Blood chemistry and swimming activity of rainbow trout exposed to supercooling and frazil ice

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Adult and juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to supercooled temperatures, frazil ice, and anchor ice in a refrigerated flume at the Cold Regions Research and Engineering Laboratory (Hanover, NH). The blood chemistry of the fish was measured before and after they were exposed to a frazil ice event. Plasma chloride, sodium and potassium levels were significantly reduced in juvenile rainbow trout after 6.5 h of exposure to supercooling, frazil ice and anchor ice. Plasma lactate did not vary but plasma glucose was increased although not in a statistically significant manner. Blood parameters of adult fish varied in a similar way as in juveniles but none of the changes were statistically significant. The swimming activity of half of the adult fish (measured by electromyogram telemetry) was significantly lower while exposed to frazil and anchor ice, and none were more active. The escape response of adult rainbow trout was decreased when they were exposed to supercooled water and frazil and anchor ice. This reduction in activity and escape response may increase the likelihood of avian or mammalian predation during subsurface ice events. These sublethal effects observed during exposure to supercooling and frazil ice suggest that further research is needed to determine how such events might impair fish survival.

1. Introduction

Several authors have shown that frazil and anchor ice can have effects on fish habitats in rivers and streams. Fish move out of pools in rivers when frazil ice fills much of them creating hanging dams (Brown et al. 1998). Anchor ice can also fill large parts of rivers excluding fish habitat and forcing fish to move to new habitats (Brown and Mackay 1995, Jakober et al. 1998, Brown *in press*). Other effects of anchor ice formations on fish are also reviewed by Brown et al. (1994). However, while it is known that frazil and anchor ice can affect fish habitat, the physiological effects of supercooling, and frazil ice on fish are unknown.

Although little evidence exists that frazil ice directly kills fish, mortalities of several juvenile trout have been noted after cold spells and frazil and anchor ice events (Brown et al. 1994). Brown et al. (1994) also speculated that frazil ice can plug the mouths and gills of fish, or abrade the gills. It has also been suggested that conditions during frazil ice events may be unsuitable for night active juvenile salmonids (Whalen et al. 1999). However, no strong direct links between frazil ice and physical damage have been found. Even if there is no physical damage to fish, stress created by these conditions may make fish avoid areas where frazil and anchor ice occur. This may be why Whalen et al. (1999) noted that few Atlantic salmon (*Salmo salar*) parr were observed on nights when anchor ice was forming or when the water was heavily laden with frazil ice. Since supercooling and frazil and anchor ice are common throughout winter below hydroelectric facilities, this topic is of particular interest.

To determine if fish are affected by supercooling or frazil and anchor ice, the activity and stress levels of rainbow trout (*Oncorhynchus mykiss*) were examined when exposed to these conditions in a large refrigerated flume. Juvenile and adult rainbow trout were exposed to ice conditions and blood was extracted to determine if fish exhibited a stress response. Electromyogram transmitters were implanted into adult rainbow trout to determine if swimming activity was affected.

2. Methods

2.1 Characteristics of the flume

The experiments were done in a refrigerated flume at the Ice Engineering Division of the U.S. Army Cold Regions Research and Engineering Laboratory. The set up of the flume and the conditions during the experiments are described in detail by White et al. (1999) and Kerr et al. (1997). Briefly, the flume is situated in a room where the temperature can be regulated between +18 and –29 °C. The flume is 0.61 by 1.22 m in cross section and 36.6 m long. It can tilt from +2 to –1° slope, has a flow capacity of nearly 0.4 m^3 per second and has a refrigerated bottom. For these experiments, the bed of the flume was insulated and covered with two layers of clean, uniform, well-rounded cobbles having a median diameter of 4 cm. Boulders with a nominal diameter of 19 cm were placed in the flume at irregular intervals to provide areas of lower velocity during the activity tests.

2.2 Experimental animals

Rainbow trout were obtained from High on a Hill Hatchery (Plainfield, NH) and held at 0-1 °C in flow-through tanks for 1 week before the beginning of the experiments. Fish were not fed during the week preceding the experiments.

2.3 Stress determined from blood analysis

The induction of a stress response by supercooling and frazil ice was assessed through the analysis of blood chemistry in juvenile and adult rainbow trout. Average body weight and fork length of juvenile fish were 37±2 g and 141±3 mm respectively (mean ± S. E.). Average body weight and fork length of adult fish were 1036±44 g and 426±7 mm respectively (mean ± S. E.).

