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Original Article

Ice cover alters the behavior and stress level of brown trout *Salmo trutta*

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Surface ice in rivers and lakes buffers the thermal environment and provides overhead cover, protecting aquatic animals from terrestrial predators. We tested if surface ice influenced the behavior (swimming activity, aggressive encounters, and number of food items eaten) and stress level (coloration of eyes and body) of stream-living brown trout *Salmo trutta* at temperatures of 3–4 °C in indoor experimental flumes. We hypothesized that an individual's resting metabolic rate (RMR, as measured by resting ventilation rate) would affect winter behavior. Therefore, groups of 4 trout, consisting of individuals with high, low, or mixed (2 individuals each) RMR, were exposed to experimental conditions with or without ice cover. Ice cover reduced stress responses, as evaluated by body coloration. Also, trout in low RMR groups had a paler body color than those in both mixed and high RMR groups. Trout increased their swimming activity under ice cover, with the highest activity found in high RMR groups. Ice cover increased the number of aggressive encounters but did not influence the number of drifting food items taken by each group. In mixed RMR groups, however, single individuals were better able to monopolize food than in the other groups. As the presence of surface ice increases the activity level and reduces stress in stream-living trout, ice cover should influence their energy budgets and production. The results should be viewed in light of ongoing global warming that reduces the duration of ice cover, especially at high latitudes and altitudes.

Key words: **aggression, climate change, energy budget, metabolic rate, winter.**

INTRODUCTION

Animals face a trade-off between seeking shelter from predation and finding foraging opportunity. In heterogeneous environments, this trade-off means that susceptible individuals should adjust their behavior according to spatial and temporal variation in shelter availability (Fischer and Lindenmayer 2006; Briones-Fourzán et al. 2007; Orpwood et al. 2010). Protection provided by overhead structures has been shown to influence activity and habitat use of animals with potential consequences for ecosystems stability (Jensen et al. 2003; Flores et al. 2012). Behavioral effects of overhead cover have been observed for many taxa from a diverse range of environments, including terrestrial velvet geckos *Oedura lesueurii* (Pike et al. 2010), freshwater crayfish *Orconectes virilis* (Fero and Moore 2014), and marine Atlantic cod *Gadus morhua* (Gotceitas and Brown 1993). During winter, freshwater systems in temperate regions are commonly covered by surface ice that reduces or completely eliminates access to aquatic prey for terrestrial predators (Meyers et al. 1992). With the ongoing climate change, however, periods with ice cover have become shorter (Gebre et al. 2014). For instance, Magnuson et al. (2000) investigated the freeze-up and ice-breakup trends for 26 rivers and lakes in the Northern Hemisphere between 1846 and 1995. On average, the ice-covered season has decreased by 1.23 days per decade. Benson et al. (2012) updated this study with additional lakes and more recent data (1855–2005) and found that freeze-up occurred 0.3–1.6 days later, whereas breakup occurred 0.5–1.9 days earlier per decade during this 150-year-long period. Given the importance of ice cover for aquatic organisms, reductions in ice cover during winter may have consequences for survival, production, energy use, and behavior (Finstad et al. 2004; Doney et al. 2012; Setzer 2012; Hedger et al. 2013).

Lack of shelter may increase stress levels and alter the physiological performance of animals (Boström and Mattila 1999; Templeton and Shriner 2004; Millidine et al. 2006; Bonnet et al. 2013). Behavioral responses to the presence or absence of

shelter may vary between individuals (Oppel et al. 2009), and such responses have been shown to be related to resting metabolic rate (RMR) (Millidine et al. 2009). The absence of ice cover in freshwater systems may thus affect energy budgets and behavioral attributes differently among individuals, which has bearing on how reductions in ice cover affect overwinter survival and production. Here, we examine the effects of ice cover on metabolic costs and behavior, including individual variation, using the study organism brown trout *Salmo trutta*, a salmonid commonly occurring in northern temperate and Arctic fresh waters.

