Lethal and Sublethal Responses of Rainbow Trout (*Oncorhynchus mykiss*) and Coho (*Oncorhynchus kisutch*) Fry to Elevated Dissolved Gas Supersaturation and Temperature

B.L. Antcliffe, I.K. Birtwell, and L.E. Fidler

Fisheries and Oceans Canada
Habitat and Enhancement Branch
Suite 200 – 401 Burrard Street
Vancouver, BC V6C 3S4

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LETHAL AND SUBLETHAL RESPONSES OF RAINBOW TROUT (*Oncorhynchus mykiss) AND COHO* (*Oncorhynchus kisutch)* FRY TO ELEVATED DISSOLVED GAS SUPERSATURATION AND TEMPERATURE

by

B. L. Antcliffe, I. K. Birtwell[1] and L. E. Fidler[2]

Fisheries and Oceans Canada
Habitat and Enhancement Branch
Suite 200 – 401 Burrard Street
Vancouver, BC, Canada V6C 3S4

[1] Fisheries and Ocean Canada, Marine Environment and Habitat Science Division, 4160 Marine Drive, West Vancouver, BC, Canada V7V 1N6

[2] Aspen Applied Sciences Ltd., 238 Kimbrook Crescent, Kimberly, BC, Canada V1A 3A7

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TABLE OF CONTENTS

ABSTRACT

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Lethal and sublethal responses of rainbow trout (*Oncorhynchus mykiss*) and coho (*Oncorhynchus kisutch*) fry to exposure to elevated dissolved gas supersaturation and temperature were compared in laboratory experiments. Sublethal behavioural responses were examined. Survivors were challenged in escape to cover tests to assess behavioural responses not apparent to observers. All treatments (114% total gas pressure "TGP", 10°C, 0.25 m; 118% TGP, 15°C, 0.25 and 0.1 m; 125% TGP, 18°C, 0.1 m) failed to produce swim bladder overinflation or rupture, or behavioural effects, assessed relative to control fish treated equally but exposed to air-equilibrated water. Ability to escape to cover was compromised significantly only at the most severe treatment; this was the only treatment that caused significant mortality. Results indicate that swim bladder overinflation was accommodated by these free-swimming fish and that lethal exposures were required to elicit a significant adverse behavioural (escape to cover) response of the survivors. Our results have implications regarding the merit of applying the accepted low-level TGP guideline to protect fish from sublethal responses to gas-supersaturated water.

RÉSUMÉ

Antcliffe, B.L., I.K. Birtwell, and L.E. Fidler. 2003. Lethal and sublethal responses of rainbow trout (*Oncorhynchus mykiss*) and coho (*Oncorhynchus kisutch*) fry to elevated dissolved gas supersaturation and temperature. Can. Tech. Rep. Fish. Aquat. Sci. 2500: 18p.

Nous avons comparé en laboratoire les réactions létales et sublétales d'alevins de truite arc-en-ciel (*Oncorhynchus mykiss*) et de coho (*Oncorhynchus kisutch*) à une exposition à une forte sursaturation en gaz dissous et à une élévation de la température. Les réponses comportementales sublétales ont été examinées. Nous avons soumis les survivants à des tests de fuite vers l'abri pour évaluer les réponses comportementales qui n'étaient pas apparentes pour les observateurs. Aucun des traitements (114 % de pression totale du mélange gazeux ou TGP, 10 °C, 0,25 m; 118 % de TGP, 15 °C, 0,25 m et 0,1 m; 125 % de TGP, 18 °C, 0,1 m) n'a provoqué de surgonflement ni de rupture de la vessie natatoire, ni d'effets comportementaux, évalués par rapport à des poissons témoins traités de la même façon mais exposés à de l'eau à l'état d'équilibre gazeux. L'aptitude à fuir vers l'abri n'a été compromise de façon significative que dans le traitement le plus sévère, et c'était le seul traitement qui causait une mortalité notable. Les résultats indiquent que ces alevins nageants s'accommodaient du surgonflement de la vessie natatoire, et qu'il fallait une exposition létale pour provoquer une réponse comportementale significative (fuite vers l'abri) chez les survivants. Nos résultats permettent de mesurer l'intérêt d'appliquer la ligne directrice acceptée sur la TGP de faible niveau pour protéger les poissons des réactions sublétales provoquées par de l'eau sursaturée en gaz.

INTRODUCTION

Dissolved gas supersaturation (DGS) can occur in water bodies as a result of natural causes (e.g., primary production, solar heating, water falls) or anthropogenic sources (e.g., thermal effluents, hydroelectric facilities). High levels of DGS, which are often expressed in terms of total gas pressure (TGP), are known to produce a harmful and even fatal condition in fish known as gas bubble trauma (GBT) (see review by Weitkamp and Katz 1980). The US Environmental Protection Agency's standard for the protection of aquatic life is 110% TGP (US EPA 1986). Canada recently developed guidelines that also recommend a maximum ΔP (pressure differential between dissolved gas pressure and interfacial gas phase pressure at the surface) of 76 mm Hg, or 110% TGP at sea-level, to protect fish from acute lethal effects of DGS (CCME 1999).

Canada also established a low-level TGP guideline that increases linearly from 24 to 76 mm Hg ΔP (sea level TGP of 103% to 110%) for water depths ranging from 0 to 1 m, respectively (CCME 1999). This guideline was developed in response to evidence that exposure of fish to ΔP less than 76 mm (below 110% TGP) may cause indirectly mortality or adverse effects through sublethal or chronic GBT, such as swim bladder overinflation or rupture. For example, Wright and McLean (1985) described very low mortality (2.5%) at ΔP ranging from 0 to 46 mm Hg (mean TGP of 105%) in a 122-d hatchery experiment with chinook salmon fry. Although the exact cause of the mortality could not be identified, it was speculated that TGP induced swim bladder overinflation and overbuoyancy were plausible causes. Cornacchia and Colt (1984) reported mortality of juvenile striped bass, which appeared to be caused by swim bladder overinflation and bubbles in the gut, at a ΔP of 42 mm Hg (106% TGP). Laboratory data produced by Shrimpton et al. (1990a, b) also showed that gas accumulation occurred in the swim bladder of juvenile rainbow trout held in air-supersaturated water starting at ΔP of 27 mm Hg (103% TGP). Others have observed sublethal or chronic responses in fish exposed to various TGP levels (e.g., Dawley and Ebel 1975; Krise et al. 1990).