Juvenile and adult rainbow trout were transferred into the flume the day before the experiment to allow them to acclimate to the flume conditions and recover from the stress of transfer. Juveniles were evenly divided into 2 sections of the flume 2 m long while adults were distributed over 2 sections 8 m long. Before refrigeration began, one group of juvenile fish and one group of adults were captured with a dip net and anaesthetized by immersion in an aqueous solution of clove oil (120 mg L^{-1}) as described by Anderson et al. (1997). A blood sample was then taken from the caudal vasculature using a heparinized syringe and the plasma was separated by centrifugation and frozen in liquid nitrogen until later analysis. These two groups of fish were used as controls. The remaining fish were exposed for 6.5 h to supercooling, frazil ice and anchor ice before being captured and blood sampled as described for the control groups. Plasma levels of potassium, chloride, sodium, glucose and lactate were later measured with an automated blood analyzer (Stat Profile 9+, Nova Biomedicals).

2.4 Activity determined from electromyogram telemetry

Adult rainbow trout were implanted with electromyogram (EMG) radiotransmitters which allow swimming activity to be quantified. Implanted fish were exposed to supercooled temperatures, frazil ice, and anchor ice. Two replicates of fish were implanted with EMG radiotransmitters using methods similar to McKinley and Power (1992) The first group (N=7, weight 1154 ±95 g; fork length 446 ±16 mm; mean ± SE) was implanted on November 19, 1998. The second group (N=5, weight 1026 ±46 g; fork length 425 ±3 mm; mean ± SE) was implanted on November 21, 1998. After fish had recovered from anesthesia, they were placed back in a holding tank and allowed to recover for three days before signals were recorded.

The fish that were implanted with EMG transmitters on November 19 were placed in the flume in the afternoon of Nov. 22. EMG signals were recorded from the evening of Nov. 22 through the afternoon of Nov. 24. The temperature in the cold room was decreased in the early morning of Nov. 23 and the fish were exposed to two periods of supercooling, one 3.3 hours long and one 4.9 hours long, with a minimum water temperature of -0.011 °C. The second replicate of fish (implanted on Nov. 21) were placed in the flume in the afternoon of Nov. 24. EMG signals were recorded from the evening of Nov. 24 through the afternoon of Nov. 26. The temperature in the cold room was decreased in the early morning of Nov. 25 and the fish were exposed to two periods of supercooling, one 3.7 hours long and one 3.2 hours long, with a minimum water temperature of -0.018 °C. During both replicates, fish were allowed to acclimate to the flume for four hours before EMG signals were used. Fish were tested in a 10 m long section of the flume which included a boulder randomly placed in each 1 m^2.

Fish were held in different sections of the flume by plastic screen dividers, which gradually accumulated anchor ice and eventually water flow through them would be blocked. To prevent this, the anchor ice was scraped off the dividers when necessary. This was done carefully so that fish were disturbed as little as possible. However, since disturbing fish at some times was inevitable, the data for a period of five minutes (starting at the beginning of divider clearing) was removed from the fish swimming activity data used to calculate differences between the test and control period. To determine if the amount fish were disturbed (as indicated by swimming activity) was related to the amount of time in supercooled water, the swimming activity data for each five minute clearing period was averaged across all fish and regression analysis was performed on these data at different amounts of time since supercooling had commenced. There were two separate supercooling events on both test days, since water temperatures rose above freezing when the refrigeration units were defrosted.

Differences in swimming activity between the control period and the test period were determined for each fish. These differences were determined with a *t*-test or with a Mann-Whitney *U*-test when data were not normally distributed. Differences were considered significant if $P<0.05$. Differences in blood chemistry between the control period and the test period were determined in a similar manner.

3. Results

Changes in blood parameters indicated that juvenile rainbow trout were stressed by the presence of supercooling and subsurface ice. Plasma chloride, sodium and potassium levels were significantly reduced in juvenile rainbow trout after 6.5 h of exposure to supercooling, frazil ice and anchor ice (Fig. 1). Plasma lactate did not vary but plasma glucose was increased although not in statistically significant manner (Fig. 1). Blood parameters of adult fish varied in a similar way as in the juveniles but none of the changes were statistically significant (Fig. 2).

Fish swimming activity was changed by the presence of supercooled water, frazil and anchor ice. Out of the 12 fish exposed to test conditions, 6 (50%) were significantly less active during the test period than the control period (Fig. 3). None of the fish were significantly more active during the test period.