Salmonids are known to increase site fidelity, daytime activity, and use of fine substrate habitats under surface ice (Linnansaari et al. 2008, 2009). Moreover, Helland et al. (2011) found that brown trout and Arctic charr *Salvelinus alpinus* reduced their RMR under simulated ice cover, and for brown trout, the presence of surface ice has been shown to reduce ventilation rates and increase their willingness to forage (Watz et al. 2013). Similarly, juvenile Atlantic salmon *Salmo salar* often have negative energy budgets in the absence of ice cover (Finstad et al. 2004), resulting in reduced overwinter survival rates (Linnansaari and Cunjak 2010; Hedger et al. 2013). Thus, the presence of surface ice, which reduces predation risk, may have positive effects on energy budgets by reducing stress and increasing foraging activity (Metcalfe and Thorpe 1992; Bull et al. 1996; Finstad et al. 2004; Watz et al. 2013).

Within salmonid populations, RMR varies, but individual differences appear permanent and repeatable over years (McCarthy 2000; Nespolo and Franco 2007) and form important precursors for individual resource consumption and allocation of energy for survival, growth, and reproduction (Metcalfe et al. 1995; Brown et al. 2004; Reid et al. 2012). RMR in salmonids has therefore attracted substantial attention by researchers. For instance, RMR has been shown to be positively related to dominance status (Yamamoto et al. 1998) and the ability to obtain a feeding territory (Metcalfe et al. 1995; Reid et al. 2012). Furthermore, the cost of aggressive interactions depends on dominance status, where subordinates have higher metabolic costs than dominants (Sloman et al. 2000; Lans 2012). Also, the presence of a conspecific can affect metabolic costs, where an individual decreases its metabolic rate in the presence of a smaller conspecific and increases its metabolic rate in the presence of a larger conspecific (Millidine et al. 2009).

At low temperatures, stream salmonids have small energetic needs and their physical abilities are impaired, reducing their scope for activity (Huusko et al. 2011; Jonsson and Jonsson 2011) and their ability to swim (Webb 1978; Rimmer et al. 1985; Graham et al. 1996) and drift-feed (Hill and Grossman 1993; Watz and Piccolo 2011; Watz et al. 2014). The overall low activity in winter may also be related to predator avoidance, as stream-dwelling fish in cold water are vulnerable to predation and must minimize exposure. As a consequence, stream salmonids in cold water reduce daytime activity (Heggenes et al. 1993; Greenberg et al. 1996; Orpwood et al. 2006), territoriality, and aggressiveness (Vehanen and Huusko 2002), even though they may still compete for instream shelter (Harwood et al. 2001, 2002; Griffiths et al. 2003; Orpwood et al. 2004). As ice cover presumably affects predation risk and may alter the nature of intraspecific interactions between trout, it is reasonable to assume that there will be differences in stress levels among individuals and depending on social context. Salmonids respond to stress by increasing gill ventilation rates (Hawkins et al. 2004) and by darkening of eye and body

coloration (O'Connor et al. 1999), and these attributes should therefore be affected by ice cover.

Here, we tested in experimental stream channels 1) if surface ice reduces stress, as indicated by ventilation rate and eye and body coloration; 2) if surface ice increases swimming activity, the level of aggression, and the rate of drift-feeding; and 3) if group composition, based on RMR, influences the behavior and stress level of each individual in the groups. We predicted that ice cover would reduce stress, increase swimming activity, drift-feeding, and aggression and that the effects would be strongest for individuals exhibiting high RMR. Thus, the present research may offer a behavioral explanation for why reduced ice cover has a negative effect on energy budgets of salmonids and reduces fish production.

MATERIALS AND METHODS
Fish

Young-of-the-year, fourth-generation, hatchery-reared brown trout provided by the Gammelkroppa hatchery (Fortum Generations AB, Sweden) were used in the experiment. The strain originated from 200 wild trout from Lake Konnevesi, Finland, and the batch used in this experiment consisted of approximately 20 000 eggs from 40 females fertilized by 40 males. During the first month after emergence, fry were fed commercial salmonid feed (Aller Performa, Aller Aqua, Christiansfeld, Denmark) during the day, and after 1 month, the feeding regime was changed so that the trout were instead fed at dawn and dusk.

Compared with wild conspecifics, the antipredator response of hatchery-reared fish is reduced but not lacking (e.g., Johnsson et al. 2001; Fritts et al. 2007). Furthermore, hatchery-reared fish tend to be diurnally active (Álvarez and Nicieza 2003) and aggressive (Metcalfe et al. 2003), which facilitates observation of aggressive behavior in laboratory experiments. These differences between hatchery-reared and wild fish might limit the generalization of our results, based on hatchery-reared fish. Yet, this difference should not be crucial for our study as wild trout with high antipredator responses should be affected even more by the presence of overhead ice cover than hatchery-reared trout with lower antipredator responses, thus making our interpretation conservative.

