing the post-capture status of fish. Impairment of reflex actions is a physical manifestation of capture stress that can be used to rapidly and accessibly evaluate the pre-release status of fish after fisheries encounters (Davis 2010); however, such tests have not previously been applied to grayling. In combination, the blood physiology and reflex impairment assessments provided an integrated evaluation of the effects of air exposure on exhaustive angling of grayling.

Methods

The study was conducted in the Lakselva watershed in Porsanger County, Finnmark, Norway, which is located near the town of Lakselv (70.0511°N, 24.9717°E). Surface water temperatures at angling locations on days samples were collected (5, 6, 7 and 25 August 2014) fluctuated between 17 and 18 °C. European grayling were captured using recreational 3–4 weight fly gear with floating lines, barbless J-hooks (sizes 10–16), and 6 lb fluorocarbon tippet. All grayling were handled in a rubberized knotless landing net. One treatment group was angled relatively rapidly (< 2 min) and blood sampled immediately to generate values of baseline blood physiology (Pankhurst 2011, Cooke *et al*. 2013). Other grayling were angled to exhaustion then assigned to one of three air exposure treatment groups developed to test the physiological alterations associated with post-capture angler behaviour scenarios, receiving 0, 10, or 120 s of air exposure to simulate time taken by anglers to photograph, measure, or otherwise observe the fish. Fish were held in the landing net in the water (for unhooking, reflex assessment) or out of the water (for air exposure). After air exposure, grayling were transferred to cylindrical black hypalon bags with mesh ends to allow water flow. The fish bags were placed in the river (*see* for example Donaldson *et al*. 2013) at areas where water velocity was sufficient (~20 cm s^{-1}) to ensure adequate oxygenation.

To assess physiological alterations associated with air exposure, grayling in the treatment groups were held for 30 min in recovery bags instead of being sampled immediately because the stress response takes time to manifest (Barton 2002, Pankhurst 2011, Cooke *et al*. 2013). The blood samples (< 1 ml) were drawn with Braun Omniflex®-F sterile 1 ml syringes with heparinized Braun Sterican® 0.60 × 60 mm hypodermic needles via venipuncture at the caudal peduncle. After being held on ice (Clark *et al*. 2011), blood was analyzed for pH as well as [lac^-], [glu], and [Na^+] with an Alere Epoc reader and a host mobile computer (Waltham, Massachusetts, USA), which is a portable point-of-care blood analysis unit that uses a BGEM test card (epoc BGEM CT-1004-00-00) for blood analysis. All pH values were corrected to temperature as in Ashwood *et al*. (1983). Presence of reflexes was assessed by manually performing tail grab and testing orientation by rolling the fish into a supine position in the water. Reflex tests were conducted in the water and at three occasions for each fish: immediately after capture, after air exposure treatment, and after 30 min holding beginning after treatment. We found that fish with impaired tail grab always had impaired orientation and therefore we combined the two into a single reflex impairment response variable. For grayling in the 0 s air exposure treatment, there was no assessment of reflexes after air exposure.

Data analysis

Analysis of variance (ANOVA) was used to compare fish sizes and fight times among treatment groups. ANOVA was also used to test whether blood-based physiological parameters pH, [lac^-], [glu], and [Na^+] differed across treatment groups. Normality of residuals for ANOVA models was assessed with the Shapiro-Wilk test and homogeneity of variance was assessed with Bartlett's test at $\alpha = 0.05$. A square-root transformation was applied to lactate values in order to maintain normality and homogeneity of variance of the residuals. Inclusion of fork length was considered for all models but dropped if not significant. The Tukey-Kramer HSD post-hoc *t*-test was performed on the final models to compare physiological values among treatment groups using the *multcomp* package (Hothorn *et al*. 2008) in the free open-source software package R (R Core Team 2014).