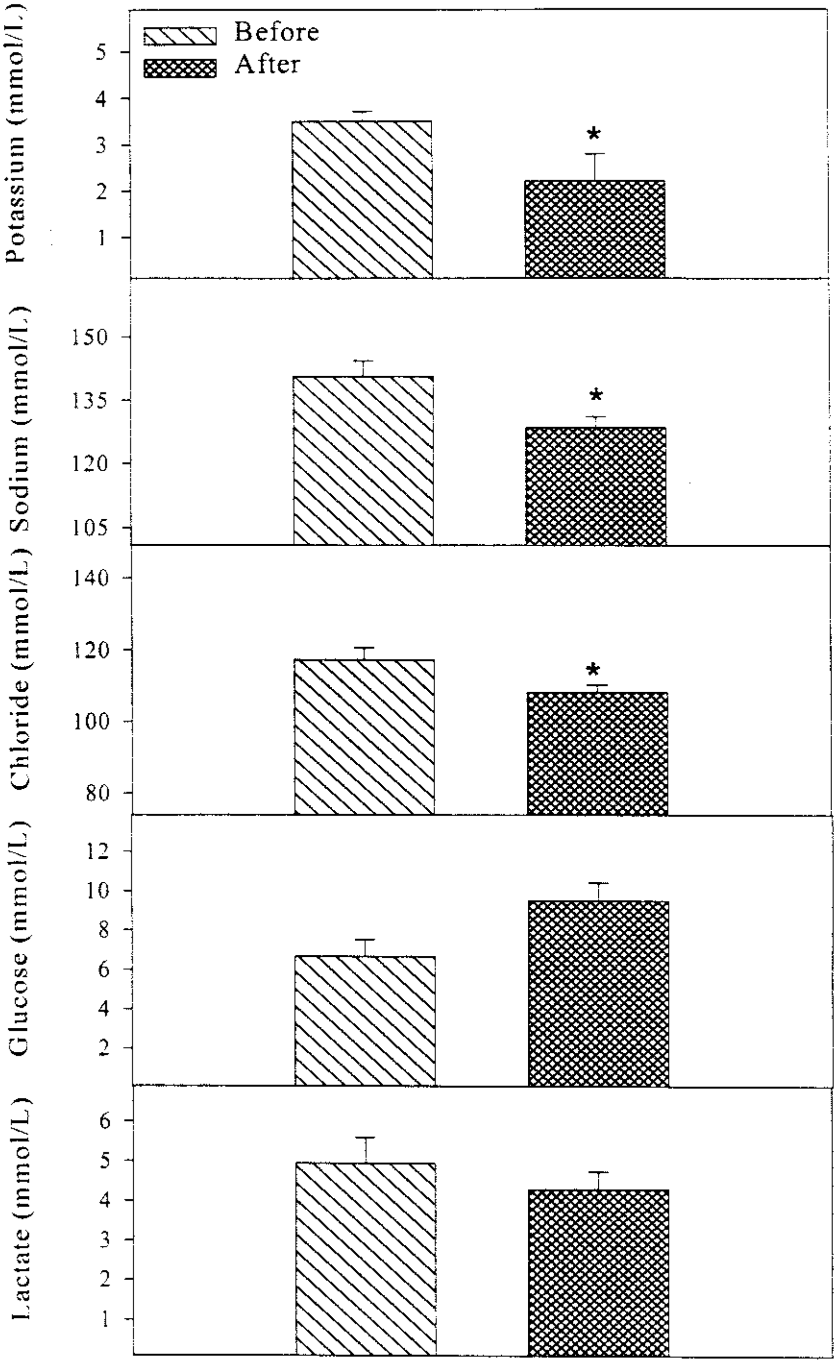

Figure 1. Plasma potassium, sodium, chloride, glucose and lactate before and after exposure of juvenile rainbow trout to supercooling, frazil and anchor ice.