In November 2013, the trout were moved to the aquarium facility at Karlstad University and held in the pool sections of 7-m-long indoor stream channels while being acclimated to laboratory conditions. The fish were fed 1 mm pellets (Inicio Plus G, Biomar SAS, Nersac, France). Photoperiod was set to 14:10 (light:dark) and water temperature to 6 °C.

In November and December 2013, the opercular beating frequency during ventilation (ventilation rate), a proxy for metabolic rate (Millidine et al. 2008), was measured after the trout had been kept individually in darkness for approximately 16 h in 200-L tanks ($n = 12$; water temperature ± standard deviation [SD]: 5.8 ± 0.6 °C). We used infrared lamps (IR illuminator No. 40748, Kjell & Co Elektronik AB, Malmö, Sweden) and a video camera (Canon XA10, Canon Inc., Tokyo, Japan), using the infrared mode, to film trout and measure resting ventilation rate in darkness. Three consecutive film sequences of at least 30 s each were used, and the median resting ventilation rate from each film was calculated (Watz et al. 2013). Pilot testing showed that 12 h was sufficient for the trout to reach their resting ventilation rate, that is, their lowest stable rate. This pilot testing also showed that trout with high resting ventilation rate did not switch to low resting ventilation rate, and vice versa, after 24 h.

The 3 film sequences of 30 s were repeated after an hour, so that each trout was screened twice (6 measurements of resting ventilation rate). The second round of measurements was performed as an extra check and to prevent false high resting ventilation rates as a consequence of activity prior to the measurements. Discrepancy between the 2 measurements was only found in 5 cases, and these trout were discarded. To establish groups of trout with different RMR, we defined 2 types of trout, one with low (below 37 beats min^{-1}) and one with high (above 43 beats min^{-1}) resting ventilation rate. In total, we screened 151 trout (Figure 1) and selected 48 trout (mean length ± SD and mean wet mass ± SD: 97.5 ± 5.7 mm and 8.7 ± 1.6 g), 24 trout with low and 24 trout with high ventilation rate, for the experiment. There were no differences in length or mass between trout with low and with high ventilation rates (Student's t-tests, t_{length} = 0.756 and t_{mass} = 0.362, degrees of freedom [df] = 46, $P > 0.05$).

Trout were size-matched in 12 groups of 4 individuals, based on their lengths (mean maximum length difference within groups ± SD: 3.1 ± 1.8 mm). We size-matched the trout to minimize confounding effects of size on fish behavior, for example, aggression and feeding. Four of the 12 groups contained trout with low RMR, 4 groups contained trout with high RMR, and 4 groups contained 2 trout with low and 2 trout with high RMR. These groups are henceforth referred to as high, low, and mixed RMR groups, respectively. There was no length difference among the 3 group types (Anova, F = 0.59, df = 2, 9, $P > 0.05$). All groups were held in separate 200-L tanks at approximately 5–6 °C and fed chironomid larvae. Three trout in one of the low RMR groups died due to technical problems with a water pump. This group was excluded, and we therefore only used 3 low RMR groups, resulting in a total of 11 groups, for the experiment. These 11 groups were assigned unique group IDs to be used in statistical evaluations. Trout were individually tagged with visual elastomer implants (Northwest Marine Technology Inc., Shaw Island, WA), injected into the dorsal and caudal fins so that each trout in a group could be visually identified.

Stream tanks

Experimental arenas measuring 1.0 × 0.7 × 0.4 m (length × width × water depth) were created in the first sections of 3 artificial stream channels. On the side of each stream, a glass window enabled us
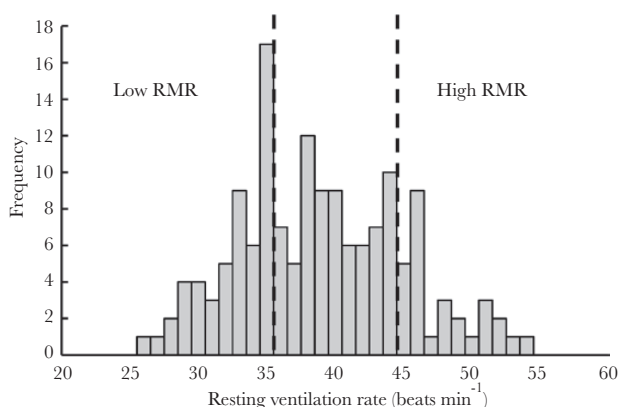