Concerns have been identified regarding the derivation of the low-level TGP guideline and its applicability to fish in natural environments. First, because swim bladder overinflation and bubbles in the gut were observed in those studies where fish were exposed to low TGP levels, they were suspected as being indirectly responsible for the low-levels of mortality observed, although the data provided only circumstantial evidence regarding the cause of mortality. Second, the unique laboratory conditions under which the thresholds for swim bladder overinflation were determined do not exist for fish in the wild. That is, Shrimpton et al. (1990a) found evidence of swim bladder overinflation and rupture in fish that were anaesthetized and restrained in very shallow water depths. There was also an apparent conflict in the exposure time required to elicit overinflation, which was 122 days for mortality in Wright and McLean (1985), whereas Shrimpton et al. (1990a) found that gas pressure build-up in the swim bladder occurred over much shorter intervals of about 15 h, before venting occurred. Finally, there are relatively few data on sublethal responses or the combined effects of elevated TGP and high temperatures.

Our objectives were to first determine whether sublethal behavioural responses, such as swim bladder overinflation or other behavioural changes, could be evoked in free-swimming fish by exposure to elevated TGP and detected by human observers. Second, we examined the relation between lethal and sublethal responses of rainbow trout and coho fry during exposure to

various combinations of TGP and temperature to determine whether sublethal responses would occur before or in the absence of mortality. Since sublethal responses such as changes in fish behaviour could be very subtle and not readily apparent to human observers without intensive and continual observation, fish that survived the exposure to elevated TGP and temperature were challenged in escape to cover tests to assess behavioural responses and their ecological consequences. This test employed the hypothesis that the condition or behaviour of fish that survived the exposure to elevated DGS and temperature would have been compromised; hence, those fish would take longer to escape to cover than would control fish. The environmental relevance and validity of using escape to cover tests to reveal the indirect effects of stressors on fish has been exemplified through the work of Sigismondi and Weber (1988).

METHODS

TEST FISH

Age-0 rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*) fry were obtained from the Vancouver Island and Big Qualicum hatcheries, respectively, and transported to our laboratory at the Rosewall Creek Hatchery in Fanny Bay, British Columbia (BC). Rainbow trout fry were reared in a 2,700-L circular fibreglass tank, and the coho in a 600-L circular fibreglass tank. Both tanks were supplied with air-equilibrated groundwater at 10°C, with flow rates of 25 $L \cdot min^{-1}$ for the rainbow trout and 10 $L \cdot min^{-1}$ for the coho. Fish were fed a maintenance ration of 2.5% wet body weight per day of commercial feed delivered uniformly between dawn and dusk using two automatic feeders to minimize variability in fish size due to the establishment of dominance hierarchies. Fish were acclimated at our laboratory for at least two weeks before experimentation, which occurred from January to April, 2001.

APPARATUS

The water supply was air-equilibrated groundwater. Control water that supplied the experimental control tanks and the escape to cover test apparatus flowed to an aeration tower to remove excess TGP that developed during water heating; this provided a TGP of about 102% for all temperatures. The experimental water was air-supersaturated after heating by exposing it to medical grade air within a pressurized PVC column (0.2 m diameter by 2.5 m height), packed to a depth of 2 m with bio-rings, which acted as mass transfer media. The operating pressure of the column was fixed by water pressure and TGP was controlled by adjusting the water level and air-flow rate using an automatic level control system. Air-supersaturated water flowed by gravity into the experimental treatment tanks, with 3 $L \cdot min^{-1}$ diverted to a PVC column (0.1 m diameter by 3.6 m height) for accurate measurement of TGP under hydrostatic pressure. The partial pressure of dissolved oxygen (pO_2) ranged from 97 to 98% atmospheric saturation for experimental control water and from 92 to 97% atmospheric saturation for treatment water. The partial pressure ratio of N_2 to O_2 ranged from 3.7 to 3.9 for air-equilibrated water and 3.9 to 4.1 for air-supersaturated water, which were near equilibrium (3.77).

Fish were exposed to elevated TGP and temperature in cylindrical fibreglass tanks with water depth of 0.1 m or 0.25 m, depending on the experiment, which provided water volumes of 10 L and 25 L, respectively. Ten pairs of tanks were aligned in series along two rows, such that

one row of control tanks received air-equilibrated water and the other row of treatment tanks received air-supersaturated water. This allowed up to nine replicates, each consisting of a group of control and treatment fish, to be tested simultaneously, while one pair of tanks was reserved for water quality. However, water heating requirements restricted the number of replicates for treatments at 15°C or higher. Flow rate to the experimental control and treatment tanks was 2.5 $L \cdot min^{-1}$.

Water flowed to the control and treatment tanks from two separate header pipes via a valve fitted with clear plastic tubing and PVC pipe (approximately one half each). The outlet end of the PVC pipe was located on the tank bottom and it was fitted with a flow restriction nozzle to control flow rates and prevent pressure losses and off-gassing along the distribution system. A centre stand-pipe controlled outflow from the surface to prevent degassing. Crossing the water delivery hoses every other pair of tanks varied the location of the control and treatment tanks, and this ensured that external factors, such as movement, noise, or lighting, affected control and treatment tanks equally. All tanks were covered with a lid constructed from black plastic material with a small viewing window covered with red cellophane to reduce disturbance from background lighting and human observers. Overhead lighting simulated the natural photoperiod.