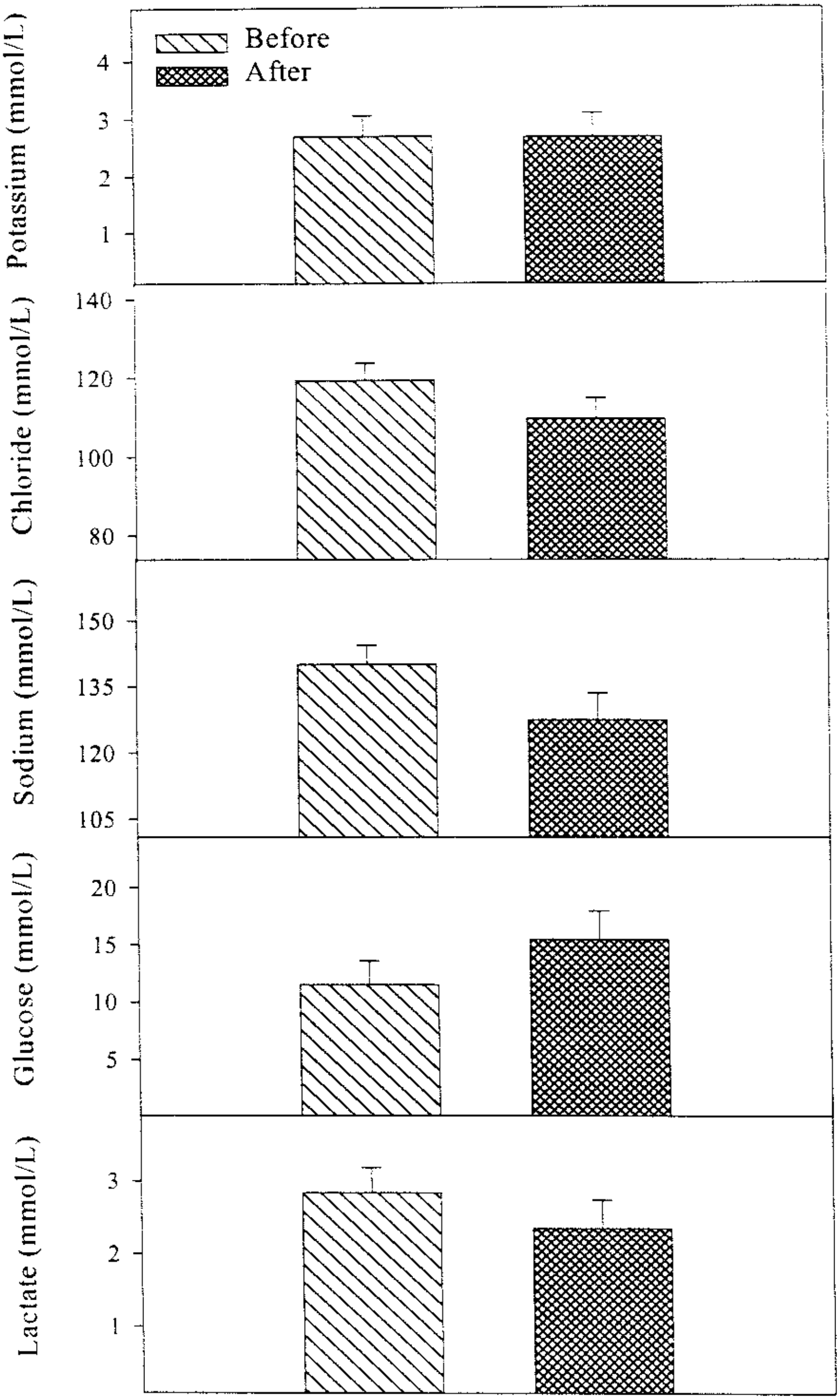

Figure 2. Plasma potassium, sodium, chloride, glucose and lactate before and after exposure of adult rainbow trout to supercooling, frazil and anchor ice.

