Effect of Dissolved Gas Supersaturation on the Survival and Condition of Juvenile Rainbow Trout (*Oncorhynchus mykiss*) under Static and Dynamic Exposure Scenarios

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EFFECT OF DISSOLVED GAS SUPERSATURATION ON THE SURVIVAL AND CONDITION OF JUVENILE RAINBOW TROUT (*Oncorhynchus mykiss*) UNDER STATIC AND DYNAMIC EXPOSURE SCENARIOS

by

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PREFACE

This report describes the results of laboratory bioassays conducted by Fisheries and Oceans Canada on the effect of dissolved gas supersaturation (DGS) on the survival and condition of juvenile rainbow trout. The province of British Columbia (BC) funded this research in support of Water Use Planning. The bioassays were designed to produce data relevant to the assessment of DGS impacts at all hydroelectric facilities throughout the province.

The experiments were originally designed to compare the direct, acute effects of DGS on juvenile rainbow trout with indirect or sublethal effects. For our purposes, susceptibility to predation was determined to be an ecologically relevant indirect or sublethal effect that could be examined at the DGS laboratory. The overall purpose was to examine whether susceptibility to predation occurred at a lower total gas pressure (TGP) threshold than direct mortality from bubble growth in the cardiovascular system or gill filaments. The hypothesis was that exposure of fish to elevated TGP would evoke behavioral changes in fish, reflecting stress from gas bubble trauma (GBT) before mortality occurred. This hypothesis was supported by Fidler (1998a), who reported that the first small bubbles in the cardiovascular system appeared hours or days before fish died from occlusion of the gill afferent filamental arteries by bubbles. The corresponding early behavioral changes may be subtle, and not apparent to human observers. However, predators may be able to detect such changes and preferentially select DGS exposed fish over control fish. If this hypothesis is valid, then predation studies are a more sensitive indicator of the impacts of exposure to DGS than mortality bioassay data.

Susceptibility to disease was thought to be an equally important indirect effect of exposure to DGS but it was eliminated from the experimental design due to a lack of suitable quarantine facilities. Further, current diagnostic tools for disease testing are limited in that they can only detect individuals that have disease, not those that are carriers of disease. However, the health of some of the dead fish and survivors from the DGS exposures was examined by testing for bacterial and viral infections, and histological changes to the gills.

It was not possible to conduct the predation bioassay component of this study because hatchery-reared predators could not be conditioned to feed upon live prey to the extent required for these tests. The predators were hatchery cutthroat trout brood stock that had been fed pellets throughout their hatchery residence. These fish had a high fat content and lacked the initiative to feed on live prey, even after considerable time and weight loss. Thus, the experimental program adopted consisted of exposure of juvenile rainbow trout to elevated DGS under static (constant exposure to steady-state TGP in shallow water) and dynamic (variable exposure to TGP due to fish use of variable water depth) conditions.

ABSTRACT

Antcliffe, B.L., L.E. Fidler, and I.K. Birtwell. 2002. Effect of dissolved gas supersaturation on the survival and condition of juvenile rainbow trout (*Oncorhynchus mykiss*) under static and dynamic exposure scenarios. Can. Tech. Rep. Fish. Aquat. Sci. 2370: 70 p.

The effect of dissolved gas supersaturation (DGS) on survival and condition of juvenile rainbow trout under static (steady-state) and dynamic exposure scenarios was assessed in laboratory experiments between January and March 2000.

The static exposure of fish to total gas pressure (TGP) of 110%, 114%, 116%, 122%, and 140% TGP at 10°C and 0.25 m water depth demonstrated an inverse dose-response relationship between TGP and the duration of exposure required to kill fish. The LT50 (time to 50% mortality) was 5.1 h for the 140% TGP exposure, compared to 55 h for the 122% TGP exposure. At 116% TGP, mortality was 42% after a nine day exposure. All fish survived exposure to 114% TGP for six days, and 110% TGP for nine days. Results are consistent with the threshold equations of Fidler and Miller (1997) that predict water surface differential pressure (ΔP) required to initiate bubble growth in the cardiovascular system or gill filaments of rainbow trout (which equates to approximately 115% to 117% TGP at sea level). Comparison of our results with those of other researchers suggests that there are differences among stocks in their susceptibility to DGS. High variability in time to mortality among our 180 fish exposed to 122% TGP indicates that large sample sizes are required to obtain a representative dose-response relationship for a given fish stock.

Dynamic exposures were used to examine the effect of fish use of variable water depths on the dose-response relationship for DGS-induced mortality. At 122% TGP and 10°C, fish were allowed access to water depths from 0 to 1 m, and from 0 to 2.5 m, using volition cages. Because fish behavior in a laboratory deep tank may not be representative of that in the wild, other dynamic exposures held fish in cages, and the cages were cycled throughout the deep tank according to a predetermined duration/depth cycle. Precise information on fish behavior in the wild was not available to design the depth cycles based on known periods of time spent above or below the TGP compensation depth (i.e., the depth at which the hydrostatic pressure negates the effects of TGP). Therefore, fish were held in cages at the surface of the deep tank (in up to 0.25 m depth) where they were exposed to 122% TGP for a duration that killed 10% of the sample. The cages were then held below the compensation depth for 3 h, before being returned to the surface. This depth cycle was repeated four times, such that cumulative mortality was from 0 to 10% during the first surface interval, and from approximately 10% to 20%, 20% to 30%, and 30% to 40% during the second, third, and fourth surface intervals, respectively. Control fish were cycled to depth at the same time as exposed fish. Similar exposures occurred at 122% TGP and 124% TGP, with time at depth intervals of 6 h. Results for dynamic and static exposures (which differed only in terms of fish use of water depth) were compared.

Fish use of water depths greater than those used in our static exposures (0 to 0.25 m) significantly delayed the onset of mortality and reduced cumulative mortality over the

exposure period examined and, therefore, had a positive effect on survival. In the 0 to 1 m volition cage, the time to initiation of mortality was about 36 h compared to 14 h for the static exposure, at the same TGP and temperature. Cumulative mortality was 22% in the 1 m deep volition cage, compared to 89% for the static exposure, over 96 h. Cyclical use of water depth, which may represent diel behavior, delayed re-initiation of mortality when fish were cycled from below the compensation depth to the surface and, in some cases, it reduced the mortality rate at the surface once mortality was re-initiated.

The absence of mortality at 122% TGP with water depths from 0 to 2.5 m provided evidence that fish used depth to compensate for the effects of gas bubble trauma (GBT). However, other researchers conducting laboratory deep tank studies found that fish were unable or unwilling to use available water depth to compensate for exposure to elevated TGP. Notwithstanding this variability, the wide range of behavioral patterns exhibited by individual fish in natural environments will likely produce very different exposure histories and, hence, variability in biological responses among fish.

Examination of fish showed that roughly half that died from exposure to TGP greater than 120% had no external signs of GBT. Many survivors also had no external signs of GBT. These findings may limit the use of external GBT monitoring programs for assessing the exposure and biological effects of DGS.

Our studies illustrate that application of the 110% TGP guideline could be conservative in certain situations, such as short exposure periods (since relatively long exposures are required to elicit mortality at low TGP, even in shallow water). It could also be conservative at 110%, or even at higher TGP levels, if fish use sufficient water depth, relative to the compensation depth, to reduce or eliminate bubble growth processes. Finally, not all species and life-history phases are equally susceptible to TGP in the 110% range. We conclude that there is a need to investigate the cause of mortality reported in the literature for exposure of fish to TGP between 110% and 115%, and to determine the conditions required to produce mortality or sublethal effects in this range. This research might narrow the range of conditions for which the 110% TGP guideline is applicable. Chronic effects associated with exposure to TGP levels below 110% were not addressed in this report.

Future efforts to assess the biological effects of exposure of fish to DGS should identify fish behavior patterns in the wild. Where necessary, exposure histories could then be simulated in laboratory DGS bioassays to determine biological effects. Dynamic GBT exposure models could also be used to predict mortality; however, they are currently limited by large inter-individual variability, differences among fish stocks in susceptibility to mortality, and lack of information available on fish behavior from which to develop relevant exposure histories.

RÉSUMÉ

Antcliffe, B.L., L.E. Fidler, and I.K. Birtwell. 2002. Effect of dissolved gas supersaturation on the survival and condition of juvenile rainbow trout (*Oncorhynchus mykiss*) under static and dynamic exposure scenarios. Can. Tech. Rep. Fish. Aquat. Sci. 2370: 70 p.

L'effet de la sursaturation en gaz dissous (DGS) sur la survie et la condition de truites arc-en-ciel juvéniles soumises à des conditons d'exposition statique (état d'équilibre) et dynamique a été évalué par le biais d'expériences en laboratoire menées entre janvier et mars 2000.

Des expositions statiques à une pression totale du mélange gazeux (TGP) de 110 %, 114 %, 116 %, 122 % et 140 % (à une température de 10 °C et à une profondeur de l'eau de 0,25 m) ont révélé une relation inverse dose-effet entre la TGP et la durée de l'exposition requise pour tuer un poisson. Le TL 50 (le temps qu'il faut pour tuer 50 % des poissons exposés) se situait à 5,1 h à une TGP de 140 %, en comparaison de 55 h à une TGP de 122 %. À une TPG de 116 %, la mortalité a atteint 42 % après une exposition de 9 jours. Tous les poissons exposés à une TGP de 114 % pendant 6 jours et à une TGP de 110 % pendant 9 jours ont survécu. Les résultats sont en accord avec les équations du seuil présentées par Fidler (1988) pour prédire la ΔP de l'eau requise pour déclencher la formation de bulles dans l'appareil cardiovasculaire ou les lamelles branchiales chez la truite arc-en-ciel (soit une TGP d'environ 115 à 117 % au niveau de la mer). La comparaison de nos résultats à ceux d'autres chercheurs semble indiquer qu'il existe des différences entre les stocks pour ce qui est de leur sensibilité à la DGS. La forte variabilité de l'intervalle de temps entre l'exposition de nos 180 truites à une TGP de 122 % et leur mort indique que de gros échantillons sont nécessaires pour obtenir une relation dose-effet représentative pour un stock donné de poissons.

Les expositions dynamiques visaient à établir l'effet de la possibilité d'accès du poisson à des profondeurs variables de l'eau sur la relation dose-effet de la mortalité induite par la DGS. À une TGP de 122 % et à 10 °C, le poisson avait accès à des cages mouillées à des profondeurs de 0 à 1 m et de 0 à 2,5 m. Comme le comportement des poissons dans un bassin expérimental profond peut ne pas être représentatif du comportement d'un poisson sauvage, d'autres expositions dynamiques ont été faites. Des poissons ont été mis en cages, qui ont été immergées dans le bassin profond selon un cycle prédéterminé de durée d'immersion et de profondeurs. Étant donné que des renseignements précis sur le comportement du poisson dans le milieu sauvage n'étaient pas disponibles pour établir les cycles des profondeurs d'après les périodes de temps connues passées au-dessus ou au-dessous de la profondeur de compensation de la TGP (c.-à-d., la profondeur à laquelle la pression hydrostatique annule les effets de la TGP), les poissons ont été gardés dans des cages maintenues à la surface du bassin profond (immergées jusqu'à 0,25 m de profondeur), où ils ont été exposés à une TGP de 122 % pendant la période de temps nécessaire pour en tuer 10 %. Les cages ont ensuite été maintenues au-dessous de la profondeur de compensation pendant 3 h, puis ramenées à la surface. Ce cycle de profondeurs a été répété quatre fois, de sorte à ce que la mortalité cumulative se situe entre 0 et 10 % pendant le premier intervalle à la surface, puis entre 10 et 20 %, 20 et 30 % et 30 et 40 % pendant le deuxième, troisième et quatrième intervalles à la surface, respectivement. Les poissons témoins ont été soumis à des cycles de profondeurs au

même moment que les poissons expérimentaux, les expositions, semblables, ayant été faites à des TGP de 122 % et 124 % et à des intervalles d'immersion de 6 h. Les résultats des expositions dynamiques et statiques (différentes seulement de par la profondeur fréquentée par les poissons) ont ensuite été comparés.

L'accès offert aux poissons à des profondeurs supérieures à celles de nos expositions statiques (0 à 0,25 m) a sensiblement retardé le déclenchement de la mortalité et la mortalité cumulative sur la période d'exposition examinée, ce qui signifie qu'il avait un effet positif sur la survie. Dans la cage d'accès libre située entre 0 et 1 m de profondeur, le poisson a commencé à mourir après environ 36 h, en comparaison de 14 h dans le cas de l'exposition statique à une TGP de 122 % à 10 °C. La mortalité cumulative se chiffrait à 22 % dans le cas de la cage d'accès libre à 1 m de profondeur, en comparaison de 89 % dans le cas de l'exposition statique sur une période de 96 h. La fréquentation cyclique de différentes profondeurs, qui reproduit le comportement nycthéméral probable, a retardé une nouvelle vague de mortalité lorsque les poissons ont été ramenés d'une profondeur inférieure à la profondeur de compensation à la surface et, dans certains cas, a réduit le taux de mortalité à la surface une fois celle-ci déclenchée à nouveau. Bien que nos expositions dynamiques aient été réalisées sur une période relativement courte (de 96 h à 7 jours), la fréquentation soutenue ou périodique de certaines profondeurs pendant des périodes d'exposition plus longues peut aussi avantager les poissons au plan de la survie.

L'absence de mortalité à une TGP de 122 % et à une profondeur de l'eau variant entre 0 et 2,5 m prouve que les poissons se servent de la profondeur pour compenser les effets de l'embolie gazeuse. D'autres études en bassins expérimentaux profonds ont toutefois révélé que les poissons ne pouvaient pas (ou ne voulaient pas) utiliser les profondeurs disponibles pour compenser l'exposition à une TGP élevée. Indépendamment de cette variabilité, la vaste gamme de schèmes de comportement adoptée par un poisson dans son milieu naturel donnera probablement des antécédents d'exposition très différents, d'où la variabilité des réponses biologiques entre les poissons.

Les résultats obtenus révèlent que plus ou moins la moitié des poissons morts d'une exposition à une TGP supérieure à 120 % ne montrait aucun signe d'embolie gazeuse. En outre, de nombreux survivants n'en portaient aucun signe externe. Ces conclusions peuvent limiter l'utilisation de programmes de surveillance de signes externes d'embolie gazeuse pour évaluer l'exposition à une DGS et ses effets biologiques.

Nos études montrent qu'il serait prudent de limiter la TGP à 110 % dans certaines situations, en particulier dans le cas d'expositions de courte durée, étant donné que des expositions relativement longues sont requises pour déclencher la mortalité à de basses TGP, même en eau peu profonde. Il serait en outre prudent à 110 %, ou même à des TGP plus élevées, de donner accès aux poissons à une profondeur suffisante par rapport à la profondeur de compensation pour réduire ou éliminer les processus de formation de bulles. En dernier lieu, toutes les espèces ne sont pas également sensibles à une TGP d'environ 110 %; celles qui le sont ne sont pas non plus également sensibles aux divers stades de leurs cycles vitaux. Nous concluons qu'il faut étudier la cause de la mortalité, signalée dans des études publiées, de

poissons exposés à une TGP de 110 à 115 % et déterminer les conditions requises pour causer la mortalité ou des effets sublétaux à ce niveau de TGP. Cela pourrait permettre de réduire la gamme des conditions pour lesquelles la limite de 110 % s'applique. Les effets chroniques liés à l'exposition à une TGP inférieure à 110 % ne sont pas couverts dans le présent rapport.

Les études futures visant à évaluer les effets biologiques de l'exposition du poisson à la DGS devraient voir à identifier ses schèmes de comportement dans le milieu sauvage. Au besoin, les antécédents d'exposition pourraient ensuite être simulés en laboratoire à l'aide de bio-essais de DGS pour déterminer les effets biologiques. Des modèles d'exposition dynamique causant l'embolie gazeuse pourraient aussi être utilisés pour prédire la mortalité, mais la forte variabilité entre les individus, les différences entre les stocks pour ce qui est de leur susceptibilité à la mortalité et le manque de renseignements disponibles sur le comportement du poisson, nécessaires pour établir les antécédents d'exposition, en limitent présentement l'utilisation.

INTRODUCTION

Dissolved gas supersaturation (DGS) occurs when the partial pressures of atmospheric gases in solution exceed their respective partial pressures in the atmosphere. DGS can result from a variety of man-made and natural causes, including water release from hydroelectric facilities, warm water discharges, solar heating of water bodies, oxygen production by aquatic plants, ingestion of air into pumping systems, supplemental oxygen in hatcheries, and natural waterfalls. At hydroelectric facilities, DGS is produced by entrainment of air as water flows over spillways or other release structures, and plunges to depth. The entrained air, in the form of bubbles, is forced into solution by hydrostatic pressure, producing elevated DGS, which is measured as total gas pressure (TGP). Downstream from hydroelectric facilities, TGP has been reported to approach one and a half times the local atmospheric pressure, or up to 150% TGP (Hildebrand 1991, Fidler and Miller 1997, U.S. ACE 1998). High levels of TGP are known to produce a harmful and often fatal condition in fish known as gas bubble trauma (GBT) (Weitkamp and Katz 1980, Fidler and Miller 1997).

In 1997, Fisheries and Oceans Canada, Environment Canada, and the Province of British Columbia developed water quality guidelines to protect aquatic biota from the effects of high DGS (Fidler and Miller 1997). The guidelines are expressed as ΔP[1] to prevent complications with the variation in TGP caused by atmospheric pressure change with altitude. The lowest guideline applies to juvenile fish in shallow water environments, where TGP-induced swim bladder overinflation can occur. Under these conditions, the guideline limits TGP to 103% of sea level atmospheric pressure at the water surface. This guideline increases linearly with depth up to a maximum of 110% of sea level atmospheric pressure for a water depth of 1 m. The maximum allowable TGP for any water depth is 110% of atmospheric pressure. This guideline protects all species of all age classes from acute mortality. The U.S. Environmental Protection Agency also has a TGP standard of 110% (EPA 1986), yet annual waivers generally allow a TGP of approximately 115% in the forebay and 120% in the tailrace of dams on the Snake River and lower Columbia River to facilitate fish passage (NMFS 1998).