Figure 1
Brown trout (n = 151) were screened for resting ventilation rate. Trout with resting ventilation rates below 37 and above 43 beats min^{-1} were classified as trout with low and high resting ventilation rate, respectively, and used in the experiment. Trout with intermediate resting ventilation rates were discarded.

to observe and film the fish. Water velocity at 60% of the depth in the middle of the arena was 4 cm s^{-1}, and discharge was 8 L s^{-1}. We used filtered copper-free water, and water temperature was controlled by a built-in chilling/heating system, which maintained temperature between 3 and 4 °C. Although water temperature is usually near 0 °C in the presence of ice in natural streams, water temperature may be well above 0 °C during cold spells at ice formation in early winter and before ice-breakup in late winter (Jakober et al. 1998; Brown et al. 2000). In the stream tanks, thawing ice did not change the water temperature by more than 0.1 °C. Gravel (0.5–3 cm) covered the bottom, and in the middle of the experimental arena, we placed a concrete block (10 × 16 cm), supported by 4 iron legs, to provide the fish with instream cover.

We froze distilled water in plastic trays (Dilling, IKEA AB, Älmhult, Sweden), and these ice blocks (70 × 60 × 6 cm) were suspended immediately above the water surface to create ice cover. Two ice blocks were used to cover the entire experimental arena. The presence of ice cover reduced light levels by approximately 50%. Fluorescent tubes (Master TL5HE 145 35W/830, Philips NV, Amsterdam, The Netherlands), positioned over the experimental arena, produced 700 Lx at the surface for 10 h each day. Trout were fed chironomid larvae by pumping (Universal 1046, Eheim GmbH & Co KG, Deizisau, Germany) them together with water through a funnel, which was connected to a hose. The opening of the hose was situated near the surface at the upstream end of the experimental arena so that prey could drift in the water column.

Experimental design and execution

The experiment was performed in January and February 2014, and 2 treatments were used in the experiment: presence and absence of ice cover. We used a repeated measures design in which each group of 4 trout was tested both with and without ice cover. Before initiating a trial, trout were starved for 48 h. The groups were tested in random order, and the stream aquarium that was used for each trial was randomly selected. Trout were carefully transferred from their holding tanks to the experimental arena the day before a trial. Ice was suspended over the experimental arena in the morning, approximately 1 h before the lights were turned on. For trials without ice cover, we put a sheet of polystyrene over the experimental arena and then removed it to disturb the fish in a similar way as in trials with ice cover. Trials were performed during the day and started at least 1 h after turning on the lights.

During a trial, we used 2 video cameras (Canon XA10, Canon Inc.). Video camera 1 was set to film the entire experimental arena, and video camera 2 was used to zoom-in and focus on each individual trout. Two chironomid larvae were fed to the trout 2 min before the start of a trial. A trial consisted of the following sequence of actions: 1) observing and filming all fish with camera 1 and filming a close-up video on 1 of the 4 trout with camera 2 for 2 min, 2) release of 2 chironomid larvae into the stream, 3) observing and filming in the same way as in (1) for 2 min, 4) release of 2 chironomid larvae into the stream, and 5) observing and filming in the same way as in (1) for 2 min. This sequence was repeated for each trout individual, and the order in which the different trout were filmed with camera 2 was randomized. With this design, a trial resulted in a 24-min film sequence of the entire experimental arena from camera 1 and four 6-min film sequences, one for each trout, from camera 2.

Data analysis and statistical model

For each trial, we used direct observations in combination with watching the films from both cameras to be able to identify the

individual trout (from their elastomer implant tag), to count the number of chironomid larvae that each trout consumed, and to quantify the types of aggressive interactions (lateral displays, chases, and nips) performed by each trout. In addition, we used the first 120 s of the close-up videos from camera 2 to assess stress coloration and measure ventilation rate and swimming activity for each of the 4 individuals.