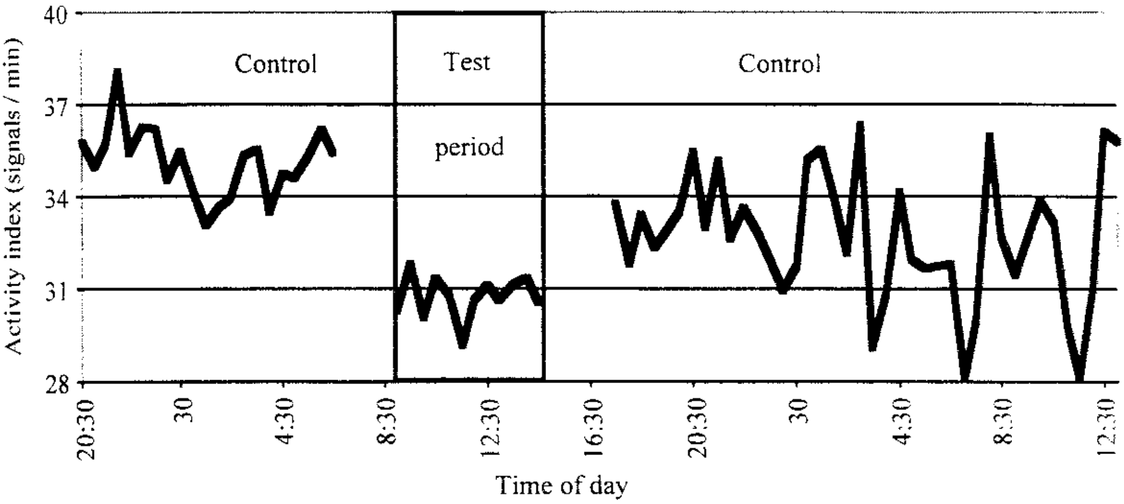

Figure 3. An example of the swimming activity of one EMG transmitter implanted rainbow trout during a test period of supercooling and frazil and anchor ice formation and during a control period. Lower levels on the activity index indicate lower swimming activity.

The escape response of rainbow trout was decreased when they were exposed to supercooled water and frazil and anchor ice (Fig. 4). The fish were less disturbed when they were approached while anchor ice was scraped off the dividers that enclosed the fish in different sections of the flume. During the first three hours of supercooling, there was a significant decrease in swimming activity as the amount of time fish were exposed to supercooling increased (Fig. 5). However if swimming activity of fish during cleaning events which occurred more than three hours after the start of supercooling are included in the analysis, there is no significant relationship. This indicates that fish may become accustomed to the ice conditions, however, data after more than three hours of supercooling are limited. During the test period, fish visual observations also showed that fish moved less as the length of supercooling increased. At first, fish would swim away as they were approached to clear the dividers but as the length of supercooling went on the fish did not appear disturbed even when approached to within 1 m.

4. Discussion

Both through changes in blood chemistry and changes in the rate of swimming activity and escape response, an effect was seen from the exposure to supercooling and frazil and anchor ice. The presence of reduced levels of plasma ions and increased plasma glucose in juvenile fish indicates that the exposure to supercooling, frazil ice and anchor ice induced a general stress response. In fish as in most animals, the exposure to constraining stimuli induces a non-specific stress response that involves the release of osmorespiratory compromise (Wendelaar Bonga 1997). The release of catecholamines also stimulates glycogenolysis which creates an increase in plasma glucose levels (Mazeaud et al. 1977).