Reflex impairment tests were conducted at three time points and therefore we had a repeated measures design necessitating the inclusion of fish ID as a random effect. Because reflex assessment results were recorded as either impaired or not impaired, we used logistic mixed effects models with logit link functions as implemented by the R package *glmmML* (Broström 2013). These models were used to determine how reflex impairment is influenced by fish length, air exposure treatment group, and assessment point (i.e., after angling, after air exposure, after 30 min). Because all fish were assessed after capture, which was prior to air exposure treatment assignment, we assume that these assessments were independent of the treatment, but it is possible that the later assessments would interact with the different treatments to have a combined effect on reflex impairment. This complex change in relationship between treatment and assessment type required a unique arrangement of the data when fitting the logistic models. We created five indicator variables for inclusion within the models: data collected for the 0 s treatment and assessed after holding, and for each of the 10 s and 120 s treatment groups paired with each of the assessments after treatment and after holding. With this arrangement, we interpreted the intercept of the model as the effect that angling (i.e., first reflex assessment) had on reflex impairment because when all indicator variables were equal to zero, the data corresponded to fish assessed after capture. With the fish length and random effect variables, the full model constituted seven variables, plus an intercept. We then eliminated different combinations of variables from this full model and used Likelihood Ratio Tests (LRT) between the full and reduced models to infer the effects of air exposure on reflex impairment. All data are presented as mean ± 1 SD.

Results

Fifty-two grayling captured for this study by two anglers were divided into four groups of 13. Grayling were 32 ± 4 cm and were played for 104 ± 51 s. Fight duration and fork length were not significantly different among the treatment groups (ANOVA: $F_{3,48} = 1.53$, $p = 0.22$; $F_{3,48} = 0.86$, $p = 0.47$). None of the grayling died during handling.

Blood pH differed between baseline and treatment groups (ANOVA: $F_{3,48} = 13.14$, $p < 0.01$) and decreased with fork length (ANOVA: $F_{3,48} = 6.42$, $p = 0.02$). Multiple comparisons of air exposure treatments indicated that whereas all treatment values were significantly different from baseline, none of the air exposure treatment groups had significantly different values from one another (Fig. 1). Similarly, plasma concentrations of [glu] and [lac$^-$] differed among treatments (ANOVA: $F_{3,48} = 5.50$, $p < 0.01$; $F_{3,48} = 104.3$, $p < 0.01$), but this was driven by low baseline values and none of the air exposure treatment groups resulted in significantly different values (Fig. 1). [Na^+] differed across treatments (ANOVA: $F_{3,47} = 3.66$, $p = 0.02$; Fig. 1), specifically [Na^+] was higher for 120 s air exposure treatment than baseline (Tukey-Kramer HSD: $t = 2.70$, $p = 0.046$). Fork length was also a significant factor contributing to variation in [Na^+] (ANOVA: $F_{1,47} = 8.48$, $p < 0.01$), with longer grayling tending to have higher [Na^+].

In all three treatment groups there were four grayling (proportion = 0.31) with impaired reflexes immediately after angling (Fig. 2). We fitted a total of five mixed effects models to the reflex impairment data and compared them using LRTs to test for significant factors (Table 1). Positive coefficients in models suggest that the corresponding variables increase the probability of impairment, whereas negative coefficients suggest a decrease. Reducing the full model by removing indicator variables and assessing the reduction with LRTs allowed us to make inferences on the significance of the main effects. The first reduced model (R1) tested whether air exposure was a significant impairment factor by eliminating all indicator variables corresponding to "after treatment" (10/AT and 120/AT) and incorporating only the "after recovery" indicator variables (0/AR, 10/AR, and 120/AR). R1 was significantly different from the full model ($p < 0.05$), suggesting that air exposure significantly impacted reflex impairment. The second reduced model, R2, tested the effect of 30 min on reflex recovery by replacing the "after recovery" indicator variables (0/AR, 10/AR, and 120/AR) variables with a composite indicator variable (Ψ; Table 1) equal to their sum for each fish. We also found R2 to be significantly different