Stress coloration was quantified by scoring the eye and body color (O'Connor et al. 1999; Suter and Huntingford 2002) on scales from 1 to 5, where a score of 1 represents fish with low stress levels (pale sclera and iris; pale body) and 5 represents fish with high stress levels (dark sclera and iris; dark body). Resting ventilation rate could not be recorded, as the trout were active during the trials. Nevertheless, we recorded ventilation rates using a film sequence of at least 30 s.

The proportion of time that the 4 fish spent swimming was calculated from the 120-s initial film sequence. A trout was considered swimming if it was moving actively or holding station by hovering over the bottom. Many trout were either actively swimming during the entire 120-s sequence or did not swim at all. Therefore, we classified the swimming activity of the trout into 4 categories: completely sedentary, moderately active, intermediately active, and completely active. Trout in the first category rested motionless on the bottom for the entire 120-s period, whereas trout in the last category swam actively during the same period. Trout in the second and third categories swam for less and more than 50% of the time, respectively, but swam for at least 1 s and rested for at least 1 s.

To test for effects of ice cover and group type on aggression, feeding, and ventilation rate, we used linear mixed models with individual fish treated as subjects and ice cover as a repeated variable. We included the factor group ID (the identity of the groups of 4), nested within group type, as a random effect in the analyses to avoid pseudoreplication. For stress coloration and swimming activity (scores and categories), we used generalized estimating equations with a multinomial distribution applied with the cumulative logit link function to test for effects of ice cover and group type, with the factor group ID nested within group type. Nonsignificant terms ($P > 0.05$), except from the nested term, were stepwise removed from all models. The least significant term was removed first. To test for differences between groups, we used repeated measures Anova and Kruskal–Wallis H-tests (scores). All statistical analyses were performed with SPSS Statistics 20 (IBM, Armonk, NY).

RESULTS

During the course of a trial, body color was relatively consistent. In contrast, eye color could shift 1 or occasionally 2 steps on the 5-graded scale. A generalized estimating equation revealed that trout had lighter body color in the presence of ice cover than in its absence, and trout in the low RMR group had lighter body color than trout in the high and mixed RMR groups (Table 1 and Figure 2). The trout exhibiting the darkest coloration in mixed RMR groups tended to have darker body color than the darkest colored trout in the low RMR groups, and a Kruskal–Wallis H-test indicated that group type affected the body color of the darkest colored trout in each group (sum from iced and ice-free conditions, $\chi^2 = 7.4$, df = 2, $P = 0.05$). This pattern was not found when comparing the body color of the lightest colored trout among groups with a Kruskal–Wallis H-test (sum from iced and ice-free conditions, $\chi^2 = 0.32$, df = 2, $P > 0.05$). This discrepancy may indicate that trout exhibiting dark stress coloration in low RMR groups

Table 1

Results from linear mixed effect models and generalized estimating equations for brown trout, detailing the dependent and significant (α = 0.05) explanatory variables for each final model

Dependent variable	Significant explanatory variables	F/χ^2	df	Significance
Mixed effect models				
Lateral displays	None			
Chases	Ice cover	6.15	1, 47.4	0.017
Nips	Ice cover	6.11	1, 51.3	0.017
Total aggressive interactions	Ice cover	5.04	1, 57.7	0.029
Prey captures	None			
Ventilation rate	None			
Generalized estimating equations				
Body color	Ice cover	7.42	1	0.006
	Group type	11.00	2	0.004
Eye color	None			
Swimming activity	Ice cover	4.24	1	0.040
	Ice cover × group type interaction	7.54	2	0.023

Trout were tested in groups of 4, where group type refers to whether the trout belonged to a group of trout with low, high, or mixed resting ventilation rates. Candidate explanatory variables were the presence or absence of ice cover, group type, ice cover × group type interaction, and stream channel. Group ID nested within group type was included in the analyses. Nonsignificant variables, with the exception of the nested term, were removed from models in a stepwise fashion to produce minimum adequate models.

were not as stressed as those in mixed RMR group. In contrast, trout exhibiting little stress coloration were not affected by conspecifics in the same way. Mean ventilation rate during the trials was not affected by group type or ice cover (Table 1), and it was not possible to control for behavior prior to or simultaneous with measuring ventilation rate.