British Columbia has embarked on a Water Use Planning (WUP) process to address existing and future impacts at hydroelectric facilities, as part of re-licensing efforts. The effects of TGP, amongst other fisheries issues, are being examined through this process. The TGP guidelines for the province of BC will serve as a reference for the various WUP studies. However, the guidelines were primarily derived from laboratory bioassay data obtained under static or steady-state exposure conditions (e.g., constant TGP and/or water depth). When trying to assess the biological effects of TGP in a watershed, the dynamics of the exposure are critical because the exposure ("dose") is determined by several factors: TGP, water temperature, and other variables, such as the ratio of partial pressure of nitrogen to oxygen, water depths used by fish, and time exposed to these conditions. Water depth is a key factor because, if fish move to depth, they are subjected to a greater hydrostatic pressure, which

[1] In this report, both ΔP and TGP % will be used to express levels of dissolved gas supersaturation (DGS). This is consistent with the recommendations contained in "Standard Methods for the Examination of Water and Wastewater", 20th Edition, APHA, AWWA, WEF (1998). Appendix 1 contains a list of important definitions and brief descriptions of some more important physical and biophysical processes involved with DGS and GBT.

correspondingly reduces the ΔP, and ΔP controls bubble growth processes (see Appendix 1, Fidler 1998a, b). The depth at which the hydrostatic pressure negates the effects of TGP (i.e., $\Delta P = 0$) is termed the TGP compensation depth. Since the TGP guidelines for BC do not account for dynamic exposure caused by fish use of different habitats and, hence, various water depths, there could be situations where TGP levels exceed the guidelines but do not cause harm to fish that use habitats below the compensation depth. Duration of exposure is another critical factor that is not reflected in the guidelines. Thus, although the guidelines provide a useful starting point for assessing effects of DGS on fish, in order to make more environmentally relevant assessments, the "dose-response" characteristics of the exposure, along with the behavior of the biota, must be considered.

Realistic assessments of the exposure and effects of DGS on fish are limited by a lack of information on the response of fish to dynamic exposure conditions. There are a few caging studies and deep-water laboratory bioassays suggesting that, even when deep water is available, it is not necessarily used completely by fish (Ebel 1971, Blahm *et al.* 1974, Meekin and Allen 1974). However, extrapolation of these results to actual exposure histories of fish in natural environments can be difficult, because fish behavior in a cage or laboratory deep tank is unlikely to be truly representative of their natural behavior in the wild, where food, predators, and other influences are present. Although some dynamic GBT mortality models are being developed for predicting mortality in fish exposed to dynamic conditions of TGP and water depth (e.g., Fidler 1998a, b, Richmond *et al.* 1998), accurate model calibration requires biological data for acute and sublethal effects, which are only partially available at this time.

To facilitate more realistic assessments of the exposure and effects of elevated DGS on fish, Fisheries and Oceans Canada undertook a series of DGS dose-response laboratory bioassays under static (i.e., constant exposure to steady-state TGP in shallow water) and dynamic (variable exposure to TGP due to fish use of different water depths) exposure scenarios. The purpose of the static exposure was to examine the effect of duration of exposure on mortality for various TGP levels, verify the bubble growth threshold equations reported by Fidler and Miller (1997), assess within-stock variability through use of replication, and assess among-stock differences by comparing results with other studies. The purpose of the dynamic exposures was to quantify the effects of DGS on fish in a manner relevant to the natural environment, where fish may occupy a range of water depths over time.

MATERIALS AND METHODS

Experimental Design

Rainbow trout were chosen as the test species because of their presence below most hydroelectric facilities throughout BC. They also appear to be one of the more susceptible salmonids to DGS (Weitkamp and Katz 1980, White *et al.* 1991, Fidler and Miller 1997).

Two primary signs of gas bubble trauma (GBT) reported in the literature are swim bladder overinflation and bubble growth in the cardiovascular system (Fidler and Miller 1997), both of which are dependent on fish size. For example, Shrimpton *et al.* (1990) found that only small fish (less than approximately 55 mm) were susceptible to swim bladder overinflation, while other researchers have shown that such small fish were more resistant to cardiovascular bubble growth (e.g., Nebeker *et al.* 1978, Jensen *et al.* 1986). Jensen *et al.* (1986) also found no significant relationship between fish size and mortality for juveniles ranging in size from approximately 80 to 200 mm fork length. Therefore, fish greater than 100 mm in fork length were used in these experiments because cardiovascular bubble growth, and the accompanying mortality, is more prevalent in this size range. Further, fish growth during the three-month experimental period was not expected to influence their susceptibility to mortality.

The static exposures were conducted in shallow water (0.25 m depth) to provide a worst-case estimate of the effects of TGP on mortality. This depth has also been used for several steady-state exposure experiments reported in the literature, thus facilitating comparison of results.

Two types of dynamic exposure scenarios were examined. First, fish were allowed access to water depths ranging from 0 to 1 m, and from 0 to 2.5 m, using volition cages. Results were compared to those for the static exposures in 0 to 0.25 m water depth, at the same TGP and temperature. The behavioral pattern of each fish determined its individual exposure history. Second, the behavior of individual fish was dictated by holding fish in shallow water cages, and moving the cages up and down throughout the deep tank according to a pre-determined depth cycle. The fish were not given access to the entire water column because it was assumed that their behavior in the laboratory deep tank would not be representative of that in the wild, regardless of size or shape of the tank, location and rate of inflow/outflow, addition of cover, or presence of predators. This is supported by the knowledge that fish behavior is highly influenced by captivity, cover, feeding, predation, water flow, temperature, noise, presence of people, and other factors, many of which cannot be controlled or duplicated in a laboratory setting. Precise information on the behavior of juvenile rainbow trout in the wild was not available at the time of this study. As a result, the depth cycle design could not be based on known periods of time spent above or below compensation depth, actual depths used, and time spent at those depths. Therefore, a range of dynamic exposure scenarios was developed, based on cyclical use of water depth, which may represent diel behavior in salmonids. The various depth cycles had different durations of time at depth, and different utilization of water depth relative to the compensation depth. The second set of dynamic exposure scenarios provided a means of examining effects of repeated bubble growth and collapse in the cardiovascular system on mortality and gill histology.

Transport and Maintenance of Experimental Fish

On December 1, 1999, approximately 4,000 juvenile rainbow trout (Badger-Tunwka stock) were transported from the Vancouver Island Trout Hatchery in Duncan, BC to the Rosewall Creek Experimental Hatchery, near Bowser, BC, using a truck fitted with an insulated tank supplied with compressed air from a portable compressor unit. Immediately before transport, the fish were vaccinated against *Furunculosis* using *Aeromonas salmonicida* bacteria

obtained from Syndel Laboratories Ltd. (Vancouver, BC). The vaccination was undertaken to help prevent infection during the experimental period. The vaccine was administered to batches of approximately 200 fish via a 60 sec immersion bath using a 1:9 dilution with freshwater. The diluted vaccine solution was aerated for about 15 sec between each batch of fish.

At Rosewall, the fish were separated equally into two 2,700 L stock tanks supplied with water at a flow rate of 42 $L \cdot min^{-1}$. This flow provided a 90% replacement time of 1.1 h, which was within the 4 h recommended by Sprague (1973). One stock tank was maintained at 10°C, and the fish from this tank were used in the experiments. Since all experiments were conducted at 10°C, temperature stress was avoided. The second (reserve) stock tank was maintained at ambient water temperature (8.5°C in December; 7.5°C in March). Fish from the reserve tank were transferred to the experimental stock tank when necessary to compensate for use of experimental fish. Initially, fish loading and flow loading densities in each stock tank were 7.3 $kg \cdot m^{-3}$ and 0.48 $kg \cdot L^{-1} \cdot min^{-1}$, respectively. The stock tanks were covered with solid black chloroplast material and black mesh (50% coverage by each).

The fish were fed a maintenance ration of 1.5% body $weight \cdot day^{-1}$ of commercial pellets. This represented 60% of the ration that the fish were fed at the Duncan Hatchery, which was designed to produce optimal growth. A reduced ration was implemented to minimize growth during the three-month experimental period. Two automatic feeders were installed on each holding tank to minimize variability in fish size due to the establishment of dominance hierarchies. The daily food ration was delivered uniformly between dawn and dusk using automatic timers. This regimen resulted in dispersal of food for 30 seconds, every 3 minutes.

Overhead fluorescent lights controlled by a timer simulated the natural photoperiod. In mid-December, the lights went on at 0700 h and off at 1700 h, and this cycle was extended in response to the natural photoperiod. During experimentation, low-level incident illumination was provided at night, behind a black curtain, to provide light for researchers carrying out the GBT assessments. The fish were acclimated to laboratory conditions for approximately seven weeks before experimentation.

Water Supply, Heating, and Generation of DGS

The water supply for all experimental apparatus and holding tanks was groundwater, pumped to an external aeration tower for atmospheric dissolved gas equilibration. The water was heated using an 8 KW (240 V) electric spa heater. After exiting the heater, the water flowed in separate directions for the control and treatment regimens. Control water flowed to an aeration tower within the laboratory to remove excess TGP that developed from heating the water, and then to either the shallow water tanks (for static exposures) or the deep tank (for dynamic exposures, see below). Treatment water flowed directly from the heater to a pressurized PVC column (20 cm dia by 2.4 m height), where it was supersaturated using medical-grade air. The column worked on the principle that, when water in equilibrium with air is pressurized, a dissolved gas deficit is created and the water will be able to absorb more gas before becoming saturated at the higher pressure. Absorption of gas within the column

was enhanced by passing the water over plastic bio-rings, which acted as mass transfer media. There was a direct relationship between the depth of packing (which was 2 m in our column) and the absorption capacity of the column (Cussler 1984).

The rate at which air was absorbed within the column at a given water flow rate depended on operating pressure and water level. The greater the operating pressure, the greater the dissolved gas deficit and absorption capacity. The lower the water level, the greater the depth of exposed packing and surface area for gas absorption. The maximum operating pressure was fixed by water pressure. Thus, TGP was controlled by adjusting the water level and air flow rate using an automatic level control system, consisting of a solenoid valve on the air supply line and an adjustable water level sensor attached to the column sight glass. The water level in the column attained a steady-state position commensurate with the air addition rate. If air flow rate increased, water level was lowered, exposing a greater depth of packing and, hence, increasing absorption capacity. Conversely, if the air flow rate was reduced, water level rose as air in the headspace was absorbed. Depth of packing and, hence, absorption capacity, was reduced accordingly. Moving the water level sensor up or down the sight glass to the level to be maintained produced the desired TGP. When the water level fell below the level of the sensor, the sensor turned the air off via the solenoid valve. With no air entering the column, the water level rose until the level sensor turned the air on, forcing the water level down. This option allowed the air flow to the column to be cycled on and off, and the water level to be maintained between the upper and lower limits of the sensor's dead-band. The dead-band was adjustable via a timer that delayed the switching of the solenoid. Supersaturated water flowed out of the bottom of the pressurized column. Approximately 3 $L \cdot min^{-1}$ of this DGS water was distributed to the TGP measuring column (a PVC column with 10 cm dia and 3.6 m height). The remaining flow was delivered to the static or dynamic exposure apparatus.

Static Exposure Apparatus

The steady-state (static), shallow water exposure apparatus consisted of ten pairs of cylindrical fiberglass tanks (35.5 cm dia by 45 cm height). Water depth was maintained at 0.25 m using an external drainpipe, which provided an experimental volume of 25 L. The tanks were aligned in series along two rows. One row of ten tanks received control water (atmospheric air-equilibrated water at 101% to 102% TGP; 10°C), while the other row received treatment water (air-supersaturated water; 10°C). Water flowed from the header pipe to each experimental tank via a valve fitted with clear plastic tubing. The outlet end of the tubing was fitted with a nozzle, to prevent pressure losses and degassing along the distribution system. The nozzle was secured to the tank bottom to prevent it from floating to the water surface, creating turbulence and potential degassing. The location of control and treatment tanks was varied by crossing the plastic hoses delivering water to each tank, every second pair of tanks. This procedure ensured that any external factors, such as movement, noise, and lighting, would affect both control and treatment tanks equally.

Nine pairs of tanks were used for experimentation, with the tenth set designated for water quality measurements. This design allowed nine replicates, each consisting of a treatment and

control tank, to be tested simultaneously. All tanks were covered with a circular lid constructed from black chloroplast material. Lids contained a small viewing window covered with red cellophane to reduce disturbance from background lighting and observers. The lids were effective in preventing fish from escaping, providing cover, and reducing disturbance from visual observations.

Flow rates to the control and treatment tanks were 1.5 $L \cdot min^{-1}$ and 2.5 $L \cdot min^{-1}$, respectively, corresponding to 90% replacement times of 36 min and 24 min, respectively (Sprague 1973). The higher flow rate to the treatment tanks was due to higher pressure in the header line. Water velocity was measured at the base of each tank, behind the flow nozzle, using a Marsh-McBirney 201D portable water current meter with a 6 sec integration time. Velocity was 5.1 $cm \cdot s^{-1}$ in the control tanks and 11.0 $cm \cdot s^{-1}$ in the treatment tanks. These velocities lie within the normal range used by juvenile rainbow trout. Fish loading densities varied among the experiments; however, they were always less than the upper limit recommended by Sprague (1973). Flow rates for the static experiments at 110% and 114% TGP were changed to achieve the desired TGP by dilution, as described below.

Dynamic Exposure Apparatus

Dynamic exposure experiments were conducted in two cylindrical 900 L flat-bottomed reinforced fiberglass tanks 3 m high and 0.6 m in diameter (supplied by Dynamic Aqua-Supply, Richmond, BC). The tanks were opaque and provided 90% visual light-transmission. Lids were made of black chloroplast material. One tank received control water (air-equilibrated water at 10°C), and the other, treatment water (air supersaturated water at 10°C). The inflow was at the base of the tank, approximately 5 cm from the bottom. The inflow pipe had a flow restrictor to control flow rates and minimize degassing. The outflow, located 5 cm from the top of the tank, reduced the potential for degassing at the surface. The 15 $L \cdot min^{-1}$ water flow rate corresponded to a 90% replacement time of 2.3 h. A water current of approximately 1 $cm \cdot s^{-1}$ was recorded at 1 m depth from the surface (Marsh-McBirney 201D portable water current meter with a 6 sec integration time). Fish loading densities varied with experiment, but were all $< 1.4\ kg \cdot m^{-3}$. Flow loading also varied, but was always $\leq 0.081\ kg \cdot L^{-1} \cdot min^{-1}$. All fish holding criteria were within standards established by Sprague (1973).

For the first set of dynamic exposures, fish were confined within the range of water depths by use of volition cages, which were cylindrical, flat-bottomed Vexor™ mesh liners inserted inside the control and treatment deep tanks. For subsequent cage experiments, cages were constructed from plastic buckets, where large sections of the sides, bottom, and top were removed and replaced with Vexor™ mesh. The cylindrical (flat bottom and top) cages measured 29 cm in diameter and 0.25 m in depth. The buckets had removable lids constructed of Vexor™ mesh and fasteners.

General Procedures for the Static Exposures

The following general procedures were used for all static exposure bioassays; specific details of each experiment are provided below.

Groups of about 200 fish were randomly netted from the stock tank and placed into a smaller 77 L holding tank. Smaller groups of ten fish were randomly selected by dip-net and placed into a 13 L plastic bucket. These fish were randomly allocated to either a treatment or control tank, using a random number generator, until all tanks contained ten fish. The procedure was then repeated in the reverse order, such that the last tank to receive the first group of ten fish was the first tank to receive the second batch of fish. Initially, 20 fish were placed into each exposure tank; however, this was later increased to 25 fish per tank, both of which maintained a fish loading density under 10 $kg \cdot m^{-3}$, as recommended by Sprague (1973). All other fish holding criteria were within the standards established by Sprague (1973).

During acclimation, the fish were not confined, to allow for recovery from handling stress. The fish were not fed for 24 h before acclimation, or during the experiments. Following acclimation, the flow of control water to the treatment tanks was terminated and supersaturated water was delivered to the tanks. The exposure began when the TGP had stabilized in the treatment tanks.

During experimentation, fish were observed every 20 to 30 min. The exception was the 116% TGP exposure, where observations were made every 24 h. Control tanks were checked simultaneously with treatment tanks to assess any mortality and to ensure equal disturbance of control and treatment fish. The time to death for each fish was recorded, along with observations of behavior. Fish were considered dead following loss of equilibrium and cessation of opercula movement. When dead fish were removed from the treatment tanks, the control tank was netted to provide the same disturbance. All fish in the control tanks survived.

Dead fish were blotted dry and weighed, then their fork lengths were determined. A section of gill arch was removed with surgical scissors and examined under a dissecting microscope at 25 to 50 X magnification. In most cases, the fourth gill arch was examined because gas bubbles initially form in this gill arch; however, other arches were also examined. The presence of bubbles and extent of bubble growth in the filaments were recorded qualitatively. Fish were then examined for external signs of GBT. Few bubbles were seen in the lateral line; however, a detailed microscopic analysis was not conducted. Given that bubbles in the lateral line are typically small and difficult to see and that they can occur on fish not exposed to supersaturated water (Dawley *et al.* 1976b), their presence was not recorded in this study. Further, researchers examining the lateral line of chum salmon for gas bubbles following exposure to DGS noted that when the whole side of the fish was wiped with paper towel to remove mucus, bubbles would instantly develop throughout the whole lateral line (Jill Korstrom, West Vancouver Laboratory, Fisheries and Oceans Canada, pers. comm.). The bubbles were a variety of shapes, including round, oblong, and tubular. If only a section of the fish was wiped near the lateral line, bubbles would form in only that section of the lateral line. When control fish (exposed to 100% TGP) were wiped, bubbles would also form in the lateral line, even though they were not seen on the surface of the fish outside the lateral line.

In some experiments, survivors were examined for bubbles in the gill filaments and external signs of GBT using the same methods as for dead fish. Fish were held in the DGS water to which they had been exposed until euthanised in a lethal dose (200 $mg \cdot L^{-1}$) of buffered tricaine methanesulfonate (MS-222), then necropsied within 15 min of death.

Static Exposure at 122% TGP

Juvenile rainbow trout were exposed to 122% TGP at 10°C and 0.25 m depth for 96 h. Nine replicates were run simultaneously, with a sample size of 20 fish per tank (20 treated and 20 control fish per replicate). The process of transferring the 360 fish from the stock tank to experimental tanks took 42 min. Fish were acclimated for 20 h. Although it was not possible to measure TGP accurately in the shallow water exposure tanks, TGP stabilized in the 120% to 121% range in the treatment water quality measuring tank approximately 60 min after the flow of DGS water commenced. This period was similar to the 99% replacement time of 53 min.

Unacclimated Static Exposure at 122% TGP

This experiment was identical to the static exposure at 122% TGP, 10°C, 0.25 m depth, with the following exceptions: there was no acclimation, there were only four replicates, and the experiment ran for 49 h. Groups of 20 fish were placed directly into treatment tanks that contained 122% TGP water at 10°C. The order in which the treatment tanks were supplied with fish was randomized; however, all 20 fish were placed into a given treatment tank simultaneously to ensure all fish had the same exposure history. Following placement of fish into a given treatment tank, the corresponding control tank was stocked with 20 fish. This experiment was conducted after the static exposure at 122% TGP, 10°C, 0.25 m, with no adjustments to the TGP generating column. Thus, TGP for these two experiments was similar.