The escape to cover tests were conducted in a fibreglass illuminated swim trough (3.25 m in length, 0.35 m in width, and 0.3 m in height) supplied with the air-equilibrated (control) water for each experiment (Fig. 1). Water depth and flow, respectively, were 0.15 m and 14 $L \cdot min^{-1}$ for tests with rainbow trout and 0.11 m and 8 $L \cdot min^{-1}$ for those with coho. A sheet of black plastic formed the overhead cover at the upstream end. A black perforated plastic screen placed 0.25 m downstream of the inflow pipe formed the back wall of the overhead cover, to block out overhead lighting. A white mesh (plastic) screen 0.25 m upstream of the outflow standpipe ensured that fish could not swim in the opposite direction of the overhead cover, and seek cover around or behind the standpipe. The overhead cover was 1.25 m upstream of the white mesh screen for the tests with rainbow trout. The distance was shortened to 0.5 m for the tests with coho (by extending the length of the overhead cover) because of their smaller size. Fluorescent lighting provided overhead illumination, and a black curtain surrounded the trough to block out extraneous light sources.

The flow rates to the stock tank, experimental control and treatment tanks, and escape to cover test trough all provided 90% water replacement times, calculated from theoretical water replacement times for constant flow situations in Sprague (1969), that ranged from 20 min to 1.8 h, which were less than the 4.5 to 7 h limit recommended by Sprague (1973) for pollutant bioassays. In all cases, fish densities and flow loading were below the upper limits of 10 $kg \cdot m^{-3}$ and 0.5 to 0.7 $kg \cdot L^{-1} \cdot min^{-1}$, respectively, as recommended by Sprague (1973).

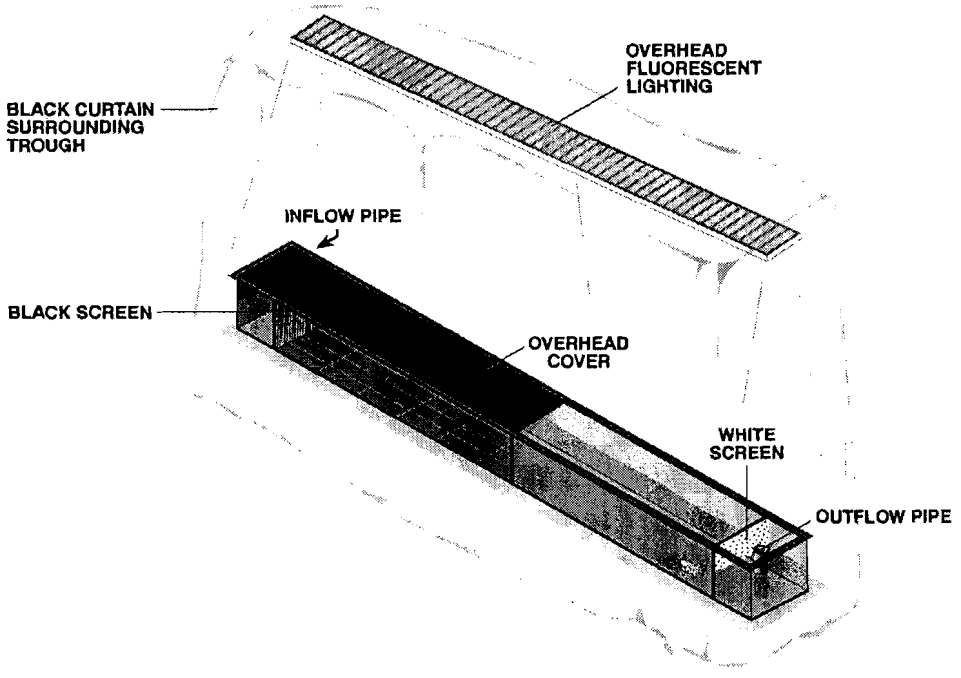

Figure 1. Diagram of the swim trough for the escape to cover tests, from side view. Key features are (1) overhead illumination, (2) black curtain surrounding trough to block out extraneous light sources, (3) fixed, black overhead cover at upstream end of the trough, and (4) a white perforated plastic screen upstream of the outflow standpipe to prevent fish from seeking cover behind the standpipe.

Two total dissolved gas meters (Common Sensing Inc., Clark Fork, Idaho; accuracy ± 0.2 mm Hg) equipped with data logging capabilities measured TGP of the air-supersaturated water every 30 min. Two meters were required for quality control. Both meters provided similar readings, hence results were averaged. A tensionometer (Model 300C, Alpha Designs, Victoria, BC) measured TGP of the air-equilibrated water. The calculations in Colt (1984) were applied to derive the specific dissolved gas parameters. Temperature was recorded every 10 min using Onset Stowaway Tidbit loggers (accuracy ± 0.2°C).

EXPERIMENTAL TREATMENTS AND PROCEDURES

There were five experimental treatments (Table 1). Rainbow trout fry were initially exposed to TGP of 114% at 10°C in 0.25 m depth for 7 days to determine whether sublethal responses could be evoked. This TGP was chosen at start because it was below the threshold for initiation of gas bubble growth in the cardiovascular system defined by Fidler (1988), meaning that direct mortality due to the formation of gas emboli in gill or other tissues should not occur, and it was within the TGP range previously observed by other investigators to induce swim bladder overinflation and rupture. That is, the effective TGP at 0.25 m would be similar to that at 111% TGP at 0.025 m in Jensen (1988) or 110% TGP at 0.01 m in Shrimpton et al. (1990a), because the hydrostatic pressure associated with every 1 m of water depth compensates for approximately 10% TGP (see Knittel et al. 1980). Our depth of 0.25 m was relevant for local

water bodies, and it ensured that fish were able to swim freely during the experiment. Since the initial exploratory test failed to produce lethal or sublethal responses, subsequent experiments were performed at increasingly higher TGP and water temperature, and eventually shallower water depth, to increase the chances of observing sublethal effects and to include lethal exposures. Higher temperatures provided maximum likelihood of swim bladder overinflation due to higher respiratory and gas transfer rates, and they were relevant for local systems. Only the highest TGP and temperature regimen was used for the coho because of their higher resistance to mortality from exposure to TGP, relative to rainbow trout (see Rucker 1975; Nebeker and Brett 1976).