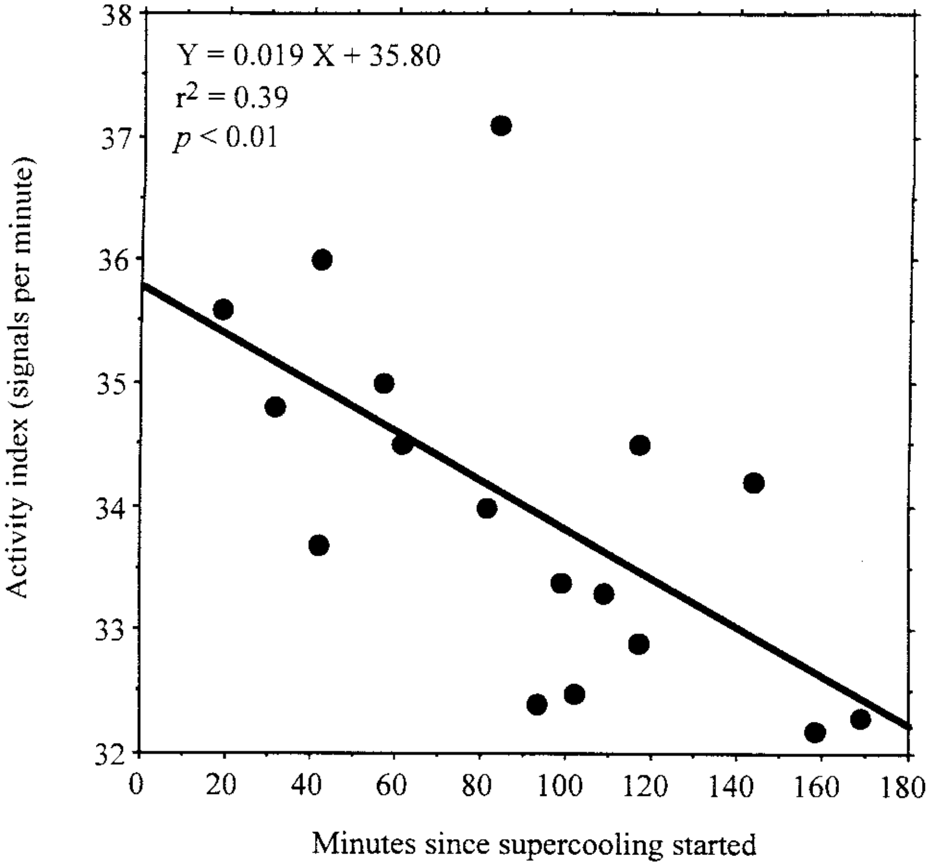

Figure 5. A regression plot of the activity index of EMG transmitter implanted rainbow trout at different periods of time after supercooling started. Mean swimming activity was calculated for all fish during five minute periods when anchor ice was cleared off dividers which enclosed fish in a refrigerated flume. Lower levels on the activity index indicate lower swimming activity.

The presence of a stress response in juvenile fish indicates that the exposure to supercooling and frazil ice was disturbing for the fish. The occurrence of repetitive supercooling and frazil ice events over the course of the winter could thus threaten the health and survival of the fish since stress is energetically demanding (Schreck 1981) and salmonids may already be experiencing difficulties meeting their metabolic needs (Cunjak et al 1987).

The lower amplitude of the alterations of blood ions in the adult fish indicates that these fish were less affected by the ice than the juveniles. A scaling of the ion losses associated with stress with the smaller fish losing more ions has also been observed by McDonald and Milligan (1997). They demonstrated that the higher gill area to mass ratio of the small fish could not explain all of the increased loss of ions and that some other differences in the abundance or permeability of the tight junctions were also involved. The higher capacity of the adult fish to maintain their ionic status under stress makes them more resistant than juveniles to physical stressors like supercooling and frazil ice. Smaller fish may also be more stressed than large fish because the frazil particles and the chunks of frazil slush are much larger in proportion to the fish.

Since juvenile fish were stressed by the presence of supercooling, frazil and anchor ice, it seems reasonable they would select habitats where these types of conditions do not occur. This was also the belief of Whalen et al. (1999) who noted that few Atlantic salmon parr were observed during snorkel sampling on nights when anchor ice was forming or when the water was heavily laden with frazil ice. It is difficult to determine what the long term effects of exposure to these conditions would be. In many streams and rivers, supercooling and subsurface ice occurs only during the freeze-up period. However, in areas where warm water effluents keep large sections of river open, such as tailwaters of hydro electric facilities, these conditions would occur commonly throughout the winter. If fish are stressed by these conditions they may perish, be in poorer condition than in other areas, or move to areas where supercooling and subsurface ice does not occur. Frazil and anchor ice also occurs commonly in areas with winter temperatures which hover around 0°C. In such climates, frazil events can occur on an almost daily basis and may be especially harsh to trout.

Many fish were less active during periods of supercooling and subsurface ice formation compared to control periods, none were more active. This decrease in activity may have broad implications for many areas of river management. This is especially true since, due to exclusions of habitat, fish are often forced to move during periods of subsurface ice formation to find more suitable habitats (Brown and Mackay 1995, Jakober et al. 1998, Brown *in press*).

Fish also moved less when approached as the amount of time they were exposed to supercooling and subsurface ice increased. Since supercooling and frazil ice events make fish less responsive to the approach of possible predators, predation is likely to increase dramatically during this period. The combination of repressed escape response and reduced activity at time when habitats are changing dramatically, leave fish particularly vulnerable to avian and mammalian predators which are not restrained by low temperatures.

Since trout are less active and appear to repress their escape response when exposed to supercooling and subsurface ice, moving into aggregations may partially offset the increased danger of predation. Many species of salmonids move into aggregations during winter. Brown (*in press*), reported that the percentage of radiotagged cutthroat trout (*Oncorhynchus clarki*) in aggregations was negatively correlated with mean weekly water temperatures in the fall and early winter. Many other authors have also noted trout in large aggregations during winter (Hartman 1965, Cunjak and Power 1986, Brown and Mackay 1995, Jakober et al. 1998). Brown (*in press*) suggested that this increasingly gregarious behaviour as temperatures decrease may reduce the chances of predation during cold winter temperatures when swimming ability is decreased (Videler 1993). Aggregation in deeper pools correlates well with a reduction in activity and repression of the escape response as well as providing some protection against frazil and anchor ice. Shoaling behaviour (such as aggregation) provides many advantages in predator avoidance (Pitcher 1986), and may partially offset the increased danger of predation fish experience during frazil ice events.