Static Exposure at 110% TGP

Juvenile rainbow trout were exposed to 110% TGP at 10°C and 0.25 m depth for approximately eight days. Unlike the other static exposures at 122% TGP, which had DGS water direct from the measuring column, the TGP of 110% was achieved by dilution of 122% TGP water with air-equilibrated water. Each treatment tank had one line delivering 122% TGP water at a flow rate of about 1.2 $L \cdot min^{-1}$, and one line delivering control water (102% TGP) at a rate of approximately 1.4 $L \cdot min^{-1}$. Each control tank was supplied by two lines delivering control water, each at approximately 1.4 $L \cdot min^{-1}$. The flows from the two lines converged a short distance from the end of the nozzles. Although this configuration limited the number of replicates that could be tested simultaneously to three, it allowed a concurrent dynamic exposure experiment to be carried out in the deep tanks (see below).

Experimental fish were randomly allocated to treatment tanks using methods described previous for the static 122% exposure with acclimation. The three replicates each consisted of 25 control and 25 treated fish. Hence, the reverse randomization process consisted of two

groups of ten fish each, followed by a final group of five fish. Acclimation was for 18 h. TGP in the treatment tank stabilized about 55 min after the addition of the DGS water.

Static Exposure at 114% TGP

This experiment was conducted by diluting 122% TGP water delivered at a flow rate of 1.8 $L \cdot min^{-1}$ with 102% TGP water delivered at a flow rate of 1.4 $L \cdot min^{-1}$, which produced an average TGP of 113.9 ± 1.0%. Replicates, sample size, control tank configuration, and transfer of fish to experimental tanks were the same as for the static exposure at 110% TGP. Acclimation lasted 20 h. TGP stabilized at 114% in the treatment tanks after about 50 min and the exposure lasted approximately six days.

Static Exposure at 116% TGP

This experiment was conducted by holding fish within the top 0.25 m layer of the deep control and treatment tanks using the Vexor™ mesh liner. Air-supersaturated water, at 116% TGP, was delivered to the treatment tank at a flow rate of 15 $L \cdot min^{-1}$. The control tank also had a flow rate of 15 $L \cdot min^{-1}$. Fish were acclimated for 19 h in cages in the control tank, using procedures described below for the dynamic exposures. The sample size was 100 fish in each of the treatment and control tanks. The fish were checked every 24 h (at 1030 h) for six days and a final observation was made on the ninth day (at 1030 h).

Unacclimated Static Exposure at 140% TGP

Fish were exposed to 140% TGP at 10°C and 0.25 m. Five replicates were run simultaneously, with a sample size of 20 fish per tank. There was no acclimation. Groups of 20 fish were placed into randomly selected control or treatment tanks, and each experiment commenced upon placement of the treatment fish into the tank containing water at 140% TGP. Fish were monitored for approximately 6 h, after which they were left unobserved for an additional 18 h to determine whether all fish would die after a 24 h period.

General Procedures for the Dynamic Exposures

The following procedures were adhered to for all dynamic exposure scenarios; specific procedures for each experiment then follow.

All fish were acclimated in cages held in the control deep tank. This procedure was necessary because the 90% replacement time for transition from control water to the air-supersaturated water in the treatment tank was considered too long for quantifying the exposure. Groups of about 200 fish were removed from the stock tank and placed in a 77 L holding tank. Batches of fish, consisting of the entire sample size for each replicate, were randomly selected and placed in a 13 L bucket. These fish were gently netted into each cage. The three control cages were stocked first, in a random order, before being lowered to 0.5 m depth for acclimation. The three treatment cages were then stocked in a random order, and placed at the surface of the deep control tank for acclimation. This method ensured that treatment fish were not

affected by hydrostatic pressure during acclimation. Dissolved oxygen was monitored during acclimation to ensure it was within acceptable levels. At the end of the acclimation, the three cages containing treatment fish were quickly lifted out of the water one at a time, and moved into the deep tank. The time taken to move each cage from the control to treatment tank was less than 5 sec. The remaining three cages in the control tank were lifted and held out of the water for about 5 sec, before being placed back into the control tank. This procedure ensured that treated and control fish were handled similarly.

During experimentation, fish were observed every 20 to 30 min in both control and treatment tanks. However, in experiments where fish were cycled to depth, the fish were not observed while below the compensation depth. When dead fish were removed from the treatment cages, the control cages were not treated the same because it was too difficult to remove the cage lids and simulate the netting of dead fish. Dead fish were examined according to the procedures for the static exposures.

Dynamic Exposure at 122% TGP in 0 to 1 m Deep Volition Cages

During their exposure to 122% TGP at 10°C, fish were allowed to swim freely from 0 to 1 m depth in the deep tank by use of the Vexor™ mesh liners. Tanks were covered with black nylon mesh to prevent escape. A black chloroplast lid was placed lightly on top of the mesh.

The acclimation process was similar to the other dynamic exposures in which fish were held in cages, except that upon completion of acclimation, the fish were slowly released from the cages into the tank lined with the Vexor™ mesh. The sample size was 120 fish per treatment and control tank, with 40 fish per cage for acclimation. The acclimation time was 23 h and the experiment followed for 96 h.

Dynamic Exposure at 122% TGP in 0 to 2.5 m Deep Volition Cages

The experimental procedure was identical to that for the dynamic exposure at 122% TGP with water depth from 0 to 1 m depth, with the exception that the depth ranged from 0 to 2.5 m, and the sample size was 100 fish per tank. Acclimation time was 17 h, and the experiment followed for 96 h.

Dynamic Exposure at 122% TGP with 3 h Intervals at Depth

Fish were held in cages at the surface of the deep treatment tank where they were exposed to 122% TGP for a duration that killed 10% of the total sample. The cages were then lowered below the compensation depth, where they were held for 3 h before returning to the surface. This depth cycle was repeated four times, such that the percent cumulative mortality was from 0 to 10% during the first surface interval, and from 10% to 20%, 20% to 30%, and 30% to 40% during the second, third, and fourth surface intervals, respectively. At the end of the experiment, the percent cumulative mortality was approximately 40%. Initially, there were 27 fish in each of the three treatment and control cages (81 fish total). Thus, for each cycle, fish were held at the surface until eight fish in total died. In some cases, two fish died together at

the end of the cycle, bringing the total count to nine fish. Hence, the cumulative mortality differed slightly from even increments of 10%. The four cycles were completed over 112 h (4.7 days).

While at the surface, the top of each cage was at 0 m and the bottom was at 0.25 m depth. At depth, the top of each cage was at 2.5 m and the bottom was at 2.75 m. The tops of the cages were just below the compensation depth for 122% TGP, which was 2.2 m. The rate at which fish were raised and lowered was 0.5 m every 10 sec. Acclimation (see general procedures) was carried out for 19 h.

Dynamic Exposure at 122% TGP with 6 h Intervals at Depth

The experimental procedure was identical to that for the dynamic exposure at 122% TGP with 3 h intervals at depth, with the exception that the fish were held at depth for 6 h, rather than 3 h. The four cycles were completed over 147 h, or approximately six days. Acclimation time was 22 h.

Dynamic Exposure at 124% TGP with 6 h Intervals at Depth

The experimental procedure was identical to that for dynamic exposure at 122% TGP with 6 h intervals at depth, with the exception that TGP was 124%. The four depth cycles were completed over approximately four days. Acclimation time was 19 h.

Disease Testing

At the end of the dynamic exposure experiment at 122% TGP with a 6 h depth cycle, 30 of the 47 survivors, along with 30 fish from the experimental stock tank, were randomly selected for disease testing. Survivors from the experiment were placed in a 77 L container filled with supersaturated water (122% TGP; 10°C). The fish from the stock tank were placed in a second container (77 L) filled with water from the stock tank. The fish were transported to the Pacific Biological Station (PBS) in Nanaimo, BC, approximately 1 h away by road.

Twenty fish that died during the 140% TGP steady exposure were also preserved in Davidson's solution immediately after death for gill histology at PBS. Some fish were fixed whole, while others had their heads severed before fixation.

At PBS, the live fish were prepared immediately for diagnostic work. For the bacteriological assessment, the posterior kidney was swabbed and cultured on Tryptic Soy agar and Shieh's medium. Direct fluorescent antibody tests (DFAT) for *Renibacterium salmoninarum* were done on the kidney tissue of all fish. For virology, twelve pools containing gill, spleen, pyloric caeca, and kidney tissue from five fish were cultured on *Epithelioma papulosum cyprini* cells for 14 days at 15°C. Finally, the fourth gill arch and the pseudobranch were examined histologically for signs of tissue damage, including edema of the lamellae and epithelial degeneration (Roberts 1989). Histological examination was performed on the gills of fixed fish.

Water Quality Measurements

TGP in the air-supersaturated water was recorded using two total dissolved gas meters (Common Sensing Inc., Clark Fork, Idaho, USA). The range and accuracy of both meters was 0 to 1500 ± 2 mm Hg for dissolved gas total pressure, and 0 to 1000 ± 2 mm Hg for pO_2. TGP measurements in control water were made using a tensionometer (Model 300C, Alpha Designs Ltd., Victoria, BC; accuracy ± 1 mm Hg, range 0 to 750 mm Hg). Calculations in Colt (1984) were applied to arrive at the dissolved gas parameters and O_2/N_2 ratios.

Temperature was recorded every 15 min by Onset Stowaway Tidbit waterproof loggers (accuracy ± 0.2°C, range -5°C to 37°C). A hatchery water quality scan was conducted on the control and treatment water delivered to the experimental apparatus before experimentation.

For the static exposure experiments conducted in the shallow water tanks, TGP was measured in the measuring column (MC) at the base of the TGP generating column. This allowed accurate TGP measurements to be taken below the compensation depth. In the 110% TGP experiment, it was possible to measure the TGP directly in the shallow water tank designated for water quality. This was not possible for higher TGP because bubble growth on the probe membrane provided an erroneously low TGP compared to the true TGP recorded in the MC. To ensure that the experimental fish were exposed to the same TGP as that measured in the MC (located at the base of the TGP column and at the start of the header line delivering water flow to the experimental tanks), a shorter (1 m) measuring column was located at the end of the row of shallow tanks (at the end of the header line). This smaller MC was served by the hose that delivered flow to the tenth treatment tank for water quality monitoring.

Degassing tests were conducted to determine whether the lower TGP readings in the shallow water tanks for TGP above 110%, relative to the true TGP reading from the MC, were due to bubble growth on the membrane or degassing at the water surface. These tests consisted of varying the flow rates and methods of shaking the TGP probes to dislodge bubbles. One test used a PVC column 0.25 m in depth and exactly the width of the probe; the nozzle was inserted at the base of the column and water spilled out over the top. This set-up prevented degassing since there was minimal available surface area over which this could occur. Dissolved oxygen and temperature measurements were also used to determine degassing.

For the experiments conducted in the deep tanks, TGP was measured in the MC at the base of the TGP generating column and proximal to the bottom of the deep tanks. Degassing tests were also conducted for the deep tanks by taking measurements at various depths and comparing results with the true TGP measured in the MC.

Data Analysis and Statistical Procedures

Treatment, Experimental Unit, and Randomization Structure

The static exposure apparatus provided for a replicated, randomized-block design. The experimental unit structure was the tank (i.e., the treatment was applied to the tank rather

than individual fish within a tank), and each pair of control and treatment tanks represented individual replicates. Fish were randomly assigned to tanks (to the extent possible without marking individual fish). The location of treatment and control tanks was blocked, meaning that the treatment and control tanks were paired, and the location of the two tanks within each pair was alternated every replicate. Statistical procedures were applied to the summary data for each tank.

For the dynamic exposures where fish were cycled to depth in cages, the three separate cages within each deep tank likely represented pseudoreplication (see Hurlbert 1984). That is, each cage represented a sub-sample rather than true replication. However, data analysis revealed a minimal tank effect (i.e., the variability among tanks was small compared to the variability among sub-samples within a tank). For example, in the two dynamic experiments with 3 h and 6 h depth intervals at 122% TGP, the variability in time to 10% mortality between the two deep treatment tanks was relatively small (SD = 1.74) compared to the variability among the three cage sub-samples (SD = 3.16, average of the two experiments). Thus, although the cages might have represented pseudoreplication rather than true replication, the effect on data analysis would have been minor, and cages were treated as replicates for some statistical analyses.

Data Analysis

The mortality data for each DGS exposure were used to prepare a cumulative mortality plot (i.e., time to mortality versus percent cumulative mortality). These plots were prepared for individual replicates and for all replicates combined (i.e., all fish from the various replicates were pooled and treated as one replicate). Plots were also prepared using the average time to mortality (averaged over all replicates) for a given percent cumulative mortality. In all cases, the response for all replicates combined (the pooled data) was similar to the average response calculated from the replicate data (see results). Hence, the pooled data were used for graphical purposes. In situations where pooled data were used for regression analyses (e.g., analysis of covariance; see below), the model included replicates as a random effect. All other statistical analyses were conducted on the replicate data.

The time required to kill 10%, 25%, 50% and 75% of the fish (LT10, LT25, LT50, and LT75, respectively) was calculated for each replicate, and for all replicates combined, using a logistic model. In those exposures where 50% mortality did not occur, the time to 40% mortality was determined (e.g., LT40).

Statistical Procedures

The static and dynamic exposures at 122% TGP were compared using several methods. The LT10, LT25, and LT40 calculated for each replicate were compared among the exposures using analysis of variance (ANOVA). This provided an assessment of the delay in mortality associated with fish use of water depth.

The effect of fish use of water depth on mortality rate was examined by comparing the mortality rate for each group that died at the water surface in a given cycle in the dynamic exposure, to the mortality rate for the corresponding cumulative mortality range in the static exposure. The ranges of cumulative mortality were approximately 0 to 10%, 10 to 20%, 20 to 30%, and 30 to 40% for the first, second, third, and fourth surface intervals, respectively. The mortality rate was determined using linear regression ($y = ax + b$), where the y variable was percent cumulative mortality, the x variable was time to mortality in hours, and the slope (a) represented the mortality rate (i.e., percent cumulative mortality per hour). The mortality rate for the second to fourth surface intervals was calculated from the beginning of the surface interval (rather than the time to death of the first fish in that interval). Differences in the slopes of the linear regression models for the static and dynamic exposures were then examined using analysis of covariance (ANCOVA), where a significant interaction term in the ANCOVA indicated a difference in slopes. Although the ANCOVA used the combined data for all replicates, a separate ANCOVA that specified replicate number as a random effect in the model was also performed. Data were not transformed, as the relationship between cumulative mortality and time to mortality was relatively linear over the small ranges of cumulative mortality that were examined. Further, logit and log transformations were applied to the data; however, they did not improve the fit of the data over all segments. For those segments where the linear fit was improved, the R^2 was only marginally higher in most cases.

ANCOVA was also applied to examine differences in slopes (i.e., mortality rate) over the entire range of 0 to approximately 40% cumulative mortality for the static and dynamic experiments at 122% TGP. The analysis was restricted to 0 to 40% cumulative mortality because the exposures were terminated after this point. The regression model used raw (untransformed) data, as they were relatively linear over this range of cumulative mortality. Further, a series of logit and log transformations did not improve the linear fit of the data over this range of cumulative mortality (i.e., the R^2 values were higher for the linear regression model on the raw data). The ANCOVA used the data for all replicates combined (time at depth included in the exposure time). A separate ANCOVA specified replicate as a random effect.

Use of linear regression on "cumulative" mortality data may violate the assumption of independence of the residuals, but the estimates are unbiased. However, the reported standard errors and p-values may have been too small. Thus, the p-values were judged more harshly (i.e., using α of less than 0.05), where regression was applied to cumulative mortality data. This had little influence on the results because the p-values were much less than 0.05 in almost all cases where the null hypothesis was rejected.

A logistic model of the form $y = a + b / \{1 + (x/c)^d\}$ was fit to the replicate data for the static and dynamic exposures at 122% TGP, where the y variable was percent cumulative mortality and the x variable was the average time to mortality for a given percent cumulative mortality. Confidence intervals (for the mean of y at the specified value of x) were examined. Other models were also fit to the data; however, the logistic dose-response model (an extension of the logit model) gave the best fit. The sigmoid function provided similar R^2 values, but there was little difference between the two models.

RESULTS

Water Quality Data

TGP and temperature data for each experiment are provided in Appendix 2. The ratio of pN_2 (including argon and trace gases) to pO_2 for the control water ranged from 3.80 to 3.94, which was near equilibrium (3.77). For the supersaturated water, this ratio ranged from 3.84 to 4.20. The ratio of O_2/N_2 in percent saturation ranged from 0.99 to 0.96 for control water and 0.98 to 0.89 for treatment water.

Additional water quality results for the control and treatment water are presented in Table 1, along with hatchery criteria for fish culture (Sigma Environmental Consultants Ltd. 1983) and the Canadian Council of Ministers of the Environment (1998) water quality guidelines for protection of freshwater aquatic life. All variables tested were within acceptable limits for fish culture and protection of aquatic life. The low concentrations of metals such as copper, aluminum, lead, and zinc indicated that the external aeration tower, water heater, aeration tank within the laboratory, and other components of the experimental apparatus did not influence water quality. The anode in the heater might have had a slight influence on zinc concentration; however, the level was below water quality guidelines or criteria in both control and treatment water. In conclusion, the control and treatment water was suitable for fish culture, and would not have caused or contributed to any mortalities recorded during the experiments.

Static Exposures

Static Exposure at 122% TGP

Mean fork length and weight of the treatment fish were 110 mm (± 14) and 13.2 g (± 5.3), respectively. There was no relationship between time to mortality and fish fork length ($p=0.7774$) (see Figure 1) or weight ($p=0.7853$) (see Figure 2), i.e., the slopes of the linear regressions did not differ significantly from zero. This was consistent with Jensen et al. (1986), who found only a weak relationship between fork length and mortality for fish greater than about 100 mm fork length.

Figure 3 shows time to mortality versus cumulative mortality for each of the nine replicate exposures, for all nine replicates (180 treatment fish) combined into one cumulative mortality plot, and for the average time to mortality for a given cumulative percent mortality (averaged over the nine replicates). The response for all replicates combined was approximately the average response for the individual replicates, particularly in the mid-range of cumulative mortality (Figure 3). The percent cumulative mortality after 96 h was 89% for all replicates combined. Percent cumulative mortality over the 96 h exposure was 100%, 95%, 100%, 75%, 90%, 70%, 80%, 85%, and 100%, for replicates 1 to 9, respectively. All control fish survived.