All treatments used fry less than 60 mm in fork length (see Table 1) because Shrimpton et al. (1990a) found that swim bladder overinflation and rupture only occurred in this size range (i.e., smaller fish incur a higher swim bladder overpressure before venting occurs). Antcliffe et al. (2002) also failed to find evidence of swim bladder effects in juvenile rainbow trout (greater than 80 mm in fork length) exposed to a range of TGPs. For the initial test at 114% TGP, three of six replicates were graded based on fork length into small (37 to 42 mm), medium (43 to 47 mm) and large (48 to 52 mm) sizes prior to placement into the experimental tanks, by anaesthetizing them in a buffered (pH=7.0) non-lethal dose (45 $mg \cdot L^{-1}$) of tricaine methanesulphate (MS-222), to examine fish size effects. There were 30 fish per experimental tank when water depth was 0.25 m, and 20 fish per tank at 0.1 m. This ensured that fish density (1.2 to 3.4 $kg \cdot m^{-3}$ for rainbow trout and 0.8 $kg \cdot m^{-3}$ for coho) and flow loading (less than 0.05 $kg \cdot L^{-1} \cdot min^{-1}$ for rainbow trout and 0.02 $kg \cdot L^{-1} \cdot min^{-1}$ for coho) were within limits in Sprague (1973).

For all treatments, fish were randomly allocated to treatment and control experimental tanks and all tanks initially received air-equilibrated water (102% TGP) at 10°C to provide an acclimation period for recovery from handling stress. For the first treatment, air-supersaturated water at 114% TGP flowed to the experimental treatment tanks following a 22 h acclimation period; it took approximately 40 min for the TGP to stabilize at 114%. For all other experiments, fish were placed directly into the exposure tanks at 102% TGP and 10°C. Temperature was then increased to either 15°C or 18°C, depending on the treatment, over the 22 h acclimation period. TGP in the treatment tanks also increased during acclimation as a result of water heating. At the end of acclimation the final TGP was achieved using the TGP generating column. Fish were not fed for 24 h prior to placement into the experimental tanks or during acclimation. They were fed a daily ration of 2.5% wet body weight $\cdot d^{-1}$ during experimentation.

During each exposure all fish were observed for signs of swim bladder overinflation or rupture, or behavioural changes, every 2 h during the day and occasionally at dusk and dawn for a minimum interval of 5 min per control and treatment tank, for all replicates. On occasion, both control and treatment fish were observed continuously for 15 min intervals. All fish in each tank were observed because individuals could not be tracked. Fish were observed for the following signs or behavioural traits: over-buoyancy (indicated by continuous swimming potentially with the head down or floating at the surface, under-buoyancy (sinking or not be able to lift off the tank bottom, loss of equilibrium (swimming or resting on their side or upside down), venting of excess gas (i.e., release of air bubbles from the mouth, vent, or opercular flaps), or any other abnormal behaviour. All behavioural changes were assessed relative to control fish that were

treated equally but exposed to air-equilibrated water at approximately 102% TGP. Fish at the surface were intentionally disturbed at least once during the exposure, by waving a hand over the tank, to determine if the fish were able to sound. Fish that died were blotted dry, weighed, measured for fork length, and examined for external signs of GBT (without magnification). Fish were carefully dissected to ascertain whether swim bladder rupture had occurred. A section of gill tissue was removed with surgical scissors and examined under a dissecting microscope at 25 × magnification.

The escape to cover tests were conducted by releasing the fish, one at a time, into the swim trough downstream of the overhead cover. Where treatment fish died during the exposure to elevated TGP and temperature, enough control fish were removed from the experimental tanks to ensure that an equal number of control and treatment fish per replicate were used in the cover test. Fish were transported individually from the experimental tanks to the trough by gently dip-netting them into a 13 L bucket half filled with either control water or supersaturated water for control and treatment fish, respectively. Individual fish were gently netted out of the bucket and placed into the swim trough in front of the white mesh screen. The time to escape to cover was recorded, along with escape trajectories. A 90 s time limit was imposed on the test. The status of the first fish used in the cover test (i.e., control or treatment) was selected randomly, and the order alternated for each successive pair of control and treatment fish. At the end of the test, fish were euthanized using a buffered (pH=7.0), lethal dose (200 $mg \cdot L^{-1}$) of MS-222 and examined for length, weight, and external signs of GBT.

STATISTICAL ANALYSIS

Survival during the exposure to TGP and temperature was analysed by comparing the proportion of dead fish between control and treatment groups using the Cochran-Mantel-Haenszel (CMH) test on the data for all replicates combined, which is an extension of the usual chi-square test for contingency tables applicable when the study design also contains a blocking factor (e.g., replicates). The null hypothesis was no association between the treatment (exposure to elevated TGP and temperature) and mortality, after adjustments for blocking (combining replicates).

Results of the escape to cover test were analysed in two ways. First, the proportion of stuporous fish (i.e., "dazed" fish that failed to swim to the cover within the 90 s time limit) for control and treatment groups were compared using the CMH test with adjustments for combining replicates. The null hypothesis was no association between the proportion of stuporous fish and the treatment. Length and weight of stuporous fish were compared between control and treatment groups using *t* tests. Second, the escape times for only those fish that actually swam to cover within the 90 s time limit were analysed using analysis of covariance (ANCOVA). The ANCOVA model was fit to the escape times for individual fish from all replicates combined and effects corresponding to individual fish were nested within replicates (to account for pooling of replicates); fish length and weight were the covariates. The null hypothesis was no difference in escape time between control and treatment fish after accounting for differences in fish length or weight on escape time. The level of significance (α) was 0.05 for all tests.