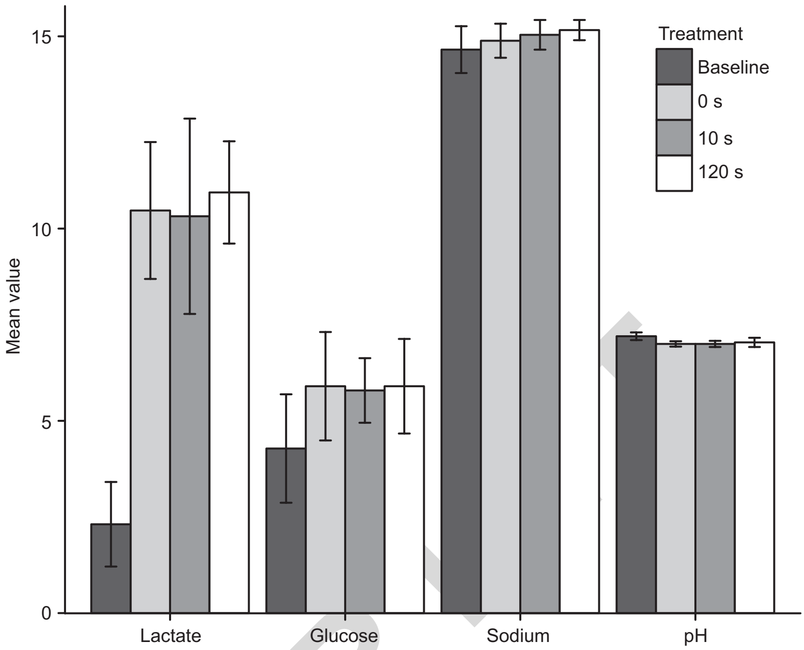

Fig. 1. Mean ± 1 SD blood parameters measured for European grayling (*Thymallus thymallus*) from a baseline group (blood sampled immediately after angling) and three air exposure treatment groups (i.e., air exposure intervals of 0 s, 10 s, 120 s). Glucose, lactate, and sodium values were measured in mmol/l whereas pH was measured logarithmically (i.e., 0–14). Sodium values were divided by 10 to fit the scale of the figure. According to the Tukey-Kramer HSD post-hoc test, baseline values are different from treatment values for glucose, lactate and pH.

from the full model, suggesting that fish from different air exposure treatment groups had different degrees of recovery after 30 min, attributable to differences in air exposure duration. The third reduced model, R3, included only the intercept, 0/AR, 10/AR, and 120/AT. We found a non-significant difference, suggesting that one or more of these variables could be deleted from this model; however, eliminating 120/AT or 0/AR produced highly significant LRTs, so these variables were included. Moreover, elimination of 10/AR generated weak evidence that the model was too reduced (model R4; Table 2; $p = 0.10$) so we chose R3 as the best model. This final model suggests that 0 s air exposure greatly reduced the likelihood of reflex impairment, and that 10 s air exposure did not have a significant effect on reflex impairment. However, 120 s air exposure significantly increased the likelihood of reflex impairment. In addition, 120 s air exposure resulted in prolonged reflex impairment given that recovery was incomplete after 30 min.