Trout increased their swimming activity under ice cover (Table 1). The mean proportion of time that the fish spent swimming under the ice was 38%, whereas it was only 21% in the absence of ice cover. Furthermore, in the presence of ice cover, 32% of the trout were completely sedentary and 18% swam for the entire 120-s sequence. In the absence of ice cover, the proportion of completely sedentary trout increased to 55% and completely active trout decreased to 9%. This pattern was most pronounced in trout from the high RMR groups, which was revealed by a significant group type × ice cover interaction term in the generalized estimating equation (Table 1). The presence of ice cover increased the mean swimming time in the high RMR groups by almost 400% compared with 56% in the mixed and no increase in the low RMR groups.

Fish were relatively aggressive, and almost every fish performed an aggressive display, chase, or nip at least once. The rate of aggressive interactions in individual trout ranged from 0 to 2.5 min^{-1}, with an overall mean of 0.3 min^{-1}. A linear mixed model revealed that the presence of ice cover increased the rate of aggressive interactions in all types of groups, and group type did not affect aggression (Table 1 and Figure 3). When analyzing the different types of aggressive interactions separately (displays, chases, and nips), linear mixed models showed that ice cover had significant effects on the number of nips and chases, but not on the number of displays (Table 1 and Figure 3).

On average, 80% of all prey were captured, and there was no effect of ice cover or group type on the number of drifting chironomid larvae taken (Table 1). However, when comparing the number

of captured prey by the most successful feeder in each group, a repeated measures Anova showed that there was a difference among group types ($F = 9.2$, df = 2, 8, $P = 0.01$), and the most
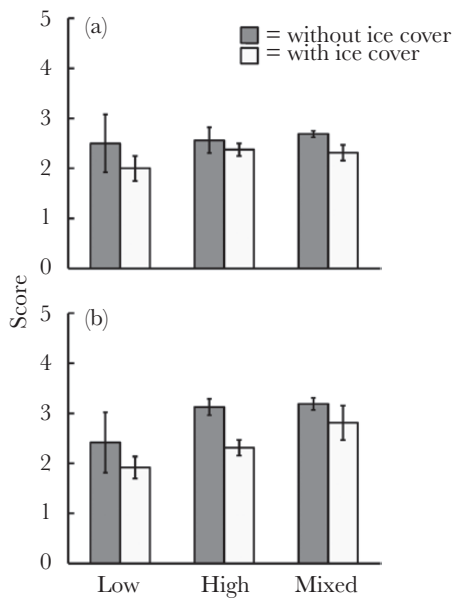
successful feeder in the low, high, and mixed groups captured on average 38%, 52% and 67% of the prey, respectively. A Bonferroni adjusted pairwise post hoc comparisons revealed that the degree of monopolization of the food was higher in the mixed than in the low RMR groups ($P = 0.01$). There were no effects of ice cover ($F = 0.01$, df = 1, 8, $P > 0.05$) or the group type × ice cover interaction ($F = 0.18$, df = 2, 8, $P > 0.05$) on the number of captured prey by the most successful feeder.