RESULTS

RAINBOW TROUT: 114% TGP, 10°C, 0.25 M

Treatment and control fish were exposed to mean (± SD) TGP of 113.9 ± 0.3% (ΔP = 106 ± 3 mm Hg) (n = 333) and 102.1 ± 0.3% (ΔP = 18 ± 3 mm Hg) (n = 27), respectively. Temperature averaged 9.9 ± 0.1°C in both treatment and control groups (n = 1110). All fish survived the 7-day exposure. No treatment or control fish exhibited any signs of swim bladder overinflation or rupture (e.g., loss of equilibrium or buoyancy control, release of excess gas), or other behavioural changes. There was a tendency for both treatment and control fish to use the bottom of the tank at night, and for fish to be more active at dawn and dusk. The fish in replicate seven (37 to 42 mm fork length) behaved differently from the larger fish in the other replicates in that they were proximal to the water surface more often, and on occasion, a few fish swam continuously around the perimeter of the tank at the water surface for several minutes. However, both the control and treatment fish exhibited this behaviour, and there were no signs of release of air bubbles from the mouth, vent, or opercular flaps of these fish. In all replicates, when fish at the water surface were intentionally disturbed, they sounded and remained on the bottom of the tank for several minutes.

RAINBOW TROUT: 118% TGP, 15°C, 0.25 M

All control fish survived the 8-day exposure (Fig. 2). Two treatment fish (3% overall) died, one from each replicate, on the final day of the exposure. One dead fish had gas bubbles in the caudal fin rays, at the base of the caudal fin, behind the dorsal fin, and in the branchial arteries, but the swim bladder was intact. The other had no external signs of GBT and the swim bladder was intact. The effect of the treatment on mortality was not significant ($p = 0.157$). There were no signs of swim bladder overinflation or rupture, or differences in fish behaviour between control and treatment fish. All fish sounded when intentionally disturbed. Mean (± SD) TGP was 117.6 ± 0.3% (ΔP = 133 ± 5 mm Hg) (n = 384) for treatment water and 102.3 ± 0.6% (ΔP = 22 ± 4 mm Hg) (n = 56) for control water. Temperature averaged 14.9 ± 0.3°C and 14.2 ± 0.3°C for treatment and control water, respectively (n = 1153).

A slightly higher proportion of treatment (19%) than control (16%) fish were rendered stuporous (Fig. 2), however, this difference was not significant ($p = 0.625$). Length ($p = 0.188$) and weight ($p = 0.071$) of stuporous fish did not differ significantly between treatment and control groups. Escape times for fish that swam to cover were highly variable for both control and treatment fish, but mean escape times were similar (Fig. 2). Escape time did not differ significantly between control and treatment fish after accounting for differences in fork length ($p = 0.938$) or weight ($p = 0.890$). Only two of the 58 treatment fish (3%) in the cover test had signs of external GBT, which were bubbles on the opercular flap. No control fish exhibited external signs of GBT.