Table 1. Water quality results for control and treatment water.

Variable	Control Water	Treatment Water	Detection Limit	Units	Hatchery Criteria[a]	CCME Guidelines[b]
Aluminum	0.0048	0.0025	0.0008	mg/L	0.1	0.1
Antimony	<0.005	<0.005	0.005	mg/L	0.001	
Arsenic	<0.01	<0.01	0.01	mg/L		0.005
Barium	0.00041	0.00025	0.00004	mg/L		
Bismuth	0.0027	<0.0004	0.0004	mg/L		
Cadmium	<0.00006	<0.00006	0.00006	mg/L	0.0003	0.00017
Calcium	9.9	8.3	0.002	mg/L		
Chloride	0.9	0.8	0.1	mg/L	250	
Chromium	<0.0006	<0.0006	0.0006	mg/L	0.04	0.001[c]
Cobalt	0.00049	<0.00003	0.00003	mg/L		
Copper	0.00049	0.00038	0.00003	mg/L	0.002	0.002
Fluoride	0.14	0.05	0.04	mg/L	1	
Hardness	31.3	27.4	1	mg/L $CaCO_3$		
Iron	<0.003	<0.003	0.003	mg/L	0.3	0.3
Lead	<0.0003	<0.0003	0.0003	mg/L	0.004	0.001
Magnesium	1.59	1.59	0.0005	mg/L		
Manganese	<0.00002	0.00002	0.00002	mg/L	0.1	
Mercury	<0.0001	<0.0001	0.0001	mg/L	0.0002	
Nickel	0.0001	0.0001	0.00001	mg/L	0.045	0.025
Nitrate-N	0.209	0.153	0.004	mg/L	1	
Nitrite-N	<0.002	<0.002	0.002	mg/L	0.015	0.06
pH	7.2	7.2			6.5 - 9.0	6.5 – 9.0
Phosphorus	<0.03	<0.03	0.03	mg/L		
Potassium	<0.4	<0.4	0.4	mg/L		
Selenium	<0.004	<0.004	0.004	mg/L	0.05	0.001
Silicon	4.86	4.94	0.004	mg/L		
Silver	<0.00005	<0.00005	0.00005	mg/L	0.0001	0.0001
Sodium	2	1.2	0.004	mg/L		
Strontium	0.0119	0.0114	0.00002	mg/L		
Sulphate	0.62	0.77	0.05	mg/L	250	
Sulphur	0.274	0.256	0.008	mg/L		
Tin	<0.003	<0.003	0.003	mg/L		
Total Alkalinity	32	29	5	mg/L	> 15 - 20	
Zinc	0.0131	0.01	0.0002	mg/L	0.015	0.03

a) Source: Sigma Environmental Consultants Ltd. (1983)
b) Source: Canadian Council of Ministers of the Environment (1998)
c) Guideline for Cr (VI)

LT10, LT25, LT40, LT50, and LT75 data are provided in Appendix 3. The time to mortality varied widely among replicates. The initiation of mortality (time to death of the first fish) ranged from 14.0 h for replicate 1 to 44.4 h for replicate 6. The mean for all nine replicates was 25.9 h. The actual time to 50% mortality varied even more widely among replicates, from 29.0 h to 77.5 h, with a mean of 54.6 h. LT50 data calculated from a logistic model were similar to the actual time to 50% cumulative mortality (see Appendix 3).

Figure 3 also compares results of the current study (rainbow trout, average 110 mm, exposed to 122% TGP, 10°C, and 0.25 m water depth) with those of Dawley *et al.* (1976a) for 180 mm steelhead trout exposed to 122% TGP (120% corrected with the addition of water vapor), 10°C, and 0.28 m water depth. Dawley *et al.* (1976a) reported earlier time to death and higher mortality rate (100% after about 29 h) than the current study. Although Dawley *et al.* (1976a) had only two replicates, each replicate contained about 80 fish.

Results of GBT assessments on fish that died from the static 122% TGP exposure are shown in Table 2. All fish that died during the experiment had tubular bubbles in the gill filaments. Considering all replicates combined, 48% (77 of 160 fish) exhibited no external GBT signs. However, 82 of 160 fish had bubbles in the caudal fin (51%). A few fish had bubbles in other fins, and one fish had bubbles around the eye. GBT assessments made on the 19 fish that survived this experiment showed that eight (42%) had bubbles in the caudal fin. Eleven fish (58%) had no external signs of GBT.

Table 2. GBT assessment results for fish that died during the 122% TGP static exposure, at 10°C and 0.25 m water depth. Percent (%) represents percentage of dead fish with various external GBT signs or no external GBT signs.

Rep.	Mortality (number of dead fish)	Bubbles in Gill Filaments (%)	Bubbles in the Caudal Fin (%)	Bubbles in the Anal Fin (%)	Bubbles in the Dorsal Fin (%)	Bubbles in or around the Eye (%)	No External GBT Signs (%)
1	20	100	50	0	0	0	50
2	19	100	53	0	5	0	42
3	20	100	55	0	0	0	45
4	15	100	33	7	7	0	67
5	19	100	58	0	0	0	42
6	14	100	29	0	0	0	71
7	16	100	50	0	6	0	44
8	17	100	53	0	0	0	47
9	20	100	65	0	0	5	35
Mean		100	51	1	2	1	48

Observations of fish behavior showed that, at the beginning of the experiment, fish swam erratically and made contact with the tank lids at night. Some of the fish held position, while others swam more actively. Fish were observed to be at various locations and used the full water depth during the experiment. A slight tendency for both control and treatment fish to use the bottom of the tank at night was noted. From the middle to the end of the exposure, the treatment fish behaved abnormally, showing characteristic signs of stress. They became lethargic and held at one location within the tank, and at various water depths for individual fish. Several fish swam sporadically and erratically just prior to their death. They would quickly dart off in various directions for approximately 30 sec to 1 min or more before losing equilibrium upon death. Dead fish eventually sank to the bottom of the tank.

Unacclimated Static Exposure at 122% TGP

The percent cumulative mortality for unacclimated exposures (for each replicate and for all replicates combined) lay within the range of variability for the acclimated exposure, also at 122% TGP, 10°C, and 0.25 m depth. Since only four replicates were conducted in the unacclimated exposures, the cumulative mortality plots for these replicates combined was compared to that for the same four replicates in the acclimated exposure (i.e., those replicates conducted in the same exposure tanks). These cumulative mortality plots were similar and exhibited several crossover points. Further analysis showed no consistent relationship between the combined response of the unacclimated and acclimated fish for each exposure tank. For example, in tanks 1 and 3, acclimated fish generally died before unacclimated fish, while in tanks 2 and 5 most of the unacclimated fish died before the acclimated fish. In all tanks, there were several points where the lines crossed over, indicating the relationship between acclimated and unacclimated was reversed.

LT data for the unacclimated exposure are listed in Appendix 3. All control fish survived. The LT10 ($p=0.0997$), LT25 ($p=0.2223$), and LT40 ($p=0.2646$) did not differ significantly among (1) the acclimated exposure with all nine replicates combined, (2) the acclimated exposure with only those four replicates conducted in the same tanks used for the unacclimated exposure, and (3) the unacclimated exposure with all four replicates combined. Thus, differences in time to mortality between the acclimated and unacclimated fish were not significant. TGP, temperature, and fish size were similar among the acclimated and unacclimated exposures. For example, mean TGP and temperature during the acclimated experiment were 122.2% (± 0.2) and 10.0°C (± 0.1), compared with 122.2% (± 0.2) and 9.8°C (± 0.1) for the unacclimated experiment (see Appendix 2). The acclimated fish had a mean length and weight of 110 mm (± 14) and 13.2 g (± 5.3), compared to 109 mm (± 14) and 13.4 g (± 5.3), respectively, for the unacclimated fish.

All dead fish had tubular bubbles in the gill filaments, and 38% of them had no external GBT, compared to 48% for the acclimated experiment. Bubbles in the caudal fin were found in 52% of the fish, compared to 53% for the acclimated experiment. Among survivors, nine (24%) had external signs of GBT (bubbles in the caudal fin), compared to 42% of the survivors in the acclimated static exposure; however, the acclimated exposure time was longer (96 h versus 49 h for unacclimated exposure).

Table 3. GBT assessment results for fish that died during the 122% TGP static exposure (unacclimated), at 10°C and 0.25 m water depth. Percent (%) represents percentage of dead fish with various external GBT signs, or no external GBT signs.

Rep.	Mortality (# of dead fish)	Bubbles in Gill Filaments (%)	Bubbles in the Caudal Fin (%)	Bubbles in the Anal Fin (%)	Bubbles in the Dorsal Fin (%)	Bubbles in or around the Eye (%)	No External GBT Signs (%)
1	12	100	58	0	25	8	25
2	8	100	25	12	0	0	63
3	8	100	63	0	12	0	37
4	14	100	57	14	0	0	36
Mean		100	52	7	10	2	38

Static Exposure at 110% TGP

All treatment and control fish survived. A random sample of 15 treatment survivors had mean fork length and weight of 110 mm (± 24) and 11.6 g (± 6.7), respectively. Of the 15 fish, 12 (80%) had no external signs of GBT and three (20%) had bubbles on the caudal fin. None of these fish had bubbles in the gill lamellae, but eight (53%) had extra-corporeal bubbles around the gill filaments.

Periodic observations showed that fish were active most of the time, positioning themselves throughout the entire water column (0.25 m depth). Occasionally, particularly at night, both control and treatment fish schooled near the bottom behind the water delivery nozzle. At times, fish in the treatment tanks showed some signs of stress (e.g., aggregating on the bottom); however, they appeared less stressed than those in the 122% TGP static exposure.

Static Exposure at 114% TGP

All control and treatment fish survived the six day experiment. A random sample of 30 survivors from the treatment tanks had mean fork length and weight of 121 mm (± 22) and 17.0 g (± 7.3), respectively. No external signs of GBT were seen in 26 fish, three had bubbles in the caudal fin, and one had an enlarged eye surrounded by bubbles. None of the 30 survivors had bubbles in the gill filaments.

Static Exposure at 116% TGP

Figure 4 shows percent cumulative mortality recorded over 24 h intervals, up to 144 h, along with results from static exposures at 110%, 114%, 122%, and 140% TGP for reference. At 116% TGP, there was no mortality after 24 h. Only one fish (1%) died after 48 h. Cumulative

mortality increased after 72 h, 96 h, 120 h, and 144 h to 5%, 9%, 17%, and 25%, respectively. No observations were made between day 6 (144 h) and day 9 (216 h); however, by day 9 (at 216 h), another 17 fish had died, for a cumulative mortality of 42%. All control fish survived. Mean fork length and weight for all treatment fish were 127 mm (± 20) and 20.4 g (± 8.7), respectively. A random sample of five survivors from the control tank had a mean fork length and weight of 127 mm (± 20) and 20.5 g (± 9.7), respectively.

Approximately half of the fish that died had external signs of GBT, primarily bubbles in the caudal fin. However, because fish were assessed once every 24 h, some GBT signs might have developed between the times of death and examination. All 57 survivors were examined for signs of GBT and 32 (56%) showed no external signs. The most prevalent GBT sign was bubbles in the caudal fin (26%). Only a few fish had bubbles in the other fins.

Unacclimated Static Exposure at 140% TGP

At 140% TGP, the initiation of mortality (time to death of first fish) was short, 2.6 h, 2.45 h, 4.55 h, 1.47 h, and 3.68 h for replicates 1 to 5, respectively, and the mortality rate was exponential (see Figure 4, for all replicates combined). Percent cumulative mortality at the end of the exposure time (5.65 h) was 55% for all replicates combined, and 75%, 50%, 60%, 50%, and 40%, for replicates 1 to 5, respectively. After 24 h, all treatment fish were dead. All control fish survived. Fork length and weight of the dead fish averaged 141 mm (± 20) and 29.8 g (± 10.3), respectively. All fish that died during this exposure had tubular bubbles in the gill filaments (Table 4). In total, 45% of fish that died showed no external signs of GBT. The most common sign was bubbles in the caudal fin (53%).

The fish used the entire water column during this experiment. Some fish exhibited sporadic swimming behavior prior to death, as seen in the 122% static exposure at 0.25 m depth, whereas others died quickly following less active swimming.

Table 4. GBT assessment results for fish that died from the 140% TGP static exposure (unacclimated), at 10°C and 0.25 m. Percent (%) represents percentage of dead fish with various external GBT signs, or no external GBT signs.

Rep.	Mortality (# of dead fish)	Bubbles in Caudal Fin (%)	Bubbles in Anal Fin (%)	Bubbles in Dorsal Fin (%)	No External GBT Signs (%)
1	15	47	0	0	53
2	10	70	20	0	20
3	12	50	0	0	50
4	10	60	0	0	40
5	8	37.5	12.5	0	62.5
Mean		53	6.5	0	45

Summary of Results for Static Exposures

Figure 4 shows results for all static exposures, and mean (± SD) LT10, LT25, LT50, and LT75 for the replicate data from the 122% TGP (acclimated) and the 140% TGP (unacclimated) experiments. Table 5 provides additional data for the static exposures.

Time to mortality for a given percent cumulative mortality was more variable for the 122% TGP exposure compared to that at 140% TGP, even when variability was expressed relative to the mean. The coefficient of variation for the LT50 was 0.284 for the 122% exposure and 0.111 for the 140% exposure. Similarly, for LT25, the coefficient of variation was 0.351 and 0.124 for the 122% and 140% exposures, respectively. For LT10, the coefficient of variation was also higher for the 122% exposure (0.363) than the 140% exposure (0.220), although the difference was less pronounced than that reported for LT25 and LT50.

Table 5. Summary of results for static exposures at 10°C and 0.25 m water depth.

TGP %	Duration of Exposure	Sample size[a]	Percent Mortality of Exposed Fish	Mean Fork Length[b] (mm) (± SD)	Mean Weight (g) (± SD)	External GBT[c] (% of Dead Fish)	External GBT[c] (% of Survivors)
110%	8 days	75	0	110 (± 24)	11.85 (± 6.7)	NA	20
114%	≈ 6 days	75	0	121 (± 22)	17.0 (± 7.3)	NA	13
116%	9 days	99	42	127 (± 20)	20.4 (± 8.7)	No data	56
122%	96 h	180	89	110 (± 14)	13.2 (± 5.3)	52	42
122%	49 h	80	53	109 (± 14)	13.4 (± 5.2)	62	24
140%	5.6 h	100	55	141 (± 20)	29.8 (± 10.3)	55	No data

a) Represents the total number of fish exposed to elevated TGP (for all replicates).
b) Weight, fork length, and GBT data are for exposed fish only.
c) Percentage of dead fish or survivors exposed to elevated TGP showing any external sign of GBT.

Dynamic Exposures

Dynamic Exposure at 122% TGP in 0 to 1 m Deep Volition Cages

Of the 120 treatment fish, 26 (22%) died during the 96 h exposure. Mean fork length and weight of these 26 fish were 125 mm (± 18) and 20.1 g (± 8.4), respectively. The first fish died after 36.5 h, compared to 14 h for the static exposure at 122% TGP and 0.25 m depth.

Percent cumulative mortality over the 96 h period was only 22% in the 1 m deep volition cage, compared to 89% in the static exposure. All control fish survived.

Ten of the 26 fish that died (38%) had no external signs of GBT, 14 (54%) had bubbles in the caudal fin, two (8%) had bubbles in the anal fin, two (8%) had bubbles in the dorsal fin, and one (4%) had bubbles around the eye. All fish that died had tubular bubbles in the gill filaments. A random sample of six survivors from the treatment tank showed that only one fish had tubular bubbles in the gill filaments and no external signs of GBT. One fish had extra-corporeal bubbles among the gill filaments, but lacked tubular bubbles within the filaments or external GBT.

Dynamic Exposure at 122% TGP in 0 to 2.5 m Deep Volition Cages

There was no mortality in the treatment or control tank over the 96 h exposure. Treatment fish moved to the bottom of the tank once they were released from the cages used for acclimation. Later, the fish used various water depths, but in the control tank, more fish used the water surface. Mean fork length and weight of a random sample of 23 survivors from the treatment tank were 135 mm (± 21) and 26.0 g (± 11.0), respectively. No external signs of GBT were found in 22 of the 23 survivors (96%) and only one fish (4%) had bubbles in the caudal fin.

Dynamic Exposure at 122% TGP with 3 h Intervals at Depth

Mean fork length and weight for all treatment fish were 109 mm (± 15) and 12.7 g (± 5.0), respectively. One fish from the control group died of unknown causes, perhaps related to caging stress, particularly the cycling up and down of cages in the deep tank. It did not show external GBT or bubbles in the gills.

Figure 5 shows time to mortality versus percent cumulative mortality for each of the three replicates, and for all replicates combined. Time at depth was included in the time to mortality. The average time to mortality curve (for the three replicates) for a given percent cumulative mortality (not shown in Figure 5) was very similar to that for all replicates combined. Figure 5 also shows cumulative mortality for all replicates combined (with time at depth subtracted) and, for comparison, the static exposure at 122% and 0.25 m (all replicates combined). All cumulative mortality plots for the 3 h dynamic exposure (both with and without time at depth subtracted) were shifted to the right of the static exposure plots, indicating that the 3 h depth intervals delayed mortality, compared to the baseline static exposure of 122% TGP at 0.25 m.

Statistical assessment of the LT10, LT25, and LT40 data (Appendix 3) support the graphical interpretations noted above. That is, the mean LT10 did not differ significantly ($p=0.8734$) between the static (29.8 h) and 3 h dynamic (28.8 h) exposures. This result was expected because both treatments were exposed to 122% TGP and 0.25 m depth over this range of cumulative mortality (0 to 10%). However, the mean LT25 differed significantly ($p=0.0049$) between the static (38.1 h) and 3 h dynamic (69.6 h) exposures. Similarly, the mean LT40

differed significantly (p=0.0006) between the static (46.9 h) and 3 h dynamic (95.2 h) exposures. Thus, the dynamic exposure significantly delayed mortality compared to the baseline static exposure. This analysis used the raw data (time at depth not subtracted) because time at depth was an integral part of the total exposure time.

Regression equations are provided in Table 6 for cumulative mortality as a function of time to mortality for the dynamic and static experiments. Ranges of percent cumulative mortality from static experiments were chosen to correspond with the four cycles of the dynamic experiments for comparison and statistical (ANCOVA) analysis. For the dynamic exposure, the percent cumulative mortality for the first surface interval (group of eight fish) was from 0 to 10%, and from 10% to 21%, 21% to 31%, and 31% to 41% for the second, third, and fourth surface intervals, respectively. The range of cumulative mortality for each surface interval was slightly different from even increments of 10% mortality because two of the 80 fish escaped from the cage during the experiment.