DISCUSSION

As predicted, the presence of surface ice influenced the behavior of brown trout. Under the ice, the trout left their holding positions on the bottom and actively swam in the stream channel to a greater extent than under ice-free conditions. The increase in activity was most pronounced in the high RMR groups. During winter, at temperatures below 8 °C, juvenile stream-living salmonids are assumed to be inactive during daytime, seeking cover within the substrate or other sheltered locations (Rimmer et al. 1983; Cunjak 1988; Heggenes et al. 1993). However, the present study demonstrated that in the presence of surface ice, the fish often cruised in the water column. Linnansaari et al. (2008) reported a similar result for the closely related Atlantic salmon, where increased daytime activity of juveniles increased with increasing ice thickness. Hence, the relationship between activity and surface ice exists for 2 species and may well be a general phenomenon for stream fish. The changed activity pattern under surface ice likely reflects the trade-off between growth and survival, where surface ice reduces predation risk (thereby increasing survival) and allows trout to spend more time searching for food (Watz et al. 2013) and interacting with conspecifics (cf. Werner and Gilliam 1984; Railsback et al.
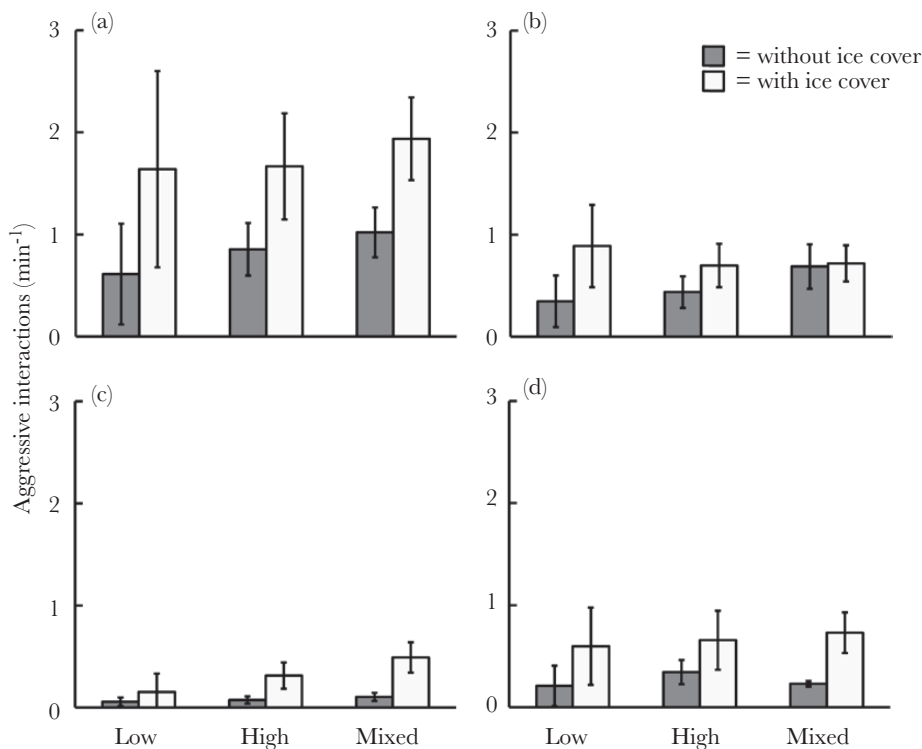

Figure 2
Mean stress coloration of brown trout (±SE) in groups with low ($n = 12$), high ($n = 16$), and mixed ($n = 16$) resting ventilation rates in the presence and absence of ice cover, (a) eye color, and (b) body color. Stress coloration was scored on a scale from 1 to 5 (1 = pale eye/body, unstressed trout; 5 = dark eye/body, stressed trout).

Figure 3
Mean aggressive actions of brown trout (min^{-1} ± SE) in groups with low ($n = 12$), high ($n = 16$), and mixed ($n = 16$) resting ventilation rates in the presence and absence of ice cover, (a) total number of aggressive interactions, (b) lateral displays, (c) chases, and (d) nips.

2005). In addition to swimming activity, aggression increased under the ice. Aggression often occurred when cruising fish swam near other individuals and thus may reflect an increase in encounter frequency among individuals. However, increased aggression may also be related to the reduced need for predator vigilance under ice cover. Both nipping and chasing, behavioral acts that more or less preclude predator vigilance, increased under ice, indicating that trout may be more willing to expose themselves when under ice due to the low risk of predation.

Earlier studies, especially on birds, viewed a high rate of resting metabolism in dominant individuals to be the cost of dominance (Senar et al. 2000), but recent evidence from a range of species has demonstrated that genetics, including epigenetic effects, may contribute to variation in RMR (Burton et al. 2011; Burton and Metcalfe 2014). It is perhaps surprising that ventilation rates during the trials did not differ among RMR group types, but this is likely because we could not control for the activity level of the fish. An additional explanation may be that individuals decrease their RMR in the presence of smaller conspecifics but increase them in the presence of larger conspecifics (Millidine et al. 2009). Thus, subordinates respond to the threat imposed by dominants by increasing their ventilation rate, whereas dominants are probably not threatened by subordinate individuals, but may instead gain protection from predators by the "dilution" effect (Milinski et al. 1997).