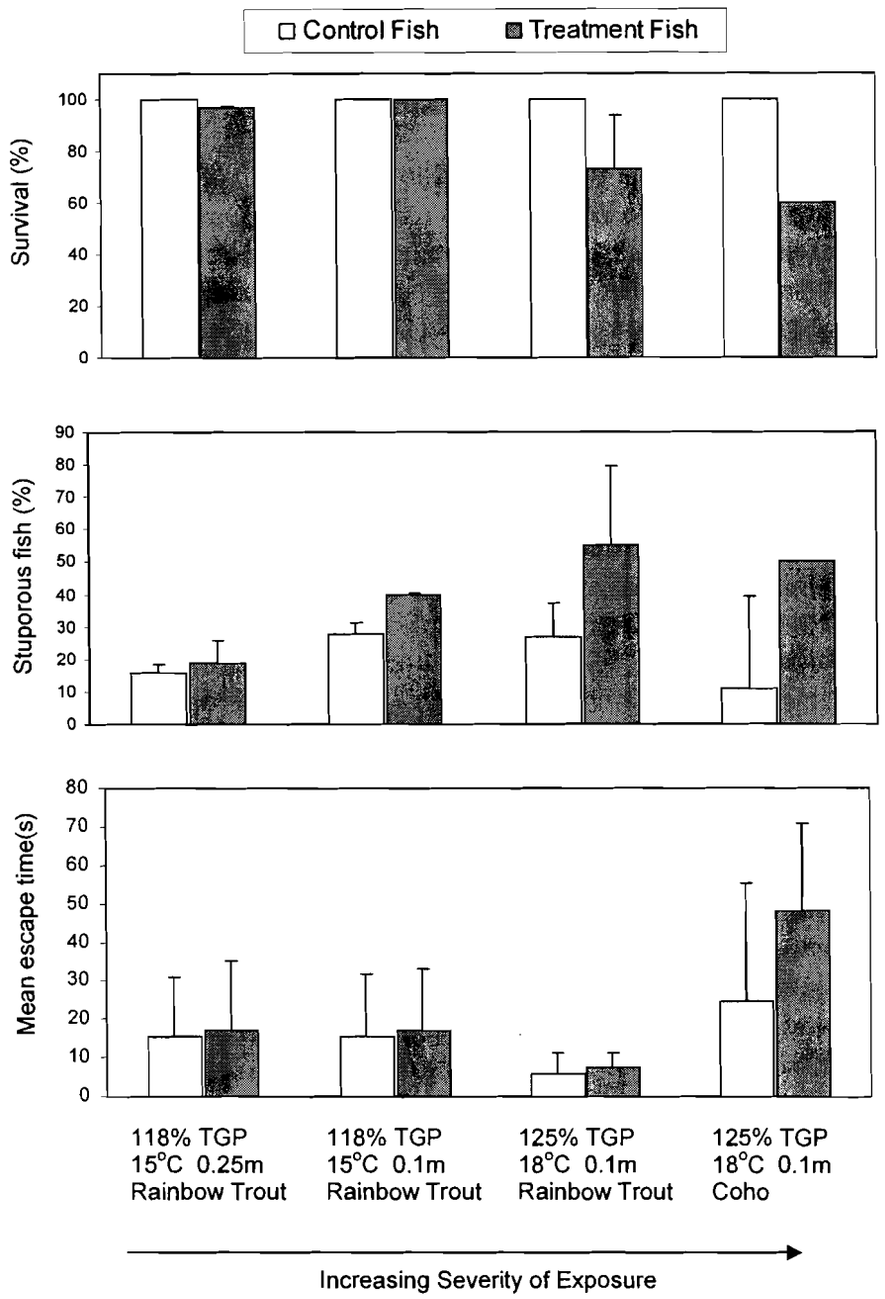

Figure 2. Survival of fish during exposure to elevated TGP and temperature and ability of the survivors to escape to cover, expressed as the percentage of fish that were rendered stuporous and did not seek cover in the escape test, and the mean escape time in seconds for all fish that swam to cover. Control fish were exposed to air-equilibrated water at the same temperature and depth as that for treatment fish. All data are for replicates combined; all statistical analyses included a blocking effect for combining replicates. Error bars for percent mortality and stuporous fish represent variation (one standard deviation) among replicates. For mean escape time error bars represent the variability (one standard deviation) in escape times for all individual fish. Asterisks indicate statistically significant differences.

RAINBOW TROUT: 118% TGP, 15°C, 0.1 M

All fish survived the 7-day exposure. There were no obvious behavioural effects or signs of swim bladder overinflation or rupture, and all fish sounded when intentionally disturbed. Treatment and control fish were exposed to a mean (± SD) TGP of 117.5 ± 0.3% (ΔP = 133 ± 3 mm Hg) (n = 340) and 102.3 ± 0.6% (ΔP = 21 ± 5 mm Hg) (n = 50), respectively. Temperature for treatment and control water averaged 14.9 ± 0.2°C and 14.2 ± 0.2°C, respectively (n = 1021).

There was no significant effect of the treatment on the proportion of fish rendered stuporous ($p = 0.180$), although more treatment (40%) than control (28%) fish were stuporous (Fig. 2). The percentages of stuporous fish were 25% and 30% for the two control replicates, and 40% each for treatment replicates. Length ($p = 0.593$) and weight ($p = 0.120$) of stuporous fish did not differ significantly between treatment and control groups. There was no significant effect of the treatment on escape time with fish length ($p = 0.833$) or weight ($p = 0.797$) as covariates. Escape times were highly variable, and most of the variation was due to individual fish rather than replicates. For example, the mean (± SD) escape times for the two treatment replicates were 16.8 ± 14.6 s and 17.2 ± 18.2 s, and 13.3 ± 16.6 s and 18.2 ± 15.4 s for control replicates. No control fish had external GBT signs, but one of 40 treatment fish (3%) had bubbles on the operculum.

RAINBOW TROUT: 125% TGP, 18°C, 0.1 M

All control fish and 73% of all treatment fish survived the 36 h exposure (Fig. 2). Survival for each of the three replicates was 50%, 90%, and 80%. In total, all 16 dead fish had bubbles in the branchial arteries or gill filaments, while only one fish had external GBT (in the caudal fin). The effect of the treatment on mortality rate was significant ($p < 0.0001$). There was no evidence of differences in fish behaviour, swim bladder overinflation or rupture, or inability to sound when disturbed. Mean (± SD) TGP was 124.7 ± 0.5% TGP (ΔP = 187 ± 5 mm Hg) (n = 65) for treatment and 102.4 ± 0.4% TGP (ΔP = 24 ± 3 mm Hg) (n = 32) for control water. Temperature averaged 17.7 ± 0.3°C and 17.3 ± 0.4°C for treatment and control water, respectively (n = 210).

The percentage of stuporous fish varied from 31%, 61%, and 80% for the three treatment replicates (55% overall), and 19%, 28%, and 40% for control replicates (27% overall). Despite this high variability, the CMH test still provided strong evidence ($p = 0.008$) that the treatment had a significant effect on being rendering stuporous. Length ($p = 0.254$) and weight ($p = 0.941$) of stuporous fish did not differ significantly between treatment and control groups. The escape time did not differ significantly between control and treatment fish using length ($p = 0.396$) or weight ($p = 0.511$) as covariates. As in the other experiments, most of the variation in escape time was related to individual fish, and very little to the different exposure tanks. Only one treatment fish (2%) had external GBT (bubbles in the caudal fin).

COHO: 125% TGP, 18°C, 0.1 M

All control fish and 70% of the treatment fish survived the 100 h exposure (Fig. 2). For each of the two treatment replicates, 6 fish (30%) died. Five of the twelve dead fish in total had external signs of GBT, including severe signs (e.g., bubbles in the mouth, on the opercular flaps,

or on the head; exopthalmia). The treatment had a significant effect on mortality rate ($p < 0.0002$). There were no signs of swim bladder overinflation or behavioural effects. All fish sounded when disturbed. Mean (± SD) TGP and temperature for treatment and control water were 125.0 ± 0.4% (ΔP = 199 ± 8 mm Hg) (n = 95) and 102.3 ± 0.5% (ΔP = 20 ± 5 mm Hg) (n = 18), respectively, and 17.5 ± 0.4°C and 17.2 ± 0.5°C, respectively (n = 599).

Significantly more treatment (50%) than control fish (11%) were rendered stuporous ($p = 0.002$). The percentages of stuporous fish varied from 0% and 21% for the two replicates for treatment fish and 50% each for the control replicates. Length ($p = 0.314$) and weight ($p = 0.778$) did not differ significantly between treatment and control groups of stuporous fish. The mean escape time for treatment fish was almost double that for control fish, however, since the escape times were highly variable, there was no significant effect of the treatment on escape time ($p = 0.221$ for length; $p = 0.219$ for weight). Six of 28 treatment fish (21%) had external signs of GBT (bubbles on the caudal fin and operculum) after the cover test. No control fish had external GBT.