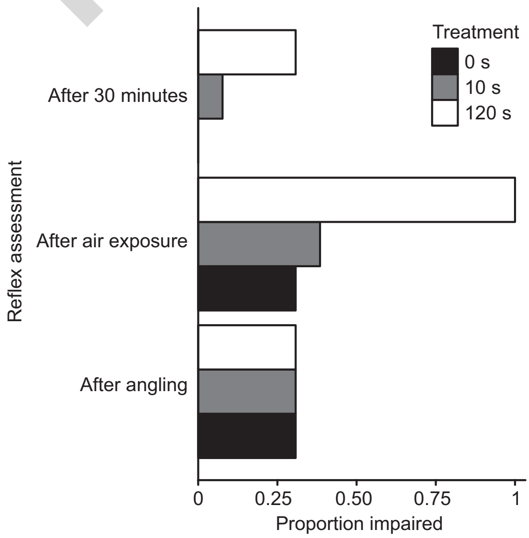

Fig. 2. Proportion of European grayling exhibiting reflex impairment after angling and exposure to one of three air exposure intervals. Reflexes were tested at three different time points: immediately after landing, after administration of the air exposure treatment (0, 10 or 120 s), and after 30 min holding in a flow-through submerged holding chamber.

Discussion

A brief air exposure interval of 10 s could be an acceptable upper threshold for anglers participating in recreational grayling fisheries. Few studies have previously studied the effects of brief air exposure intervals on fish in recreational fisheries, instead focusing on longer intervals (≥ 30 s). Yet, brief intervals are the most relevant to stakeholders because anglers and managers need to strike an appropriate compromise with respect to best handling practices (Cook *et al*. 2015). This is also one of the first studies to evaluate changes in reflex impairment of individual fish at different time points, allowing us to evaluate impairment and revival of fish exposed to the experimental stressors.

The degree of exhaustion and stress should increase the longer the individual is exposed to the stressor (e.g., fight time or air exposure interval; Gustaveson *et al*. 1991, Ferguson and Tufts 1992, Arends *et al*. 1999), theoretically until it plateaus at a maximum. Longer air exposure intervals in this study were anticipated to increase the magnitude of the secondary stress response of European grayling (e.g., Ferguson and Tufts 1992), but such a pattern was not observed. Indeed, grayling did not have different values of circulating [glu], [lac^-], [Na^+], or blood pH among air exposure treatment groups. Only after 120 s air exposure did [Na^+] differ significantly from baseline values. Thorstad *et al*. (2003) identified an increase in plasma [Na^+] of Atlantic salmon (*Salmo salar*) after angling, and postulated that it may have been related to size differences among treatment groups. Correspondingly, we identified size as a significant factor influencing [Na^+] in our model. However, increased [Na^+] may actually be attributable to [lac^-] production during extended anaerobiosis caused by air exposure, which creates an osmotic gradient in the blood (Wood *et al*. 1983). In our study, higher [Na^+] in the 120 s air exposed grayling relative to baseline was the only indication that long air exposure increased physiological stress relative to angling without air exposure.

Long air exposure after exercise (120 s) significantly increased the probability of reflex impairment whereas the brief air exposure interval (10 s) did not when compared to fish that did not receive air exposure. Furthermore, grayling air exposed for 120 s did not fully recover reflexes, whereas those exposed for 0 or 10 s recovered reflexes to a greater extent. Lack of response to tail grab can be influenced by muscular inhibition resulting from exhaustion of white muscle (Raby *et al*. 2012) used for burst-type exercise and fuelled by ATP, phosphocreatine, and glycogen (Milligan and Wood 1986, Dobson and Hochachka 1987, Wood 1991, Milligan 1996, Kieffer 2000). Intramuscular fuels were not measured and therefore exhaustion of ATP, glycogen, or phosphocreatine may have explained the reflex impairment observations better than the metabolic end products of the secondary stress response that we measured (Kieffer 2000). Impairment of the orientation reflex is more related to cognitive impairment resulting from insufficient oxygen delivery to the brain (Raby *et al*. 2015) and has been determined to represent a further departure from homeostasis that is more predictive of post-release mortality than loss of the tail-grab response (Raby *et al*. 2015).