Table 6. Linear regression equations for the static and 3 h dynamic exposures.

Exposure	Range of Cumulative Mortality	Linear Regression Equation	R^2
Static: 0.25 m (122% TGP)	0 to 10 %	y = 0.94 x – 15.2	0.762
	10 to 21 %	y = 1.56 x – 28.5	0.987
	21 to 31 %	y = 0.92 x – 8.9	0.940
	31 to 41 %	y = 1.37 x – 29.2	0.917
3 h Dynamic: (122% TGP)	0 to 10 %	y = 1.23 x – 18.9	0.974
	10 to 21 %	y = 0.40 x – 0.1	0.969
	21 to 31 %	y = 0.34 x – 1.6	0.907
	31 to 41 %	y = 0.48 x – 11.6	0.893

The ANCOVA on the data for the static and 3 h dynamic exposures over the range of 0 to 10% cumulative mortality showed that the interaction term yielded by the ANCOVA was not significant (p=0.219), meaning that the slopes of the regressions did not differ significantly. The interaction term was significant over the range of 10% to 21% (p<0.0001), 21% to 31% (p<0.0001), and 31% to 41% (p<0.0001) cumulative mortality. These results indicate that the slopes (rates of mortality) were significantly reduced in the 3 h dynamic exposure compared to the static exposure, over these ranges of cumulative mortality. When replicate was explicitly included in the ANCOVA model as a random effect, the conclusion was the same. The interaction was not significant over the range of 0 to 10% cumulative mortality (p=0.327), but it was significant over the range of 10% to 21% (p<0.0001), 21% to 31% (p<0.003), and 31% to 41% (p<0.001) cumulative mortality. Further, the ANCOVA indicated that the replicate effect was significant in the range of 10 to 21% cumulative mortality (p=0.004), but not significant in the other ranges of cumulative mortality (p=0.615; p=0.004; p=0.523; p=0.233; respectively, for 0 to 10%, 21% to 31%, and 31 to 41%).

The mean time to 10% mortality at the surface for all cycles was 24.8 h, but increased from 23.3 h for the first cycle to 25.3 h for the second cycle, 26.8 h for the third cycle, and then decreased to 23.8 h for the fourth cycle (Table 7). This time at depth corresponded to 13%, 12%, 11%, and 13% of the total time for cycles 1 to 4, respectively, with a mean of 12%.

When the fish came to the surface in the second and subsequent cycles, the time to initiation of mortality (time to mortality of the first fish) was considerably shorter than that required in the first cycle, and with each successive depth cycle there was a greater delay in the re-initiation of mortality. For example, the time to initiation of mortality was 17 h for the first cycle, compared to 2.4 h, 5.8 h and 8.6 h for the second to fourth cycles, respectively. Further analyses showed that once the first fish died in a given surface interval, the mortality rate for the remaining fish at the water surface was reduced, compared to the static exposure, in the range of 10% to 21% and 21% to 31% cumulative mortality, but not in the range of 31% to 41%.

There were no external GBT signs in 18 of the 32 fish that died (56%). Seven fish (22%) had bubbles in the caudal fin, two (6%) had bubbles in the anal fin, and five (16%) had bubbles in the dorsal fin. All dead fish had tubular bubbles in the gill filaments.

Dynamic Exposure at 122% TGP with 6 h Intervals at Depth

Time to mortality versus cumulative mortality for the 6 h dynamic exposure, for replicates and all replicates combined (with and without time at depth subtracted from the exposure time), are shown in Figure 6, along with data for the static exposure at 122% and 0.25 m depth (all replicates combined), for comparison. The average time to mortality (for the three replicates) for a given percent cumulative mortality for the dynamic exposure was not shown; however, it was very similar to that for all replicates combined. The 6 h dynamic exposures (both with and without time at depth subtracted) were shifted to the right of the static exposure, indicating that fish use of water depth delayed mortality. Mean fork length and weight for all treatment fish were 117 mm (± 12) and 16.4 g (± 5.0), respectively. All control fish survived.

The mean LT10, LT25, and LT40 data are provided in Appendix 3. The mean LT10 did not differ significantly between the static (29.8 h) and 6 h dynamic (32.2 h) exposures ($p=0.6785$). However, both the mean LT25 ($p=0.0006$) and the mean LT40 ($p<0.001$) were significantly shorter for the static exposure than the dynamic exposure with 6 h depth intervals. The mean LT25 was 38.1 h for the static exposure, compared to 86.6 h for the 6 h dynamic exposure. The mean LT40 was 46.9 h for the static exposure and 131.2 h for the 6 h dynamic exposure. This analysis did not subtract time at depth. These results support the graphical interpretation above, as they show that repeated excursions to depth for 6 h intervals significantly delayed mortality, compared to the baseline static exposure of 122% TGP at 0.25 m depth.

Table 7. Data for dynamic exposures, where fish were held in cages and cycled from the surface to below the compensation depth in the deep tank.

Exposure	Cycle	Time to 10% Mortality at Surface (h)	Percent of Time at Depth per Cycle	Number of Dead Fish per Cycle	Time to Initiation of Mortality per Cycle (h)	Percent of Time to Initiation of Mortality Relative to First Cycle
122% TGP;	1	23.3	13	8	17.0	
3 h at depth	2	25.3	12	8	2.4	14
	3	26.8	11	8	5.8	33
	4	23.8	13	8	8.6	51
	Mean	24.8	12			
122% TGP;	1	30.3	20	9[a]	18.2	
6 h at depth	2	34.3	18	8	11.4	63
	3	24.6	24	9[a]	13.9	76
	4	34.8	17	8	16.0	88
	Mean	31.0	20			
124% TGP;	1	22.4	27	8[b]	14.5	
6 h at depth	2	19.0	32	8	12.8	88
	3	15.8	38	8[b]	8.8	61
	4	19.4	31	8	9.7	67
	Mean	19.2	32			

a) Two fish died together at the end of the 1st and 3rd cycles.

b) When the cages were brought to the surface at the end of cycles 1 and 3, they contained one and two dead fish, respectively. These mortalities were disregarded because exposure time could not be quantified.

Regression equations are provided in Table 8 for cumulative mortality as a function of time to mortality for the dynamic and static experiments. Ranges of percent cumulative mortality from static experiments were chosen to correspond with the four cycles of the dynamic experiments for comparison and statistical (ANCOVA) analysis.

The ANCOVA on the data for the range of 0 to 10% cumulative mortality showed that the interaction term was not significant ($p=0.380$), meaning that the slopes of the regressions did not differ significantly for the static and 6 h dynamic exposure. The interaction term was significant (and hence the slopes, or mortality rate differed) over the range of 11% to 21% ($p<0.0001$), 21% to 32% ($p<0.0001$), and 32% to 42% ($p<0.0001$) cumulative mortality. A similar conclusion was reached when replicate was explicitly included in the ANCOVA model as a random effect. The interaction was not significant over the range of 0 to 11% cumulative mortality ($p=0.426$), but it was significant over the range of 11% to 21%

($p<0.0001$), 21% to 32% ($p<0.01$), and 32% to 42% ($p<0.001$) cumulative mortality. The replicate effect was not significant in each of the four ranges of cumulative mortality ($p=0.354$; $p=0.080$; $p=0.716$; $p=0.308$; respectively).

Table 8. Linear regression equations for the static and 6 h dynamic exposures.

Exposure	Range of Cumulative Mortality	Linear Regression Equation	R^2
Static: 0.25 m (122% TGP)	0 to 11%	$y = 0.98 x - 16.2$	0.771
	11 to 21 %	$y = 1.36 x - 23.3$	0.953
	21 to 32 %	$y = 0.92 x - 9.3$	0.954
	32 to 42 %	$y = 1.46 x - 33.1$	0.877
6 h Dynamic: (122% TGP)	0 to 11 %	$y = 0.83 x - 14.8$	0.942
	11 to 21 %	$y = 0.31 x - 1.4$	0.937
	21 to 32 %	$y = 0.41 x - 13.4$	0.734
	32 to 42 %	$y = 0.29 x - 1.6$	0.841

The times to 10% mortality during the water surface intervals were 30.3 h for the first, 34.4 h for the second, 24.6 h for the third, and 34.8 h for the fourth cycles (Table 7), with a mean cycle length of 31.0 h. This pattern differed from that for the 3 h at depth experiment, in that the time to 10% mortality was shortest for the third cycle. Time at depth for the 6 h dynamic exposure corresponded to 20%, 185%, 24%, and 17% of total time for cycles 1 to 4, respectively with a mean of 20%.

The time to initiation of mortality (time to death of the first fish) was 18.2 h for cycle 1 and considerably shorter for subsequent cycles (Table 7). The re-initiation of mortality with each successive depth cycle also took longer, 11.4 h for the second cycle (63% of that in the first cycle), 13.9 h for the third cycle (76%), and 16.0 h for the fourth cycle (88%). Further analyses showed that once the first fish died, the mortality rate for the remaining fish at the water surface was reduced, compared to the static exposure, in the range of 11% to 21% cumulative mortality, but not in the other ranges.

Of the 34 fish that died, 11 (32%) had no external signs of GBT, 26 (59%) had bubbles in the caudal fin, three (9%) had bubbles in the anal fin, eight (34%) had bubbles in the dorsal fin, and one had bubbles around the eye. All dead fish had tubular bubbles in the gill filaments.

Comparison of Dynamic Exposure with 3 h and 6 h Intervals at Depth

The logistic model was fit to the replicate data for the static and dynamic exposures (with 3 h and 6 h intervals at depth - time at depth not subtracted), as shown in Figure 7. There was overlap among the 95% confidence intervals for the three exposure regimens up to approximately 15% cumulative mortality. Above this range of cumulative mortality, there

was no overlap between the static exposure and dynamic exposures. The confidence intervals for the 3 h and 6 h dynamic exposures overlapped until approximately 25% cumulative mortality. When the logistic model was fit to the data for all replicates combined, the confidence intervals were narrower, and there was no overlap in confidence intervals among the three exposure regimens above 10% to 15% cumulative mortality. These findings further support the conclusion that use of water depth by fish significantly delayed mortality.

Further analysis indicated that the LT10 did not differ significantly ($p=0.8605$) among the three exposures shown in Figure 7, as was expected. The mean LT25 differed significantly among the three exposures ($p=0.0007$), but the mean LT25 did not differ significantly between the 3 h and 6 h at depth dynamic exposures ($p=0.3215$). The mean LT40 also differed significantly among the three exposures ($p<0.001$), and pair-wise comparisons showed that all three exposures differed significantly. That is, the static exposure differed significantly from each of the dynamic exposures (p values provided above), and the dynamic 3 h at depth exposure differed significantly from the dynamic 6 h at depth exposure ($p=0.0223$).

Although the LT10 did not differ significantly, the average initiation of mortality (over all replicates) varied among the static exposure (25.9 h), the dynamic exposure with 3 h depth intervals (18.4 h), and the dynamic exposure with 6 h depth intervals (21.6 h). For all replicates combined, the first fish in the 122% TGP static exposure died after 14 h, compared to 17 h for the second fish and almost 20 h for the third fish.

Linear regression equations for time to mortality as a function of percent cumulative mortality over the range of 0 to 42% cumulative mortality for the static and 3 h and 6 h dynamic exposures are shown in Table 9 (all replicates combined). The ANCOVA on these data showed that the interaction term was significant ($p<0.0001$), meaning that slopes were not equal. ANCOVA on each pair of exposures showed that the interaction term was significant for the static and 3 h dynamic data ($p<0.0001$), the static and 6 h dynamic data ($p<0.0001$), and the 3 and 6 h dynamic data ($p<0.0001$). Results of the ANCOVA with replicate explicitly stated in the model as a random effect provided a similar conclusion (based on p-values).

Table 9. Linear regression equations for the static and all dynamic exposures.

Exposure	Range of Cumulative Mortality	Linear Regression Equation	R^2
Static: 0.25 m (122% TGP)	0 to 42 %	$y = 1.18 x - 19.3$	0.983
3 h Dynamic: (122% TGP)	0 to 42 %	$y = 0.37 x - 0.7$	0.979
6 h Dynamic: (122% TGP)	0 to 42 %	$y = 0.29 x - 1.2$	0.979

Results presented in Table 7 provide further comparison of the static and dynamic exposures at 122% TGP. On average, the percentage of time at depth during a given cycle in the dynamic experiments was 12% for the 3 h at depth exposure and 20% for the 6 h at depth exposure. The percent cumulative mortality was 41% for both the 3 h and 6 h at depth dynamic exposures; however, the 3 h dynamic exposure took 112 h to produce that mortality, compared to 147 h for the 6 h dynamic exposure. The time required to produce 41% cumulative mortality in the static exposure at 122% TGP was about 51 h.

Dynamic Exposure at 124% TGP with 6 h Intervals at Depth

One fish from the control group died, appearing very bloated and lying on the bottom of the cage for a long period without moving. It did not have bubbles in the gills or on the external surfaces, and it exhibited abnormal behavior during the experiment. When the cage returned to the surface after the third cycle to depth, the fish was found to be dead. The cause of death was unknown. Mean fork length and weight for all treatment fish were 112 mm (± 13) and 13.7 g (± 5.0), respectively.

Time to mortality versus percent cumulative mortality for the 124% TGP dynamic exposure (time at depth not subtracted) is shown in Figure 8, along with data for the other static and dynamic exposures. LT10, LT25, and LT40 data are provided in Appendix 3. Even though TGP was higher in this exposure, mortality was still delayed relative to the baseline, static regimen. When the 6 h time interval at depth was subtracted from the exposure time, the cumulative mortality still was less then the static baseline case, indicating delayed mortality resulting from use of water depth.

Linear regression was applied to the data for the 124% TGP exposure for all replicates combined, to estimate the slope (mortality rate) for each group of fish dying at the water surface. The slopes were 0.92, 0.47, 0.61, and 0.44 for cumulative mortality ranges of 0 to 10%, 10% to 20%, 20% to 30%, and 30% to 40%, respectively. The mortality rate was, therefore, reduced in the second and subsequent cycles relative to the first cycle, suggesting that time at depth reduced the mortality rate when fish returned to the water surface. However, Figure 8 shows that, once mortality was re-initiated in the second and subsequent surface intervals (i.e., once the first fish died), the mortality rate was similar to that in the first interval (and to that in the static exposure). Also, the mortality rate for each group of fish at the water surface (once the first fish died) was generally faster in the 124% TGP experiment, compared to the 3 h and 6 h dynamic exposures at 122% TGP.

The time to 10% mortality at the surface was 22.4 h for the first cycle, 19.0 h for the second cycle, 15.8 h for the third cycle, and 19.4 h for the final cycle, with a mean of 19.2 h (Table 7). This time at depth corresponded to 27%, 32%, 38%, and 31% of the total time for cycles 1 to 4, respectively, with a mean time of 32%.

The time to initiation of mortality (time to death of the first fish) was 15 h for cycle 1 (prior to any time at depth), compared to 12.8 h for cycle 2 (88% of the time for cycle 1), 8.8 h for cycle 3 (61%) and 9.7 h for cycle 4 (67%).

In this experiment there were two occasions when dead fish were found after cages were brought to the water surface, at the start of cycles 1 and 3. All fish were alive before the cages were sent to depth. These fish were not included in the analysis because their time to mortality could not be quantified.

All dead fish had tubular bubbles in the gill filaments. No external signs of GBT were seen in 15 of the 32 fish that died (47%); however, 14 (44%) had bubbles in the caudal fin, four (13%) had bubbles in the anal fin, three had bubbles in the dorsal fin, and one had a severe case of exophthalmia. Extra-corporeal bubbles were noted between the gill filaments and on the outside surfaces of the fish when held in cages at the surface.

Summary of Results for Dynamic Exposures

Table 10 and Figure 8 summarize the results of the dynamic and static exposures at 122% TGP and 10°C.

Table 10. Results for all static and dynamic exposures at 122% TGP and 10°C.

Exposure and Mean TGP (± SD)	Depth or Depth Cycle	Duration of Exposure	Sample Size [a]	Percent Mortality of Exposed Fish	Mean Fork Length (mm) (±SD)	Mean Weight (g) (±SD)	External Signs of GBT [b] (%)
Static 122.2 ± 0.2%	0 to 0.25 m	96 h	180	89	110 (± 14)	13.2 (± 5.3)	52 (D)
Dynamic 122.2 ± 0.2%	0 to 1.0 m	96 h	120	22	127 (± 19)	20.7 (± 8.6)	62 (D)
Dynamic 122.3 ± 0.2%	0 to 2.5 m	96 h	100	0	135 (± 21)	26.0 (± 11)	4 (S)
Dynamic 122.3 ± 0.5%	3 h depth intervals	112 h	78	41	109 (± 15)	12.7 (± 5.0)	44 (D)
Dynamic 122.3 ± 0.3%	6 h depth intervals	147 h	81	42	117 (± 12)	16.4 (± 5.0)	68 (D)

a) Represents the total number of fish exposed to elevated TGP (for all replicates).
b) Percentage of fish exposed to elevated TGP (D=dead fish; S=survivors) showing any external sign of GBT.

Disease Survey Results

Bacteriological tests conducted on survivors from the 122% TGP dynamic exposure (6 h depth cycle) showed non-pathogenic bacterial growth in four of the 30 fish examined. Two of the 30 fish were infected with a *Flexibacter*–like bacterium. All direct fluorescent antibody tests (DFATs) for *Renibacterium salmoninarum* were negative. Non-pathogenic bacterial growth was detected in four of the 30 fish taken from the stock tank, which held the supply of fish used in the various TGP experiments. All DFATs for *Renibacterium salmoninarum* were negative for fish from the stock tank.

The virology results were negative for all six pools of tissue (containing gill, spleen, pyloric caeca, and kidney tissue) taken from fish surviving the 6 h dynamic exposure. All six pools of tissue analyzed for fish from the stock tank were also negative for viruses.

Gill histological assessments of survivors showed normal results for 11 of 26 fish examined. There was a slight separation of the epithelial layer of the gill lamellae in 13 fish and moderate epithelial separation and vacuolated spaces between the filaments of the pseudobranch in two fish. Results for fish from the stock tank showed that 12 of the 25 fish tested were normal and 13 had slight separation of the epithelial layer of the gill lamellae. No necrosis (cell death) was reported for any fish examined. No pathological changes were apparent for 20 fish that died during the static 140% TGP exposure on April 4, 2000. Some of these fish were fixed whole, while others had their heads severed before fixation.