DISCUSSION

Despite that our experiments were conducted in shallow water and under conditions of high water temperature to provide a worst-case assessment of lethal and sublethal responses of rainbow trout and coho fry to DGS and temperature, high TGP levels (125%) were required to produce mortality of rainbow trout and coho fry over the exposure period (see Table 1). All treatments, however, including the most severe treatments at 125% TGP, failed to produce evidence of sublethal responses, such as ruptured swim bladders or signs of overinflation, or obvious behavioural changes. There were no signs, such as loss of equilibrium or buoyancy control, or venting of excess gas via release of bubbles from the mouth, opercular flaps, or vent. All fish were able to sound when frightened. There was aberrant behaviour in a few of the small rainbow trout less than 42 mm in fork length exposed to 114% TGP at 10°C, including swimming continuously at the surface around the perimeter of the tank and use of shallower water depths. However, this behaviour was also observed in the control fish; hence, it was likely related to fish size rather than exposure to elevated TGP. This behaviour was not exhibited by any of the coho fry, which ranged from 30 to 40 mm fork length. The incidence of external GBT was low (less than 3%) for surviving fish, and for dead fish it ranged from 2 to 6% for the rainbow trout and 42% for the coho.

Our results are consistent with those of Jensen (1988), who reported no evidence of swim bladder overinflation or rupture in free-swimming steelhead trout fry (approximately 25 mm fork length) exposed to 111% TGP at 10°C for 5 days in 0.025 m depth and a partial pressure of dissolved oxygen (pO_2) of 100% atmospheric saturation (i.e., normal O_2/N_2 ratios), or slightly lower pO_2 of 75% atmospheric saturation. The pO_2 levels in our experimental treatment water ranged from 92 to 97% atmospheric saturation. Jensen (1988) did, however, observe ruptured swim bladders in a small percentage (2.6%) of the steelhead trout fry under very low water pO_2 (50% atmospheric saturation) and high N_2 (127%). Aside from swim bladder rupture, Jensen (1988) found no other signs of swim bladder overinflation (e.g., fish swimming head down at the surface, releasing air bubbles, or concentrating on the tank bottom), at any water pO_2.

Table 1.—Mean (± SD) lengths and weights of control (C) and treatment (T) fish for all treatments. All control fish were exposed to TGP of approximately 102% at the same temperature and depth as that for treatment fish. The sample size (N) represents the number of exposed fish in each of the control and treatment groups.

	Fork length (mm)		Weight (g)	
	C	T	C	T
Rainbow trout exposed to 114% TGP at 10°C in 0.25 m for 7 d (9 replicates):				
Replicates 1-6 (N=270):	44 (± 2)	44 (± 2)	1.0 (± 0.2)	1.0 (± 0.2)
Replicate 7 (N=30):	39 (± 3)	38 (± 3)	0.7 (± 0.2)	0.7 (± 0.2)
Replicate 8 (N=30):	45 (± 1)	46 (± 1)	1.1 (± 0.2)	1.2 (± 0.2)
Replicate 9 (N=30):	48 (± 2)	49 (± 1)	1.3 (± 0.2)	1.4 (± 0.2)
Rainbow trout exposed to 118% TGP at 15°C in 0.25 m for 8 d (2 replicates):				
All replicates (N=60):	53 ± 4	54 ± 4	1.6 ± 0.3	1.7 ± 0.3
Rainbow trout exposed to 118% TGP at 15°C in 0.1 m for 7 d (2 replicates):				
All replicates (N=40):	54 ± 4	53 ± 3	1.7 ± 0.4	1.6 ± 0.3
Rainbow trout exposed to 125% TGP at 18°C in 0.1 m for 36 h (3 replicates):				
All replicates (N=60):	52 ± 3	51 ± 5	1.3 ± 0.4	1.4 ± 0.4
Coho salmon exposed to 125% TGP at 18°C in 0.1 m for 100 h (2 replicates):				
All replicates (N=40):	35 ± 2	36 ± 2	0.4 ± 0.2	0.5 ± 0.2

Our results differed from those of Shrimpton et al. (1990a) where swim bladder rupture was observed in a low percentage of rainbow trout fry exposed to TGP slightly above 110% in 0.01 m water depth at 8-16°C. However, Shrimpton et al. (1990a) relied on measurement of increased gas pressure within the swim bladder to document the overinflation effect, and this methodology required the fish to be anaesthetized and restrained within a small chamber without access to the surface. Under these conditions, gas could passively diffuse from the water into the blood and then into the swim bladder, with no opportunities for venting or buoyancy control through normal swimming movements. The anaesthetic may have prevented voluntary venting of excess gas through the pneumatic duct, allowing the swim bladder pressure to rise above that which would normally occur, causing rupture in some fish. Other studies by Shrimpton et al. (1990b) showed that fish could use hydrodynamic forces associated with swimming and water depth to offset swim bladder-induced overbuoyancy. Thus, the 0.25 m depth and use of free-swimming fish in our experiments may have prevented any observable effect of swim bladder overpressures. Since water pO_2 was not measured in Shrimpton et al. (1990a), it is unknown

whether their results were for conditions of low oxygen saturation. Regardless, the laboratory conditions under which the fish were held in Shrimpton et al. (1990a) were not relevant to free-swimming fish in natural environments.