Reflex impairment is an ecologically relevant metric for evaluating the impacts of stressors such as fisheries interactions, in part because inability to recover can increase instances of post-

Table 1. The coefficients and LRT *p* values for the five mixed effects models fitted to the reflex impairment data (*see* Methods for explanation of indicator variables). *p* values are for likelihood ratio tests comparing the full model to the reduced models. Reduced models that are significantly different from the full model (α = 0.05) indicate that removing terms has an effect on model performance. The best reduced model, R3, is therefore not significantly different from the full model and indicates which indicator variables are important to the model (*see* Results for interpretation). AR = after 30 min recovery, AT = after air exposure, FL = fork length, Ψ is an indicator variable that corresponds to summing the 0/AR, 10/AR, and 120/AR variables for each fish.

Model	Intercept	FL	0/AR	10/AT	10/AR	120/AT	120/AR	Ψ	*p*
Full	–5.49	0.14	–14.73	0.52	–2.12	15.37	–0.12	–	–
R1	–4.71	0.14	–10.84	–	–2.51	–	–1.03	–	< 0.001
R2	–5.55	0.14	–	0.53	–	13.49	–	–1.30	< 0.05
R3	–0.98	–	–10.39	–	–2.23	12.08	–	–	0.41
R4	–1.11		–9.49	–	–	11.62	–	–	0.10

release predation (Raby *et al*. 2014). The waters where we angled grayling were also home to large predatory northern pike (*Esox lucius*), and previous studies have identified increased predation of released fishes associated with reflex impairment resulting from fisheries interactions, including increasing reflex impairment with longer air exposure intervals (e.g., Danylchuk *et al*. 2007, Cooke *et al*. 2014). Releasing fish with impaired reflexes may increase mortality rates, particularly in areas with high predator density. Given our results, a more integrated study that incorporates tracking (e.g., with biotelemetry) of reflex impaired fish released by anglers would be necessary to evaluate the extent to which reflex impairment, post-release mortality, and post-release predation influence survival in recreational grayling fisheries.

All grayling were played, netted, handled, and recovered in a similar manner to ensure that these factors were standardized. Grayling were air exposed in nets rather than by hand in an effort to minimize epithelial damage and stress associated with handling. However, we observed fish struggling within the nets, behaviour that can also lead to abrasion and increased stress much the same way that handling does (e.g., Barthel *et al*. 2003, Colotelo and Cooke 2011), while also increasing the degree of muscular exhaustion and anaerobiosis relative to calm fish (e.g., van Raaij *et al*. 1996, Brownscombe *et al*. 2013). Holding fish prior to sampling is a challenge when using physiological metrics in the field because it can confound findings (Langkilde and Shine 2006, Cooke *et al*. 2013), which is why we opted for recovery bags for holding the fish. Nonetheless, it is possible that recovery bags may have increased stress and could explain why no significant differences were observed among air exposure treatment groups in terms of lactate, pH, and glucose (Gustaveson *et al*. 1991).

Conclusion

Even in the absence of notable increases in physiological stress detectable in blood, increased reflex impairment associated with protracted air exposure justifies elimination or considerable reduction of air exposure to short intervals (< 10 s) by anglers targeting European grayling at summer water temperatures. Fishery sustainability concerns have led to consideration of some catch-and-release management policies for maintaining grayling populations (e.g., Aas *et al*. 2000). However, little if any research has previously been conducted to determine the effects of recreational angling on grayling, even though other species in the order Salmoniformes (e.g., rainbow trout, Atlantic salmon, sockeye salmon) have received considerable attention from recreational fisheries researchers (Cooke and Suski 2005). Because limited data have previously been collected to quantify the response of European grayling to angling, the actual value of catch-and-release for conservation of this species is not fully understood. Therefore, the results of this study are particularly relevant for grayling conservation initiatives. Our finding that air exposure can increase probability of reflex impairment is pertinent to grayling fisheries given that released grayling are often held out of the water by anglers for photographs or general admiration. Further research efforts directed at identifying thresholds for air exposure with a specific emphasis on short air exposure intervals will be useful for generating best practice recommendations that anglers can use to maintain the welfare status of fish in recreational fisheries.

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