Degassing Tests

Degassing test results (Appendix 4) showed that TGP was consistently lower in the shallow water tanks than the measuring column (MC) at the flows used in the experiments. TGP readings in the shallow tanks were highly influenced by the extent to which the TGP probe was shaken. When the TGP probe was shaken lightly or gently tapped every couple of minutes to dislodge bubbles, the measurement was lower by 0.8% to 1.5% TGP than the true TGP recorded in the MC (under hydrostatic pressure). Vigorous shaking of the probe caused measured TGP in the shallow tanks to drop substantially (by up to 4.8% TGP), even after shaking had stopped. Higher flow rates reduced the error in the TGP readings. However, only at very high flow (e.g., 10 $L \cdot min^{-1}$) was the error between the true and measured TGP reduced considerably. This flow rate caused the water level in the shallow tanks to increase, and this might have collapsed bubbles on the probe, contributing to a more accurate reading. The 0.25 m PVC column (designed to minimize surface area for degassing) provided a similar inaccuracy to the shallow water tanks, suggesting that degassing did not occur and that errors were related to bubbles on the probe. The dissolved oxygen and temperature measurements in the shallow tanks also suggest that degassing did not occur (i.e., at 122% TGP, dissolved oxygen ranged from 113% to 114% saturation among the treatment tanks, and the variation in temperature was 0.1°C).

The difference between true TGP at depth and TGP recorded at the surface of the deep tank ranged from 0.3% to 0.6% TGP, which was less than the error determined for the shallow

tanks. This difference suggests that the erroneous TGP recorded in the shallow tanks might have been caused by both degassing (primarily from agitation of the water surface caused by shaking the probe) and inaccurate measurements caused by bubble formation on the probe. Both these factors might have contributed to the measurement error at the surface of the deep tank; however, the extent of degassing in the deep tank would likely be less than in the shallow tanks. This was because water withdrawal from the deep tank was from the surface, rather than at depth, and the deep tank water volume was much greater than the shallow tank. Assuming there was no degassing in the deep tank, then the bubble formation on the probe membrane would have accounted for the lower TGP reading (by 0.3% TGP to 0.6% TGP) at the surface of the tank relative to that at depth. Applying this reasoning to the 122% TGP experiments in the shallow tanks (where the recorded TGP ranged from 0.8% to 1.5% TGP lower than the true value), potential degassing could be estimated at 0.2 to 1.2% TGP. This loss would have been relatively small and within the variation typically reported in the literature. Further, degassing should have been less at lower TGP than at 122% TGP. At 110% TGP, the probe produced accurate TGP readings in the shallow tanks.

Additional tests showed that TGP in the measuring column (at the start of the header line delivering flow to the experimental tanks) was the same as that measured in the shorter (1 m) measuring column located at the tenth pair of tanks designated for water quality monitoring (at the end of the header line). This suggested consistency in TGP levels from start to end of the experimental tank set-up and within individual tanks.

DISCUSSION

General Comments

In the static, shallow water exposures, both control and treatment fish used various water depths from the surface to the bottom of the tanks. Therefore, TGP measurements were not corrected for the effects of hydrostatic pressure compensation by the fish. If treatment fish had remained on the bottom of the tanks for the entire exposure they would have been exposed to 119.5% TGP (correction factor of 2.5% TGP). Similarly, for exposures in the deep tanks, fish used various water depths available to them; hence, the TGP data were not corrected for hydrostatic pressure.

All fish that died during the static and dynamic exposures to elevated DGS were examined for the presence of gas bubbles in the gill filaments and external signs of GBT. Every fish that died had evidence of bubble growth in the gill filaments. Bubbles were likely present in other areas of the vascular system (e.g., heart, branchial arteries), but these areas were not examined. Therefore, we concluded that the cause of death in all fish exposed to elevated DGS was bubble formation in the cardiovascular system, causing blockage of blood flow and death (Fidler 1988, Fidler 1998a, b). Signs of tissue hypoxia (e.g., lethargy) in some fish exposed to 120% and 140% TGP support our conclusion regarding cause of death (i.e., gas bubbles in the circulatory system would reduce blood flow to tissues, causing hypoxia). It is unlikely that the external signs of GBT observed in dead fish would have contributed to death

because the severity was low, and the most prevalent sign was bubbles in the caudal fin. Further, there was no evidence of sub-dermal emphysema in any of the dead fish. Extra-corporeal bubbles between the gill filaments were present after some exposures, particularly when the fish were held in cages at the water surface in the 124% TGP dynamic exposure. These bubbles could have contributed to death by leading to blockage of respiratory water flow over the gills (Fidler 1988).

Only two of the 1073 control fish for all experiments died, and these fish (which died in the cages used in the dynamic exposures) did not have bubbles in the gills or external signs of GBT. The cause of death for the two control fish was unknown.

Static Exposure of Juvenile Rainbow Trout to Elevated DGS

Results of our static TGP exposures, along with those reported in the literature, demonstrate an inverse dose-response relationship between TGP and the duration of exposure required to produce mortality in fish. At high TGP (e.g. 135% to 140%) in shallow water, the time to mortality is typically very short (e.g., hours). At a TGP of approximately 115% in shallow water, the time to mortality is in the order of days to weeks. For example, in our experiments at 0.25 m water depth and 10°C, the time to 50% cumulative mortality was 5.1 h at 140% TGP, compared to a cumulative mortality of 42% after a nine day exposure to 116% TGP. No fish died from exposure to 110% or 114% TGP.

The duration of our static exposures at 110% and 114% TGP (eight and six days, respectively) may not have been long enough to elicit mortality, since literature data show that long exposure periods (e.g., 20 days or more) are required to elicit mortality at low TGP (e.g., in the 110% to 115% range), even in shallow water. For example, Meekin and Turner (1974) exposed 230 mm steelhead trout to a TGP of 111% and found that the time to 50% mortality was about 486 h. However, their groundwater supply provided a partial pressure ratio of nitrogen to oxygen that was higher than equilibrium, which tends to increase the lethality of a given ΔP to fish (Jensen *et al.* 1986, Fidler 1988). Hence, the results of Meekin and Turner (1974) would represent a maximum impact for 111% TGP, and may not be representative of pN_2/pO_2 conditions normally expected below hydroelectric facilities. Mesa *et al.* (2000) reported that no chinook salmon died when exposed to 113.4% TGP and 12°C for 22 days. This finding was based on two trials, one in 1995 using 144 chinook (136 mm), and the other in 1997 using 128 chinook (147 mm). Another difference between the Meekin and Turner (1974) and Mesa *et al.* (2000) data, in addition to pN_2/pO_2 ratios, may be a greater susceptibility of steelhead and rainbow trout to DGS compared to chinook (Nebeker *et al.* 1978, Weitkamp and Katz 1980).

The results from the static exposures at 110%, 114%, and 116% TGP are consistent with the bubble growth threshold equations reported in Fidler (1988). These equations predict that the water ΔP required to initiate bubble growth in the cardiovascular system or gill filaments of rainbow trout is about 116 mm Hg (or 115% TGP at sea level) for water depth of 0 m, and ΔP of 134 mm Hg (117.7% TGP at sea level) for a depth of 0.25 m. They also predict that the water ΔP required to initiate sub-dermal emphysema and extra-corporeal bubble growth

between the gill filaments in rainbow trout ranges from 83 mm Hg (110.9% TGP) at 0 m depth to 113.4 mm Hg (113.4% TGP) at 0.25 m depth, at sea level. Observations that fish in our exposures started dying at 116% TGP (and not at 110% or 114% TGP), and that every fish that died during the exposures had tubular bubbles in the gill filaments, support the threshold water ΔP required for bubble growth in the cardiovascular system or gill filaments. That some of the survivors in the 110% and 114% exposures had bubbles in the fins or other external surfaces also supports the predicted threshold ΔP required to initiate sub-dermal emphysema and extra-corporeal bubble growth.

The comparison of our results with those reported by Dawley *et al.* (1976a) for 180 mm steelhead trout exposed to 116% and 122% TGP at 10°C in water depth of 0.28 m suggests that there might be differences among stocks in susceptibility to DGS. The rainbow trout used in our experiments were more resistant to 116% and 122% TGP, as they exhibited a lower mortality rate and a lower overall percent cumulative mortality compared to the fish used by Dawley *et al.* (1976a). For example, Dawley *et al.* (1976a) reported 65% mortality after 175 h at 116% TGP, whereas our experiments showed 42% mortality after 216 h. However, the time to initiation of mortality was similar (approximately 48 h). Although the fish used by Dawley *et al.* (1976a) were larger than our fish, Jensen *et al.* (1986) found only a weak relationship between fish size and mortality in this size range, up to about 200 mm. Hence, other factors likely contributed to the observed differences. The higher pN_2:pO_2 ratio in our supersaturated water (3.84 to 4.2), relative to equilibrium (3.7), would not have been a contributing factor because a higher ratio would increase the lethality of a given ΔP to fish, meaning our fish would have lived even longer at equilibrium (Jensen *et al.* 1986, Fidler 1988).

Factors that might have contributed to the reported differences in susceptibility to DGS between our fish and those of Dawley *et al.* (1976a) include fish handling, disease, and overall level of stress before experimentation. The fish in our stock tank were free of any pathogenic bacteria or viruses and, hence, were not under any additional stress during the TGP experiments due to disease. The use of a hatchery source for fish also likely reduced stress due to captivity. In contrast, Dawley *et al.* (1976a) had collected wild fish from the Columbia River and held them in captivity, which might have led to increased stress on the fish.

Comparisons can also be made with data provided by Mesa *et al.* (2000) for juvenile steelhead (217 mm) exposed to 120% TGP in 0.28 m water depth at 12.2°C. The first replicate of Mesa *et al.* (2000) was similar to the results of Dawley *et al.* (1976a), whereas the fourth replicate mirrored our results for all replicates combined, and the second and third replicates were intermediate in value. Results of Knittel *et al.* (1980) for 127 mm steelhead trout exposed to about 121% TGP, at 10°C and 0.1 m depth were also similar to ours, with an LT50 of about 47 h. Dawley and Ebel (1975) exposed 124 mm steelhead trout to 122.9% TGP at 0.25 m depth and 15°C and reported an LT100 of about 25 h. The higher water temperature in this study would have contributed to increased mortality.

The results from our 122% TGP static exposure and those from Mesa *et al.* (2000) indicate considerable variability in susceptibility to DGS among individual fish within a stock. In our experiments, this variability was greater for the 122% exposure than for the 140% TGP exposure. This high variability means that large sample sizes are required to obtain a representative description of the susceptibility of a given fish stock to DGS.

Much of the published literature for GBT bioassays was based on small sample sizes. Knittel *et al.* (1980) used 20 fish per treatment and reported on only a single repetition of each experiment. Fickeisen and Montgomery (1978) had sample sizes of ten fish in single repetition bioassays. Nebeker *et al.* (1978) and Blahm *et al.* (1976) also reported sample sizes of ten fish. Stroud and Nebeker (1976) had a random sample of 38 fish divided into four lots (i.e., four replicates of nine or ten fish each). Nebeker *et al.* (1976) had four replicates of ten fish per sample, while Rucker (1976) used between eight and 50 fish per replicate, depending on fish size (numbers of replicates were not reported). Dawley *et al.* (1976a) reported sample sizes ranging from 24 to 29 fish with two replicates of each treatment. Meekin and Turner (1974) did not report specific numbers in their steelhead trout experiments, only a range of 5 to 100 fish. In summary, most of the literature involves small sample sizes and limited replicates. This may explain crossovers of mortality curves that show the effect of fish size on susceptibility to DGS (e.g., Meekin and Turner 1974) or attempt to show an effect of water temperature on GBT susceptibility Nebeker *et al.* 1979).

In our experiments, there were no consistent trends in time to mortality among individual tanks that would suggest an effect of experimental apparatus on variability among replicates. For example, in the 140% TGP exposure, the first fish that died in replicate 4 did so much quicker than in any other replicates, yet this replicate had the second lowest percent cumulative mortality at the end of experiment. This was also evident for the 122% exposures. In addition, our gas-generating column produced very stable TGP, unlike other designs (e.g., pumps that bleed air into an orifice, or those with varying water pressure). These findings suggest that variability was due to the fish themselves, rather than variations in water quality among tanks. Mesa *et al.* (2000) also reported substantial variation in cumulative mortality among the four trials conducted in 1997, where juvenile steelhead trout were exposed to 120% TGP at 12°C.

Our studies confirm that rainbow trout, along with steelhead and, possibly, mountain whitefish (White *et al.* 1991) are the salmonid species most sensitive to DGS-induced mortality.

Acclimation

There was no statistically significant difference in time to mortality between the acclimated and unacclimated experiments, even though fish sizes and exposure conditions were the same. The mortality response of unacclimated fish lay within the range of variability seen for the acclimated fish, and no consistent relationship between the pairs of acclimated and unacclimated replicates was found. It was expected that unacclimated fish would die sooner than acclimated fish, due to increased stress from movement into the exposure apparatus and

the sudden introduction to 122% TGP. There was, however, less variability in the time to mortality for unacclimated fish compared to acclimated fish, particularly at the LT10 and LT25 levels. Increased stress associated with lack of acclimation may decrease the tolerance of fish to DGS, thereby reducing the variability in time to mortality. Regardless, we recommend an acclimation process for future experiments because it is an integral part of experimental design and, in the case of TGP, it is more representative of exposure conditions in the wild.

Effect of Fish Use of Water Depth on Survival

Fish use of water depth had a significant and substantial effect on the survival of fish exposed to TGP in the 122% to 124% range. In the volition cage experiments, fish use of a range of water depths significantly delayed the initial onset of mortality and the percent cumulative mortality over a 96 h exposure. However, acceleration of mortality rate at the end of the 96 h exposure in the 1 m deep volition cage experiment suggests that the exposure was not adequate to represent the total mortality that might have occurred over a longer period. Regardless, over 96 h, the additional 0.75 m of water depth reduced mortality considerably from the 89% reported in the static exposure at 0.25 m and 122% TGP (Figure 8).

Cyclical use of water depth demonstrated in the dynamic exposures with 3 h and 6 h intervals at depth, which may represent diel behavioral patterns, also delayed mortality when fish were cycled from below the compensation depth back to the surface. The delay in mortality was longer than the actual time spent at depth (i.e., time to mortality for a given percent cumulative mortality for the dynamic exposures with time at depth subtracted was still delayed relative to the baseline, static case). Hence, there was a delay in re-initiation of mortality when fish returned to the surface.

Use of water depth reduced the mortality rate when fish returned to the surface, over the entire duration at the water surface. However, once the first fish died in a given interval at surface, the mortality rate for the rest of the fish in that interval was not consistently reduced relative to the static exposure. For example, the mortality rate (after the first fish died) was only reduced in the first and second surface intervals in the 3 h dynamic exposure, and the first interval in the 6 h dynamic exposure at 122% TGP. In the 6 h dynamic exposure at 124% TGP, the mortality rate (once mortality was re-initiated) in the second and subsequent surface intervals was similar to that in the first interval (and to that in the static exposure). Thus, in the 124% TGP exposure, the primary benefit of depth compensation was the delay in time to re-initiation of mortality when the fish returned to the water surface. However, in the 122% TGP dynamic exposures with 3 and 6 h intervals at depth, the benefits of depth compensation included both a delay in time to re-initiation of mortality when the fish returned to the water surface and a reduction in the mortality rate (once the fish started dying), at least initially.

Some or all of the reduction in mortality rates for the dynamic exposures (calculated for the entire surface interval) could be accounted for by a delay in re-initiation of mortality (i.e., time to death of the first fish). The effect was greatest for exposure to 124% TGP.

Compensation depth differed between the 122% and 124% TGP dynamic exposures. It was 2.2 m for the 122% TGP (for an effective TGP% of zero), compared to 2.4 m for the 124% TGP exposure. At depth, the fish were located between 2.5 m and 2.75 m. Thus, fish were further below the compensation depth in the 122% TGP exposure; hence, these fish would experience a greater rate of bubble collapse than those closer to the compensation depth (Appendix 1). This might explain the shorter delay in re-initiation of mortality when fish returned to the water surface, reported for some cycles of the 124% TGP exposure compared to the 122% TGP exposure (6 h intervals at depth, third and fourth intervals at surface, Table 7). It might also explain why, in the 124% TGP exposure, the time to initiation of mortality in the first and second cycles was similar, even though there was a 6 h interval at depth between the two intervals at surface. That is, the fish were close to the compensation depth, hence there was no change in bubble growth or size.

A portion of the time required to reach initial mortality in the first exposure of fish to elevated TGP involves equilibrating internal tissues and body compartments with the external water TGP (Fidler 1998a, b). Consequently, taking fish to depth (any depth, for any duration of time) does not recover that component of the time to initiation of mortality. The time to equilibration is thought to be relatively short, on the order of a few hours. Hence, this might have accounted for the shorter time to initiation of mortality in the first and second cycles in the 124% TGP exposure, which was only 1.75 h, if there was, in fact, no reduction in bubble size due to insufficient compensation depth.

In the 122% TGP dynamic exposures, where fish were below the compensation depth, the use of water depth likely caused some reduction in the rate of bubble growth and, hence, bubble size, while at depth. However, the data suggest that the time at depth would not have completely collapsed any gas bubbles to their original nucleation site size (i.e., where they were before the experiment – see Appendix 1). The evidence for this was the time to initiation of mortality in the second and subsequent cycles, which was considerably less than that required in the first cycle (Table 7). The differences were much larger than the equilibrium time (a component of the first cycle only). Thus, when the fish returned to the surface, any pre-existing bubbles likely continued their growth cycle from macroscopic bubbles rather than the microscopic nucleation sites from which their initial growth began.

The increase in time to onset of mortality from the second to fourth cycles in both dynamic exposures at 122% TGP suggests that each sequential interval at depth provided greater reduction in bubble size, and increased the duration of time required to re-initiate mortality once above the compensation depth. However, as cycles progressed, the remaining fish would be more resistant to DGS, and this may explain why it took longer for the onset of mortality in later cycles.

The length of time spent below the compensation depth also affected the time to re-initiation of mortality at the water surface, which was greater for both of the 6 h at depth exposures compared to the 3 h at depth exposure. Again, this effect could be explained by the effect of hydrostatic pressure on the bubble growth process. The longer time at depth likely provided a

greater reduction in bubble size and, hence, a greater delay in re-initiation of mortality at the water surface.

When fish were returned to the water surface, and once mortality was re-initiated, those in the 124% TGP dynamic exposure died at a faster rate than fish in the 122% TGP dynamic exposure (Figure 8). The higher TGP likely explained this difference, since the mortality rate for the first surface interval was higher for 124% than for 122% TGP. The difference in fish position relative to compensation depth may have contributed; however, once mortality was re-initiated at the water surface, the prior depth compensation may not have affected the subsequent mortality rate.