The skin and eye darkening observed in subordinate individuals is influenced by the melanocyte-stimulating hormone, a pituitary peptide that elicits chromatophore dispersion and increases the number of chromatophores (Höglund et al. 2000; Höglund et al. 2002). By changing color, subordinate fish are able to signal to dominants that they pose no threat, which appears to inhibit attacks from dominant conspecifics (Beeching 1995). O'Connor et al. (1999) found that both skin and eye darkening reflect low social status. Moreover, skin darkening is a general indication of stress (Ruane et al. 2005) and correlates with plasma cortisol levels (Iger et al. 1995). In our experiment, we found that skin color was easier to interpret and gave more consistent results than eye color when looking for signs of stress. The pale body coloration observed for fish under surface ice supports the hypothesis that fish living under ice cover are less stressed than those in streams without surface ice. As the presence of ice cover also increased the level of aggression, it seems likely that the low stress level under the ice was mostly the result of a reduced predation risk (Linnansaari et al. 2008; Watz et al. 2013), rather than an indication of social stress. Ice cover reduced the stress coloration of trout from each of the 3 RMR groups tested, indicating that predation risk during winter may be an important factor shaping behavior and affecting physiology regardless of fish type.

We found little support for our hypothesis that ice cover would affect fish with high RMR rates to a higher degree than fish with low RMR, as the only significant interaction effect between group type and ice cover was for swimming activity. Moreover, contrary to our expectation, neither group type nor the presence of ice cover affected the number of drifting prey taken by individual fish. This may be because the number of prey used for each trial was low relative to the feeding capacity of the fish. There was, however, an effect of group type on the ability of a single individual to monopolize the food. Single individuals in the mixed RMR groups were better able to monopolize the food than fish in the other groups. This difference may be because the mixed groups consisted of fish with the most variable RMR and probably also the highest variation in the degree of aggressiveness. The result is consistent with the concept of the "ideal free distribution" (Brown 1969; Fretwell

and Calver 1969). If animals are of equal competitive ability, the intake rates for all competitors should also be identical, but not if their competitive abilities differ (Parker and Sutherland 1986).

Ice cover acts both as a shelter from terrestrial predators and reduces the amount of light reaching the water (Watz et al. 2013). To a certain degree, reduced light intensity is known to stimulate salmonid activity (Valdimarsson et al. 1997; Valdimarsson and Metcalfe 2001). For instance, Young (1999) reported that during summer, both the proportion of active fish and the distance moved correlated negatively with light intensity. This is consistent with the crepuscular peaks in activity in brown trout described by, for example, Greenberg and Giller (2001) and Ovidio et al. (2002). Accordingly, individual growth of brown trout has been found to correlate positively with the length of the twilight period when light levels are low (Jensen et al. 2000). Thus, an increase in swimming activity under surface ice may be a response not only to overhead cover but also to the lower light levels, which would be lowest if the ice is covered by snow. Interestingly, light has been shown to be inversely related to activity level also for several nonsalmonid stream fishes (Prenda et al. 2000). It may be that predation risk in streams is typically low at twilight when light levels are low, providing fish with the opportunity to feed. In our study, there was no snow on the ice and the presence of ice only reduced light levels from 700 lx to approximately 350 lx, a light level corresponding to an overcast day and not twilight or night. The effects of light intensity on salmonid behavior within this range of light intensities is very small or nonexisting (Valdimarsson et al. 1997), and thus, it seems likely that the effect of ice on trout behavior is a response to overhead cover, more so than a response to light (Watz et al. 2013).

The results of our study are limited to winter conditions where light can penetrate the ice, and in complete darkness under snow-covered ice, it is possible that, for example, aggression is reduced by the lack of visual contact between fish. Also, our results might have been influenced by the use of hatchery-reared fish, although this probably made our assessment of the effects of ice cover to be conservative, because wild fish have stronger antipredator responses than hatchery-reared conspecifics.

The effects of global warming are expected to be strong at high latitudes and altitudes, posing a major threat to the resident species (Parmesan 2006; Post et al. 2009; Molnar et al. 2011). The length of the annual period with surface ice on rivers and lakes is decreasing (Gebre et al. 2014) and is expected to do so during the rest of this century (IPCC 2013). Aquatic animals in this region are adapted to live under ice during large portions of the year. A reduction in the duration of periods with ice cover will probably affect energy budgets of fish and other animals negatively (Finstad and Forseth 2006; Doney et al. 2012). Our findings show that under surface ice, trout extend their activity period to include daytime and exhibit less signs of stress, which may explain why salmonid production is higher in ice-covered rivers than in open stretches of rivers (Hedger et al. 2013). Global warming reduces the duration of periods with ice cover in high altitude and latitude areas, and this reduction is expected to influence the winter activity and production of salmonid fishes negatively.

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