Many salmonids make behavioural changes in the fall and early winter which allow them to avoid or at least decrease their exposure to frazil and anchor ice. Many species of trout move out of shallow, faster habitats and into deeper, lower velocity habitats in the fall or early winter (Chisholm et al. 1987, Baltz et al. 1991, Heggenes et al. 1993, Brown and Mackay 1995, Jakober et al. 1998). Shallow, turbulent mountain streams are often fully mixed throughout the water column with the result that suspended frazil is encountered at all depths. In deeper, slower water, the frazil tends to accumulate into buoyant flocs that rise to the surface. Because full mixing is less likely in deeper, slower water, anchor ice is also less likely to occur in deeper areas than in shallower habitats. Thus, movement towards deeper habitats should decrease the amount of frazil ice that fish are exposed to in the water column.

As temperatures decrease in the fall and early winter many salmonids move into areas kept warm by groundwater influx (Cunjak and Power 1986, Brown and Mackay 1995, Brown *in press*). These groundwater inflows often provide refuges from exposure to frazil and anchor ice (Power et al. 1999). However, as Brown (*in press*) warns, these groundwater sources must have sufficient influx of warm water during winter, or during very cold periods they may become supercooled and uninhabitable by fish.

We suggest that further work be carried out to determine the response of fish to supercooling and frazil ice events. Activity of trout in tailraces of dams should be examined to determine if adult fish move out of these areas when multiple frazil events occur, as should the behaviour and success of juvenile fish in these areas. The behaviour of avian and mammalian predators must also be examined to see if they are more numerous or more successful in tailraces than in other areas. Studies are needed on the effects of supercooling and subsurface ice on other species of fish, especially fish that are not hatchery reared. Wild fish may respond quite differently than hatchery fish. In our work, the hatchery fish were exposed to supercooling and frazil ice for the first time so they may have acted differently than wild fish, or been more stressed. However, the fish we tested should represent the large number of fish which are stocked into rivers and streams each year. Tests should also be done to determine if fish become less stressed after multiple exposures to supercooling and frazil ice. The fish may become accustomed to the conditions and act more normally. Stress levels of juvenile fish in different types of substrates or cover should also be examined as well as determining how fish respond to longer term ice events when much of the channel is filled with anchor ice for periods of several days.

In conclusion, many effects of supercooling and frazil and anchor ice were found. Juvenile fish exhibited a stress response, and adult fish were less active and had a reduced escape response. Supercooling and subsurface ice conditions may drastically increase the likelihood of predation to fish. These effects may also make areas where supercooling and subsurface ice occur of lower suitability than other areas. This important, and chronically overlooked area of fisheries management merits much more attention and future research.

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6. References

Anderson, W. G., R. S. McKinley, and M. Colavecchia. 1997. The use of clove oil as an anesthetic for rainbow trout and its effects on swimming performance. *North American Journal of Fisheries Management* 17:301-307.

Baltz, D. M., B. Vondracek, L. R. Brown, and P. B. Moyle. 1991. Seasonal changes in microhabitat selection by rainbow trout in a small stream. *Transactions of the American Fisheries Society* 120:166-176.

Booth, J. L. 1979. The effects of oxygen supply, epinephrine and acetylcholine on the distribution of blood flow in trout gills. *Journal of Experimental Biology* 83: 31-39.

Brown, R. S. *In press*. Fall and early winter movements of cutthroat trout, *Oncorhynchus clarki*, in relation to water temperature and ice conditions in Dutch Creek, Alberta. *Environmental Biology of Fishes*.

Brown, R. S., and W. C. Mackay. 1995a. Fall and winter movements of and habitat use by cutthroat trout in the Ram River, Alberta. *Transactions of the American Fisheries Society* 124:873-885.

Brown, R. S., G. Power, R. S. McKinley, and S. Beltaos. 1998. Effects of hanging dams, surface ice break-up, and flooding on fish. *Proc. of the 14th International Symposium on ice*. Potsdam, NY, Volume 1, p. 175-181.