Overall, results showed that fish use of water depth had a significant, beneficial effect on survival of fish exposed to DGS. The reason for this was likely the effect of hydrostatic pressure on nucleation sites and bubble growth processes. Our findings also illustrate that the benefits of depth compensation must be examined in terms of both time at depth and water depth relative to compensation depth. Further, it is likely that the beneficial effect of fish use of water depth would be even greater than that shown in our experiments when greater depths are available, such as in deeper river systems, e.g., the Columbia River.

Although the beneficial effect of fish use of water depth cannot be ignored in assessing exposure and effects of TGP on survival, it is important to note that there could be situations where mortality is irreversible after a fish exposed to TGP moves to deeper water or to air-equilibrated water (Meekin and Turner 1974; Weitkamp 1976). This occurred in our dynamic exposure to 124% TGP, where, on three occasions, fish that were alive at the water surface died after being moved to depth. This phenomenon is most likely to occur when fish occupy habitats above the compensation depth for long enough to lead to an advanced state of bubble growth in the vascular system. If fish then moved down to the compensation depth, bubbles would cease to grow but then could be of sufficient size to block blood flow and result in death. Even if the fish moved below the compensation depth, the rate of bubble collapse might be too slow to permit adequate oxygen exchange. No fish died at depth in our 122% TGP dynamic exposures, perhaps because these fish were held further below the compensation depth, thereby causing a greater rate of bubble reduction, or because the rate of bubble growth at the surface was not fast enough at the lower TGP of 122%.

Evidence of Depth Compensation

The absence of fish mortality in the 122% TGP dynamic exposure with water depths from 0 to 2.5 m provided evidence that fish use water depth to compensate for TGP exposure. These results differ from those of Dawley *et al.* (1976a, b), who exposed chinook salmon and steelhead trout to 120% TGP in laboratory tanks 2.5 m deep and found that, for both species, some individual fish were unable or unwilling to use the available water depth to compensate for elevated TGP. Both species tended to school and occupy an average water depth of about 1 m; however, Dawley *et al.* (1976b) stated that "individual fish apparently moved substantially from the observed mean depth of the test lot", and that "less hydrostatic compensation was derived due to depth disposition than expected when the mean depths of

the fish groups were considered." In our volition cage experiment with 0 to 2.5 m depth, the fish appeared to school at water depths greater than 1 m. However, fish exhibited different behavior in the 0 to 1 m volition cage experiment. On first glance, fish appeared to be evenly distributed throughout the entire water column; however, upon closer examination, it was evident that some fish remained relatively stationary and occupied the same water depth for long periods, whereas others swam continuously and used a variety of water depths ranging from the surface to 1 m depth. The behavior of the active fish supports the observation by Dawley *et al.* (1976b) that individual fish moved randomly throughout their space. Those fish that remained stationary, above the compensation depth, may have been in some advanced state of bubble growth and, hence, tissue hypoxia, thereby deterring expenditure of energy required to move to deeper water. Others have found that fish will occupy sub-optimal habitat detrimental to survival, even when presented with the opportunity to move into optimal habitat (e.g., Kruzynski and Birtwell 1994).

Our results for the 2.5 m deep volition cage experiment also differed from those of Ebel (1971), who found that juvenile fall chinook salmon incurred 56% mortality over seven days in volition cages 4.5 m deep under TGP conditions ranging from 127% to 134% and water temperatures ranging from 17.1 to 19.4°C. Although conditions were considerably more severe than those in our experiments, it should be noted that 4.5 m provided more than adequate compensation depth for GBT, had the fish used it. Clearly, there may be behavioral differences among species that influence or determine how they use compensation depth when exposed to high TGP. Alternatively, there may be a TGP level above which certain species cannot make beneficial use of compensation depth. For example, in water with a TGP greater than 130%, bubble growth in the cardiovascular system occurs rapidly. If fish, due to behavioral traits or other reasons, spend enough time near the water surface, they may become afflicted with bubbles in the cardiovascular system or external surfaces to the extent that they can no longer perform properly due to tissue hypoxia or other effects. In this situation, they may lose the ability to return to depth for relief and, hence, become stranded at the surface until they die.

In terms of cardiovascular bubble growth, it is important to understand compensation depth requirements in relation to bubble growth thresholds. The absence of fish mortality in the 122% TGP dynamic exposure with water depths from 0 to 2.5 m provided clear evidence that fish used depth to compensate for TGP exposure. However, the extent of depth compensation required to eliminate mortality over this exposure period would have been relatively small. As discussed above, at 122% TGP, the compensation depth to produce an "effective" ΔP of zero (i.e., no bubble growth or collapse) would be 2.2 m. However, only 0.7 m would be required for a ΔP of 114 mm Hg, the threshold ΔP for cardiovascular bubble growth. Thus, fish in our experiments only had to stay below 0.7 m to eliminate mortality over the 96 h exposure period at 122% TGP.

Notwithstanding variability in the extent to which fish use depth compensation when exposed to DGS in laboratory studies, the wide range of behavioral patterns exhibited by individual fish in the wild will produce very different exposure histories and, hence, variability in biological responses among fish. Extrapolation of behavioral responses of fish in our volition

cage experiments to natural environments is difficult because the behavior of the fish in the deep tanks was highly influenced by the presence of people, noise, lighting, and other disturbances. Therefore, it was unlikely that fish behavior in the deep tank would have been representative of that in the wild, especially since our tanks lacked the presence of cover, predators, food supply, and other elements of a natural ecosystem.

Assessment of GBT

The ecological significance of external signs of GBT in fish exposed to DGS has received considerable attention in the literature (e.g., Dawley *et al.* 1976a, Stroud and Nebeker 1976, Nebeker *et al.* 1976, Nebeker and Brett 1976, Weitkamp and Katz 1980, Mesa *et al.* 1996, 2000). A recent review of this subject by Mesa *et al.* (2000) showed that the strength of the linear relationship between progression and severity of all GBT signs and mortality were weak for both chinook salmon and steelhead trout exposed to 120% TGP. The relationships were, however, relatively strong for both species at 130% TGP. Nevertheless, Mesa *et al.* (2000) concluded that the efficacy of GBT monitoring programs remained limited by variability in persistence of GBT signs, variability among individuals in development of GBT signs, inconsistent relationships of GBT signs to mortality, and lack of knowledge of the relationship between exposure history and development of GBT signs in wild fish.

In our experiments, approximately half of the fish that died from exposure to TGP greater than 120% lacked external signs of GBT. The primary reason for lack of external signs of GBT in dead fish at higher TGP is that the rate of bubble growth in the cardiovascular system and gill filaments is faster than the rate of growth of emphysema on external surfaces. Thus, above a TGP of 120%, it is expected that death will probably occur from cardiovascular bubble growth before external body emphysema becomes widespread.

In general, it is expected that, at death, external body emphysema will be present at TGP between 110% and 120%. The rationale for this is that at TGP below 120%, the rate of bubble growth in the cardiovascular system is slow enough that it may be accompanied by emphysema of external body surfaces and other signs of GBT. However, it was in this range of TGP that Mesa *et al.* (2000) found a weak correlation between the severity of GBT signs and mortality. This may limit the use of GBT monitoring programs as indicators of mortality in this range of TGP.

Monitoring the prevalence and severity of GBT signs as indicators of exposure to DGS, rather than biological effect, is also limited because the absence of GBT signs does not infer lack of exposure. For example, in our experiments at 116% and 122% TGP, many survivors were found to have no or few external signs of GBT. At higher TGP, there is a higher probability that the dead fish will have no external signs of GBT. Thus, although the presence of GBT indicates that the fish have been exposed to DGS, the converse is not always true.

One reliable predictor of GBT mortality in fish is bubble growth in the gill filaments. Fidler (1998a, b) examined real time bubble growth in the gill filaments of surgically altered juvenile fish exposed to DGS and found that death generally occurred when the gill filaments

became approximately 80% occluded with bubble growth. Since non-lethal sampling methods are currently not available to monitor bubble growth in the gill filaments or vascular system, these signs cannot be used as early warning signals of mortality. Further, direct mortality may not be the endpoint of concern for all resource management agencies. Few authors have examined the correlation between external GBT signs and sublethal effects, which might prove more promising than lethality. An exception is Birtwell *et al.* (2000), who found a significant relationship between external signs of GBT and susceptibility to predation in juvenile chum salmon exposed to 120% and 130% TGP at 20.7°C.

Evidence of Sublethal Effects

Death from exposure to DGS and the presence of external GBT signs have been studied extensively and reviewed by Weitkamp and Katz (1980) and Fidler and Miller (1997). Mortality has been the primary consideration in the establishment of DGS guidelines. Indirect or sublethal effects of DGS exposure generally have been given relatively little attention in the literature, yet are equally important, because bubble growth in the cardiovascular system (and, possibly, sub-dermal emphysema and extra-corporeal bubble growth) are initiated long before death occurs in fish exposed to DGS (Fidler 1998a, b). The significance of our results with respect to susceptibility to predation, disease, and gill histology is discussed below.

Susceptibility to Predation

Susceptibility to predation is a relevant sublethal effect of exposure to DGS because it is known that behavioral changes in fish occur long before death from cardiovascular bubble growth (Weitkamp and Katz 1980). Thus, it is expected that larger predators will be able to detect these behavioral changes and preferentially select these fish over fish of the same size not exposed to dissolved gas supersaturation. Birtwell *et al.* (2000) found that juvenile chum salmon were vulnerable to predation when they were exposed to warm seawater (20.7°C) and TGP of 120% for 24 h and 130% for 12 h; the results for the 48 h exposure at 115% TGP and 20.7°C were not significant. Birtwell *et al.* (2000) speculated that the fish were rendered more susceptible to predators through a performance deficit caused by gas bubbles reducing the sensory capabilities and blood circulation of the fish. Mesa and Warren (1997) found that chinook salmon were more vulnerable to predation after exposure to DGS at 130%. They suggested that increased vulnerability was likely due to fish having a significantly higher proportion of their lateral lines and gills occluded with bubbles compared to control fish. However, their predators (squawfish, a physoclist fish) appeared to be affected by shallow water confinement, and had to swim continuously during the experiments to avoid overbuoyancy. Birtwell *et al.* (2000) stated that it was plausible that bubbles within the circulatory system play a significant role in susceptibility to predation because the exposed fish used in his predator challenge tests did not display the same severity of GBT signs as those in Mesa and Warren (1997). Mesa *et al.* (1994) reviewed the published predator challenge tests and attributed the causes of increased susceptibility to predation to the failure to detect the predator, increased consciousness, slow decision-making and fast-start performance, and loss of ability to school effectively.

In our experiments, fish exposed to 122% TGP exhibited several signs that would appear to render them more susceptible to predation. For example, fish had a tendency to swim very sporadically before dying, and in the wild, this erratic swimming behavior would likely attract predators. In addition, some of the fish would roll over prior to or immediately upon death, thereby flashing their white ventral side while drifting or sinking to the bottom of the tank. This activity would also attract predators. Finally, many of the fish became lethargic after being exposed to 122% TGP. This was likely caused by tissue hypoxia due to bubble growth and reduced gas transfer in the tissues. This lethargy would ultimately affect swimming ability and, therefore, feeding and escaping abilities.

Susceptibility to Disease

The only significant pathologic finding from the disease survey was a light infection with a *Flexibacter*-like bacterium in two of the 30 fish that survived the 122% dynamic exposure with a 6 h depth interval. None of the 30 fish tested from the stock tank had this infection. The TGP exposure might have contributed to this infection; however, the additional stress associated with caging and moving the cages up and down in the water column might have contributed as well. The significance of this result is unknown, and a comparison with the literature is limited because the effects of DGS exposure on susceptibility to disease have not been well studied (White *et al.* 1991). Reasons for this include the lack of suitable quarantine facilities and limitations of the current diagnostic tools, which can only detect individuals that have disease, not carriers. However, Antcliffe *et al.* (1997a, b) suggest that TGP might have been a contributing factor to the elevated cumulative disease survey index for mountain whitefish caught below several hydroelectric facilities, a pulp mill, and a smelter on the Columbia River near Castlegar, BC.

Gill Histology

Survivors of the TGP experiment did not appear histologically different from fish selected from the stock tank (unexposed fish). The cause of the epithelial separation in the gill lamellae could not be determined, but it was unlikely attributable to dissolved gas supersaturation, because these changes were also observed in the fish that had not been exposed to DGS. Some degree of epithelial separation might have been due to vascular pressure from severing the head after euthanasia.

Histological results indicate that bubble growth in the gill filaments did not cause structural damage to gill tissue. That is, of the 20 fish examined that died during exposure to 140% TGP, none had histological signs of gill damage, even though they would have had gas bubbles in the gill filaments at the time of death (as did all other fish that died during this exposure). The lack of pathology on histological examination of the fish was consistent with the findings in Baath (1989), where histology was not found to be an effective method of evaluating pathologic changes resulting from chronic low-level gas supersaturation (e.g., 101% to 103% TGP).

Effects of Experimental Apparatus

The deep tank apparatus provided similar results to those obtained in the shallow water apparatus, for the same water depth. For example, the mortality rate for the first 10% of the population did not differ significantly among the static exposure at 122% TGP, the 3 h dynamic exposure, and the 6 h dynamic exposure. The only difference among the exposures over this range of cumulative mortality was that the static exposure occurred in the shallow water tanks, and the dynamic exposure in cages at the top of the deep tank. The fish in all three exposures had access to 0 to 0.25 m of water depth.

The variability among replicates in the static exposure regimen was higher than that in the dynamic exposures in the deep tanks, perhaps because the deep tank, which housed all replicates in the same tank, provided a more constant environment compared to nine individual shallow water tanks, each with it's own inflow line. Another possible explanation is that the differences in variability were due to fish behavior. Since the black Vexor™ mesh on the sides and bottom of the cages would have provided some form of cover, the cages might have reduced fish movement in the deep tank bioassays. This would tend to reduce variability among individuals in terms of exposure and, hence, time to mortality.

TGP Measurement Problems and Degassing

We measured TGP under hydrostatic pressure to eliminate errors associated with TGP measurements taken above the compensation depth. At levels above 110%, TGP measured in the shallow water experimental tanks (above the compensation depth) was always lower than the "true" TGP measured under hydrostatic pressure in a separate measuring column. The lower TGP measurement could have been due to bubble formation on the membrane of the probe (which can cause an erroneously low TGP readings because the bubbles inhibit gas transfer to the interior of the probe), and/or degassing at the water surface. Tests were conducted to determine whether degassing occurred; however, the results were highly variable and inconclusive. If degassing did occur, the amount would have been relatively small, hence the TGP data were not corrected for it.

Significance of Results with Respect to DGS Guidelines

The effects of exposure of fish to DGS can be divided into acute and chronic responses, depending on DGS levels (Fidler and Miller 1997). Acute GBT generally involves DGS in excess of 110% TGP and can result in direct mortality or high levels of stress in fish due to bubble formation in the cardiovascular system, sub-dermal emphysema on body surfaces (including the lining of the mouth), and extracorporeal bubble formation in gill lamella of large fish or in the buccal cavity of small fish. Chronic GBT usually involves TGP levels between approximately 103% and 110% TGP, and can result in overinflation of the swim bladder or bubble formation in the gut, mouth, buccal cavity, gill cavity, and yolk sac in larval fish (Fidler and Miller 1997). Mortality is generally low for chronic GBT, and requires long exposure times.

The DGS guidelines developed for the Province of BC protect against both acute and chronic GBT. The 110% TGP guideline protects all species and life-history phases from acute GBT, whereas the low-level guideline, which ranges from 103% to 110% TGP depending on water depth, primarily protects fish from the effects of swim bladder overinflation. This latter effect tends to occur in small fish only (typically fry less than about 55 mm), which were not used in our experiments. Hence, further discussion of the significance of our results with respect to the DGS guidelines for BC pertains only to the 110% TGP guideline for acute effects. A review of chronic GBT (e.g., swim bladder overinflation) and the low-level TGP guideline for BC is being prepared elsewhere.

The 110% TGP guideline for BC was established because it was the lowest TGP level shown in the literature to cause direct mortality in fish. However, the application of the acute TGP guideline of 110% would be conservative in a number of situations. It would be conservative for short exposures, since the literature data indicate that long exposure periods are required to elicit mortality at low TGP, even in shallow water. It would also be conservative at 110%, or even at higher TGP levels, if fish use sufficient water depth, relative to the compensation depth, to reduce or eliminate bubble growth processes. Finally, not all species and life-history phases are equally susceptible to TGP in the 110% range. Salmonid eggs, embryos, and pre-swim-up larvae are thought to be relatively resistant to this TGP level, but the swim-up fry stage may not be (Nebeker *et al.* 1978, Alderdice and Jensen 1985). Juvenile and adult fish are susceptible, with lethal signs occurring at TGP levels at or above 110%.

The threshold ΔP required to initiate bubble growth in the cardiovascular system of juvenile rainbow trout (one of the more sensitive salmonid species) exceeds the primary water quality guideline of 76 mm Hg, or 110% TGP for sea level conditions. Thus, even though the guideline was set at 110%, the cause of mortality reported in the literature at TGP levels of 110% to 115% (in data sets used to develop the BC guideline) has not been identified. The mortality in this TGP range was often accompanied by extensive emphysema of external body surfaces and other signs of GBT. Therefore, it is speculated that the mortality may be caused by a combination of emphysema of the lining of the buccal cavity and extra-corporeal bubbles between gill filaments (Fidler 1988).

Our research emphasizes the need to investigate the causes of mortality reported in the literature for exposure of fish to TGP between 110% and 115% TGP. Specifically, the conditions and exposure times required to produce effects in this range need to be identified, along with the species and life-history phases that are susceptible in this range of TGP. If extra-corporeal bubble growth plays a role, then the effects may, in part, be artifacts of the experimental enclosures used in laboratory experiments. For example, in some studies (e.g., Fidler 1988), holding containers were small and the fish had no room to burst swim or swim at high velocities. Such swimming, which is available to fish in the wild, may dislodge any extra-corporeal bubbles, and perhaps prevent mortality in the 110% to 115% TGP range. Other factors must also be considered, such as temperature or lower pN_2/pO_2 ratios associated with use of groundwater in laboratory bioassays (Meekin and Turner 1974). This investigation would help to narrow the range of conditions for which the 110% TGP guideline would be applicable.

Although our results showed the beneficial effect of fish use of water depth (even relatively small increments in water depth, or water depths at or just below the compensation depth for short periods of time) on survival, our experiments were conducted over relatively short exposure periods, ranging from 96 h to seven days. In some systems, such as the Columbia River, summer spill events cause elevated TGP for many weeks at a time. Thus, for longer exposures, it is not known whether depth compensation will simply delay mortality, ultimately resulting in the same cumulative mortality over a longer period, or whether it will reduce the total mortality over the exposure duration. However, if the primary mechanism for increased survival from depth compensation is the effect of hydrostatic pressure on nucleation sites and bubble growth processes, then sustained or periodic use of water depth by fish over long term exposures should also provide benefits to survival.