The high TGPs (118% and 125%) and temperatures (15°C and 18°C) used in our studies were expected to force a higher rate of swim bladder inflation compared to that in Jensen (1988) or Shrimpton et al. (1990a), thereby providing the maximum likelihood of evoking swim bladder overinflation and observing venting of gas, or rupture. However, the higher TGPs could have caused the swim bladder to vent more frequently, or the fish to sense the overbuoyancy more quickly and vent the swim bladder voluntarily. Yet, if the fish were venting the swim bladder more frequently at the higher TGPs, there should have been some evidence of bubbles being expelled from the mouth, vent, or opercular flaps, unless they were swallowed and transferred into the gut. The latter case would be consistent with observations of Cornacchia and Colt (1984), who found air bubbles in the gut of juvenile striped bass exposed to dissolved gas supersaturation. Bubbles in the gut might also affect buoyance, but we found no evidence of this.

Another consideration is that at low TGP (i.e., below 110%), increases in swim bladder pressure might be so slow that venting does not occur automatically, causing rupture. However, after accounting for differences in water depth, the inflation rate associated with the 110% TGP in Shrimpton et al. (1990a) should have been similar to that in the long-term (72-day) experiments of Jensen (1988) at 111% TGP and that in our experiment at 114% TGP in 0.25 m, yet the latter two experiments found no swim bladder overinflation or rupture under normal oxygen saturation. Thus, results indicate that swim bladder overinflation was accommodated by these free-swimming fish under normal oxygen saturation, and that the swim bladder overpressures and ruptures reported elsewhere were likely due to other factors, such as reduced pO_2 in the case of Jensen (1988) and laboratory methodology and/or unmeasured pO_2 in Shrimpton et al. (1990a).

ESCAPE TO COVER TESTS

There was a significant effect of the treatment on ability to escape to cover only at the most severe exposure (125% TGP), and the effect was on the ability of the rainbow trout and coho to initiate movement towards the cover, rather than on the time to swim to cover. That is, a significantly higher portion of treatment fish failed to escape to cover within the 90 s time limit imposed on the test, while the escape time for fish that swam to cover did not differ significantly between control and treatment groups of fish. If the condition of the rainbow trout was compromised during exposure to 118% TGP and 15°C in 0.1 m or 0.25 m, then it had no ecological consequence in terms of their ability to escape to cover.

The significant cover test response was likely related to the formation of gas emboli in the cardiovascular system because only TGP differed for control and treatment fish (temperature and all other factors such as netting stress were the same), there was no evidence of sublethal responses such as swim bladder overinflation or behavioural effects during the exposure, and the cover test response was only significant in those treatments where substantial mortality occurred during the exposure. Further, gas emboli were present in the branchial arteries or gill filaments of all dead fish. Gas emboli form in the blood or tissues once the blood and tissue TGP levels

rise to threshold levels, and they will continue to expand in size and number until they eventually block blood flow in gill filaments or other vessels, causing death by hemostasis (see Knittel et al. 1980). Prior to reaching lethal levels, some of the surviving fish in which bubble growth had begun would experience a significant impairment of blood flow to the tissues and oxygen transfer, potential systemic hypoxia, respiratory acidosis, and necrosis from build up of toxic wastes (Newcombe 1974). This would likely have a debilitating effect on their performance, and presumably on their ability to escape to cover.

Our escape to cover test results were generally in agreement with predator challenge tests conducted by Birtwell et al. (2001), where juvenile chum salmon were rendered more vulnerable to predation following exposure to 20.7°C seawater at 120% TGP for 24 h and 130% TGP for 12 h, but there was no significant effect after a 48 h exposure to 20.7°C seawater at 115% TGP. Similarly, we detected a significant response in our escape to cover tests when fish were exposed to 125% TGP at 18°C for 36 h (rainbow trout) or 100 h (coho), however, the response was not significant at 118% TGP and 15°C for a 7 to 8-day exposure. Kruzynski et al. (1994) also found a significant correlation between the escape to cover and the predator challenge test (i.e., fish that took longer to escape to cover were also rendered more susceptible to predation). Thus, it is plausible that our fish would have been rendered more susceptible to predation at the most severe treatment (125% TGP), yet we saw no evidence of sublethal effects, such as swim bladder overinflation or obvious effects on fish behaviour.

The effect of aquatic variables that render fish more conspicuous (e.g., by a change in behaviour) or debilitate them so they become easier to catch is an obvious cause for concern because predators tend to attack conspicuous prey or those in substandard condition (Landeau and Terborgh 1986; Temple 1987; Mesa et al. 1994). For example, depending on strategies employed, some predators will selectively prey upon those with modified or abnormal behaviours (Kruzynski et al. 1994) and reduced performance capabilities (Bams 1967). Research has shown that fish can survive exposure to a range of potentially lethal compounds or circumstances, but they may be physiologically and behaviourally compromised as a consequence. However, our fish were not behaviourally compromised at sublethal exposures to TGP and temperature.

IMPLICATIONS FOR DGS GUIDELINES

Our results have important implications for the development and application of DGS guidelines. First, they suggest that data on swim bladder rupture from Shrimpton et al. (1990a) used, in part, to derive the Canadian low-level DGS guideline for protection of aquatic biota from sublethal or chronic effects are not relevant for free-swimming fish in natural environments. Although there remains the evidence of Jensen (1988), where swim bladder rupture occurred in fish exposed to elevated TGP under low water oxygen saturation, these conditions are most relevant for hatchery or enhancement facilities where inadequate water exchanges rates or overcrowding may reduce oxygen saturation. They would not be expected to occur in natural environments where the primary sources of DGS promote normal oxygen saturation (e.g., natural waterfalls, water flow over hydroelectric release structures, primary production, thermal effluents). Second, our finding that there were no significant sublethal responses to exposure to elevated DGS and temperature until the exposure resulted in significant mortality of the rainbow trout and coho fry does not support application of the low-level DGS guideline for these

behavioural effects. However, other species (e.g., juvenile striped bass) may be more susceptible to swim bladder overinflation and formation of bubbles in the gut (see Cornacchia and Colt 1984). In conclusion, our results should be taken into account on a site-specific basis when considering whether to apply the more conservative TGP guideline for protection of aquatic biota from sublethal effects of gas supersaturated water.

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