Brown, R. S., S. S. Stanislawski, and W. C. Mackay. 1994. Effects of frazil ice on fish. In T. D. Prowse (editor) Proceedings of the Workshop on Environmental Aspects of River Ice, Saskatoon, Sask., 18-20 August 1993:261-278. NHRI Symposium No. 12. National Hydrology Research Institute, Environment Canada, Saskatoon, Sask.

Chisholm, I. M., W. A. Hubert, and T. A. Wesche. 1987. Winter stream conditions and use of habitat by brook trout in high-elevation Wyoming streams. *Transactions of the American Fisheries Society* 116:176-184.

Cunjak, R. A., Curry, R. A., and Power, G. 1987. Seasonal energy budget of brook trout in streams: Implications of a possible deficit in early winter. *Transactions of the American Fisheries Society* 116: 817-828.

Cunjak, R. A. and G. Power. 1986. Winter habitat utilization by stream resident brook trout *Salvelinus fontinalis* and brown trout *Salmo trutta*. *Canadian Journal of Fisheries and Aquatic Sciences* 43:1970-1981.

Hartman, G. F. 1965. Role of behavior in the ecology and interaction of underyearling coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada* 22:1035-1081.

Heggenes, J., O. M. W. Krog, O. R. Lindas, J. G. Dokk, and T. Bremnes. 1993. Homeostatic behavioural responses in a changing environment: brown trout (*Salmo trutta*) become nocturnal during winter. *Journal of Animal Ecology* 62:295-308.

Jakober, M. J., T. E. McMahon, R. E. Thurow, & C. G. Clancy. 1998. Role of stream ice on fall and winter movements and habitat use by bull trout and cutthroat trout in Montana headwater streams. *Transactions of the American Fisheries Society* 127: 223-235.

Kerr, D. J., H. T. Shen, and S. F. Daly. 1997. Anchor ice formation and growth on gravel channel bed. *Proc. 9th Workshop on River Ice*, Fredericton, N. B., 24-Sept. 1997:153-171.

Mazeaud, M. M., Mazeaud, F., and Donaldson, E. M. 1977. Primary and secondary effects of stress in fish: Some new data with a general review. *Transactions of the American Fisheries Society* 106: 201-212.

McDonald, G., and Milligan, L. 1997. Ionic, osmotic and acid-base regulation in stress. *In*: Fish stress and health in aquaculture. G. K. Iwama, A. D. Pickering, J. P. Sumpter and C. B. Schreck, *editors*. Society for Experimental Biology Seminar Series 62. Cambridge University Press, Cambridge.

McKinley, R. S. and G. Power. 1992. Measurement of activity and oxygen consumption for adult lake sturgeon (*Acipernser fulvescens*) in the wild using radiotransmitter EMG signals. Pages 307-318 *in* I. G. Priede and S. M. Swift, editors. Wildlife telemetry: remote monitoring and tracking of animals. Ellis Horwood, New York.

Pitcher, T. J. 1986. Function of shoaling behaviour in teleosts. Pp. 294-337. In: T. J. Pitcher (ed.) The Behaviour of Teleost Fishes, The Johns Hopkins University Press, Baltimore.

Power, G., R. S. Brown, and J. G. Imhof. 1999. Groundwater and fish – insights from northern North America. *Hydrological Processes* 13:401-422.

Randall, D. J., and Perry, S. F. 1992. Catecholamines. *In*: Fish Physiology, Volume XIIB. W. S. Hoar, D. J. Randall and A. P. Farrell, *editors*. Academic Press. New York.

Schreck, C. B. 1981. Stress and compensation in teleostean fishes: Response to social and physical factors. *In*: Stress and Fish. A. D. Pickering, *editor*. Academic Press. New York.

Videler, J. J. 1993. Fish Swimming. Chapman & Hall Inc., New York. 260pp.

Wendelaar Bonga, S. E. 1997. The stress response in fish. *Physiological Reviews* 77:591-625.

Whalen, K. G., D. L. Parrish, and M. E. Mather. 1999. Effect of ice formation on selection of habitats and winter distribution of post-young-of-the-year Atlantic salmon parr. *Canadian Journal of Fisheries and Aquatic Sciences* 56:87-96.

White, K. D., S. F. Daly, and J. J. Gagnon. 1999. Simulating Winter Environments for Aquatic Life in the CRREL Refrigerated Flume. *Proc. 10th Workshop on River Ice*, Winnipeg, Manitoba.