Despite concerns regarding long-term exposure to DGS, our results are directly relevant to situations where spill events occur for shorter durations, such as in coastal and southern interior regions of BC, where spills due to winter rain events are of relatively short duration (e.g., few days). In addition, water temperature in these watersheds would be similar to that used in our experiments (i.e., 10°C). Thus, for these facilities, fish use of water depth would likely delay mortality long enough to reduce overall cumulative mortality over the duration of the spill event. However, the behavior of individual fish in the wild will ultimately determine their exposure history and, hence, susceptibility to DGS. The extent to which various fish species and life-history phases use water depth should, therefore, be examined to provide a complete and relevant assessment of the impacts of DGS on fish and fish habitat.

Significance of Results with Respect to Prediction of Biological Effects

Future efforts to examine the biological effects of exposure of fish to elevated DGS should focus on identifying fish behavior patterns in the wild, that is, fish use of specific water depths to determine exposure histories. Those exposure histories could then be simulated in laboratory bioassays (under the appropriate TGP and temperature conditions, and duration of exposure) to assess mortality and sublethal effects. In situations where it would be necessary to simulate a wide array of fish behavioral patterns to ascertain whether a population was impacted by elevated DGS, the approach could be coupled with the application of dynamic GBT exposure models to predict mortality (e.g., Fidler 1998a, b; Richmond *et al.* 1998). That is, the data from laboratory simulations for a given TGP exposure history could be used to calibrate and validate these models. However, these models are currently limited by large inter-individual variability and differences among fish stocks in terms of susceptibility to mortality, and by the limited amount of information available on fish behavior from which to develop exposure histories.

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REFERENCES

Alderdice, D.F. and J.O.T. Jensen. 1985. An explanation for the high resistance of incubating salmonid eggs to atmospheric gas supersaturation of water. Aquaculture 49: 85-88.

Antcliffe, B.L., D. Kieser, G. Lawrence, W.L. Lockhart, D.A. Metner and J.A.J. Thompson. 1997a. Monitoring of mountain whitefish, *Prosopium williamsoni* from the Columbia River system near Castlegar, British Columbia: final assessment of fish health and contaminants, July 1996. Can. Tech. Rep. Fish. Aquat. Sci. 2184: 79 p.

Antcliffe, B.L., D. Kieser, J.A.J. Thompson, W.L. Lockhart, D.A. Metner and R. Roome. 1997b. Monitoring of mountain whitefish, Prosopium williamsoni, from the Columbia River system near Castlegar, British Columbia: health parameters and contaminants in 1994. Can. Tech. Rep. Fish. Aquat. Sci. 2142: 101 p.

APHA, AWWA, and WEF. 1998. Standard Methods for the Examination of Water and Waste Water. 20th Edition. American Public Health Association, American Water Works Association, and the Water Environment Federation.

Baath. 1989. The influence of gas supersaturation in the water on infectious diseases of the rainbow trout. 1989. Fish Health Protection Strategies, 1989, pp. 231-248.

Birtwell, I.K., J.S. Korstrom, M. Komatsu, B.J. Fink, L.I. Richmond and R.P. Fink. 2000. The susceptibility of juvenile chum salmon (*Oncorhynchus keta*) to predation following sublethal exposure to elevated temperature and dissolved gas supersaturation in seawater. Can. Tech. Rep. Fish. Aquat. Sci. (In press).

Blahm, T.H., R.J. McConnell and G.R. Snyder. 1974. Effect of Gas Supersaturated Columbia River Water on the Survival of Juvenile Salmonids. Final Report - Part 1. Natl. Mar. Fish. Serv. Environmental Field Station, Prescott, Oregon.

Blahm, T.H., B. McConnell and G.R. Snyder. 1976. Gas supersaturation research, National Marine Fisheries Service Prescott Facility - 1971 to 1974 In: Gas Bubble Disease, proceedings of a workshop cosponsored by Battelle, Pacific Northwest Laboratories, and U.S. Atomic Energy Commission, held at Richland, Washington. Edited by Fickeisen, D.H., and Schneider, M.J. CONF-741033. Technical Information Center, Office of Public Affairs, Energy Research and Development Administration. Oak Ridge, TN. pp. 11-19.

Canadian Council of Ministers of the Environment. 1998. Canadian Water Quality Guidelines for the Protection of Aquatic Life. Task Force on Water Quality Guidelines. Ottawa, Canada.

Colt, J. 1984. Computation of dissolved gas concentrations in water as functions of temperature, salinity, and pressure. Spec. Publ. No. 14. American Fisheries Society, Washington, DC.

Cussler, E.L. 1984. Diffusion: Mass Transfer in Fluid Systems. Cambridge University Press; Cambridge, United Kingdom. 525 p.

Dawley, E.M. and W.J. Ebel. 1975. Effects of various concentrations of dissolved atmospheric gas on juvenile chinook salmon and steelhead trout. Fish. Bull. 73: 787-796.

Dawley, E.M., M.H. Schiewe and B.H. Monk. 1976a. Effects of long-term exposure to supersaturation of dissolved atmospheric gases on juvenile chinook salmon and steelhead trout in deep and shallow test tanks. In Gas Bubble Disease, proceedings of a workshop cosponsored by Battelle, Pacific Northwest Laboratories, and U.S. Atomic Energy Commission, held at Richland, Washington. Edited by Fickeisen, D.H., and Schneider, M.J. CONF-741033. Technical Information Center, Office of Public Affairs, Energy Research and Development Administration. Oak Ridge, TN. pp 1-10.

Dawley, E., B. Monk, M. Schiewe, F. Ossiander and W. Ebel. 1976b. Salmonid bioassay of supersaturated dissolved air in water. US Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN. 39 p.

Ebel, W.J. 1971. Dissolved Nitrogen Concentrations in the Columbia and Snake Rivers in 1970 and Their Effect on Chinook Salmon and Steelhead Trout. NOAA Tech. Rept. NMFS SSRF-646. 7 p.

EPA (U. S. Environmental Protection Agency). 1986. Quality criteria for water. EPA, Report 440/5-86-001, Washington, D.C.

Fickeisen, D.H. and J.C. Montgomery. 1978. Tolerances of fishes to dissolved gas supersaturation in deep tank bioassays. Trans. Am. Fish. Soc. 107(2): 376-381.

Fidler, L.E. 1988. Gas Bubble Trauma in Fish. Ph.D. Thesis, Department of Zoology, University of British Columbia, Vancouver, British Columbia.

Fidler, L.E. 1998a. Laboratory physiology studies for configuring and calibrating the Dynamic Gas Bubble Trauma Mortality Model. Contract report prepared for Battelle Pacific Northwest Division, Richland, Washington by Aspen Applied Sciences Inc., Kalispell, Montana under Contract DACW68-96-D-0002, Delivery Order 6. U.S. Army Corps of Engineers, Walla Walla, WA.

Fidler, L.E. 1998b. The configuration, preliminary calibration, and operation of the Dynamic Gas Bubble Trauma Mortality model. Contract report prepared for Battelle Pacific Northwest Division, Richland, Washington by Aspen Applied Sciences Inc., Kalispell, Montana under Contract DACW68-96-D-0002, Delivery Order 8. U.S. Army Corps of Engineers, Walla Walla, WA.

Fidler, L.E. and S.B. Miller. 1997. British Columbia Water Quality Criteria for Dissolved Gas Supersaturation - Technical Report. Contract report to the B.C. Ministry of Environment, Department of Fisheries and Oceans, and Environment Canada. Aspen Applied Sciences Ltd., Cranbrook, British Columbia.

Hildebrand, L. 1991. Lower Columbia River Fisheries Inventory - 1990 Studies. Vol. 1, Main Report. Contract report by R.L. & L. Environmental Services Ltd., Edmonton, Alberta to B.C. Hydro, Environmental Resources, Vancouver, B.C.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54(2):187-211.

Jensen, J.O.T., J. Schnute and D.F. Alderdice. 1986. Assessing juvenile salmonid response to gas supersaturation using a general multivariate dose-response model. Can J. Fish. Aquat. Sci. 43:1694-1709.

Knittel, M.D., G.A. Chapman and R.R. Garton. 1980. Effects of hydrostatic pressure on steelhead survival in air-supersaturated water. Trans. Am. Fish. Soc. 109: 755-759.

Kruzynski, G.M. and I.K. Birtwell. 1994. A predation bioassay to quantify the ecological significance of sublethal responses of juvenile chinook salmon (*Oncorhynchus tshawytscha*) to the antisapstain fungicide TCMTB. Can. J. Fish. Aquat. Sci. 51:1780-1790.

Meekin, T.A. and R.L. Allen 1974. Summer chinook and sockeye salmon mortality in the upper Columbia River and its relationship to nitrogen supersaturation. Wash. Dept. Fish. Tech. Rep 12: 127-153.

Meekin, T.A. and B.K. Turner. 1974. Tolerance of salmonid eggs, juveniles, and squawfish to supersaturated nitrogen. Wash. Dept. Fish. Tech. Rep. 12: 78-126.

Mesa, M.G., T.P. Poe, D.M. Gadomski and J.H. Petersen. 1994. Are all prey created equal? A review and synthesis of differential predation on prey in substandard condition. J. Fish Biol. 45(Suppl. A): 81-96.

Mesa, M.G., J.J. Warren, K.M. Hans and A.G. Maule. 1996. Progression and severity of gas bubble trauma in juvenile chinook salmon and development of non-lethal methods for trauma assessment. In: Gas Bubble Trauma Monitoring and Research of Juvenile Salmonids. 1996 Annual Report of the U.S. Geological Survey, Biological Resources Division, Columbia River Research Laboratory, Cook, Washington. Prepared for the Bonneville Power Administration, Portland, Oregon.

Mesa, M.G., and Warren, J.J. 1997. Predator avoidance ability of juvenile chinook salmon (*Oncorhynchus tshawytscha*) subjected to sublethal exposure of gas-supersaturated water. Can. J. Fish. Aquat. Sci. 54: 757-764.

Mesa, M.G., L.K. Weiland and A.G. Maule. 2000. Progression and severity of gas bubble trauma in juvenile salmonids. Trans. Am. Fish. Soc. 129: 174-185.

Nebeker, A.V., J.D. Andros, J.K. McCrady and D.G. Stevens. 1978. Survival of steelhead trout (*Salmo gairdneri*) eggs, embryos, and fry in air-supersaturated water. J. Fish. Res. Bd. Can. 35(2): 261-264.

Nebeker, A.V. and J.R. Brett. 1976. Effects of air-supersaturated water on survival of Pacific salmon and steelhead smolts. Trans. Am. Fish. Soc. 105: 338-342.

Nebeker, A.V., A.K. Hauck and F.D. Baker. 1979. Temperature and oxygen-nitrogen gas ratios affect fish survival in air-supersaturated water. Water Res. 13: 299-303.

Nebeker, A.V., D.G. Stevens and R.K. Stroud. 1976. Effects of air-supersaturated water on adult sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Can. 33: 2629-2633.

NMFS 1998. Biological Opinion 1998. Operation of the Federal Columbia River Power System including the smolt monitoring program and the juvenile fish transportation program: A supplement to the biological opinion signed on March 2, 1995 for the same projects. United States Department of Commerce, Silver Springs, Maryland. 120 p. + Appendices.

Richmond, M.C., W.A. Perkins, and T.D. Scheibe. 1998. Two-Dimensional Hydrodynamic, Water Quality, and Fish Exposure Modeling of the Columbia and Snake Rivers. Part 1: Summary and Model Formulation. Draft Final Report submitted to U.S. Army Corps of Engineers, Walla Walla District. Battelle Pacific Northwest Division, Richland, Washington.

Roberts, R. 1989. Fish Pathology. 2nd ed. Bailliere Trindall, Toronto, p. 363-364.

Rucker, R.R. 1976. Gas bubble disease of salmonids: variation in oxygen-nitrogen ratios with constant total gas pressure. In: Gas Bubble Disease, proceedings of a workshop cosponsored by Battelle, Pacific Northwest Laboratories, and U.S. Atomic Energy Commission, held at Richland, Washington. Edited by Fickeisen, D.H., and Schneider, M.J. CONF-741033. Technical Information Center, Office of Public Affairs, Energy Research and Development Administration. Oak Ridge, TN. pp. 85-88.

Sigma Environmental Consultants Limited. 1983. Summary of Water Quality Criteria for Salmonid Hatcheries. Prepared for the Department of Fisheries and Oceans by Sigma Environmental Consultants Ltd. SECL 8067. Revised Edition. October, 1983.

Shrimpton, J.M., D.J. Randall and L.E. Fidler. 1990. Factors affecting swim bladder volume in rainbow trout (*Oncorhynchus mykiss*) held in gas supersaturated water. Can. J. Zool. 68: 962-968.

Sprague, J. B. 1973. The ABC's of pollutant bioassays using fish. Biological Methods for the Assessment of Water Quality, ASTM STP 528, American Society for Testing and Materials. pp. 6-30.

Stroud, R.K. and Nebeker, A.V. 1976. A study of the pathogenesis of Gas Bubble Disease in steelhead trout (*Salmo gairdneri*). In: Gas Bubble Disease, proceedings of a workshop cosponsored by Battelle, Pacific Northwest Laboratories, and U.S. Atomic Energy Commission, held at Richland, Washington. Edited by Fickeisen, D.H., and Schneider, M.J. CONF-741033. Technical Information Center, Office of Public Affairs, Energy Research and Development Administration. Oak Ridge, TN. pp. 66-71.

U.S. ACE 1998. 1998 Total Dissolved Gas Annual Report. U.S. Army Corps of Engineers, North Pacific Division.

Weitkamp, D.E. 1976 Dissolved gas supersaturation: live cage bioassays at Rock Island Dam, Washington. In Gas Bubble Disease, proceedings of a workshop cosponsored by Battelle, Pacific Northwest Laboratories, and U.S. Atomic Energy Commission, held at Richland, Washington. Edited by Fickeisen,D.H., and Schneider, M.J. CONF-741033. Technical Information Center, Office of Public Affairs, Energy Research and Development Administration. Oak Ridge, TN. pp. 24-36

Weitkamp, D.E. and M. Katz, M. 1980. A review of dissolved gas supersaturation literature. Trans. Am. Fish. Soc. 109: 659-702.

White, R.G., G. Phillips, G. Liknes, J. Brammer, W. Conner, L. Fidler, T. Williams and W. Dwyer. 1991. Effects of Supersaturation of Dissolved Gases on the Fishery of the Bighorn River Downstream of the Yellowtail Afterbay Dam. Montana Cooperative Fishery Research Unit, Montana State University, Bozeman, Montana. Final report to the U.S. Bureau of Reclamation.

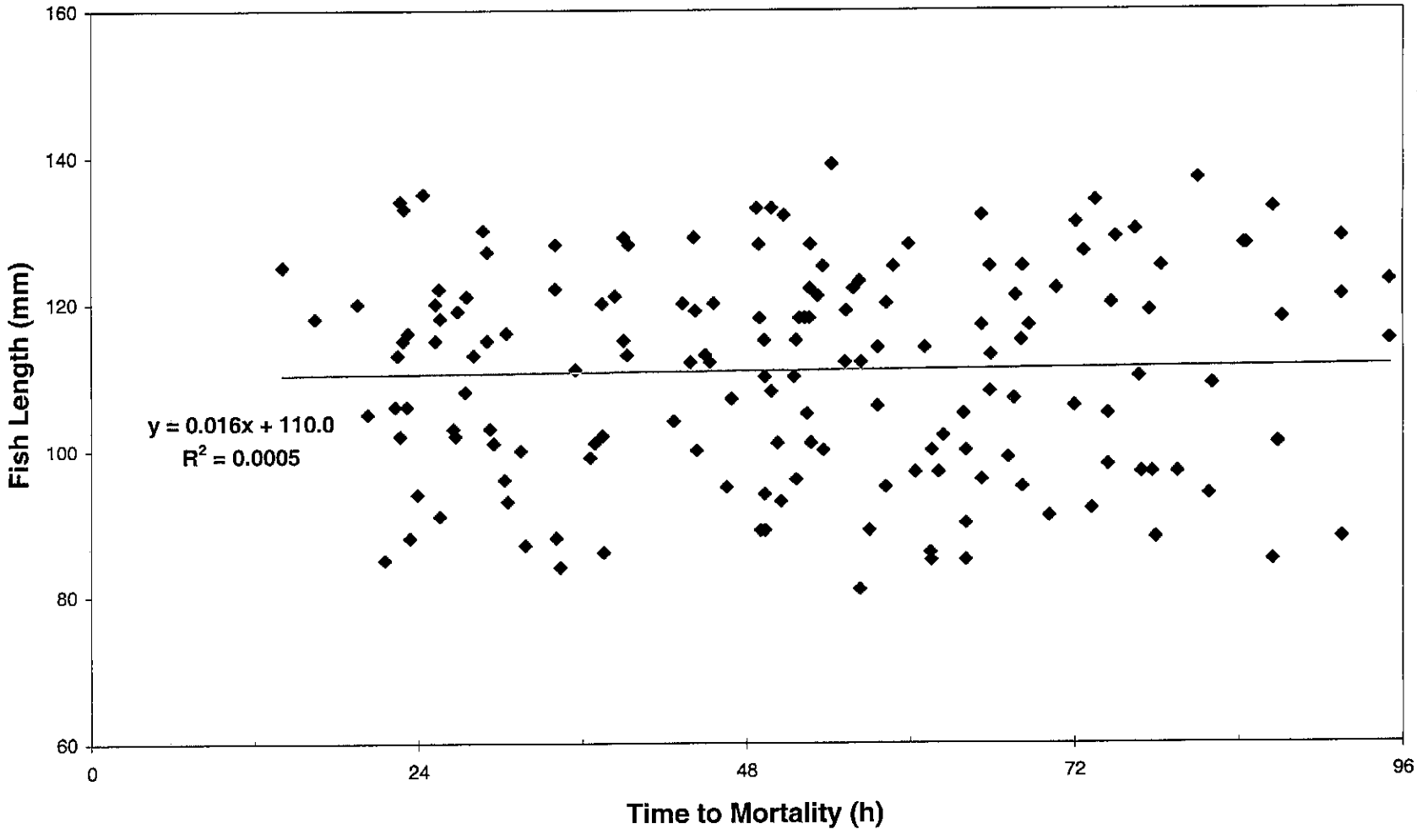

Figure 1. Time to mortality as a function of fish length for the static exposure of juvenile rainbow trout to 122% TGP at 10°C and 0.25 m water depth.

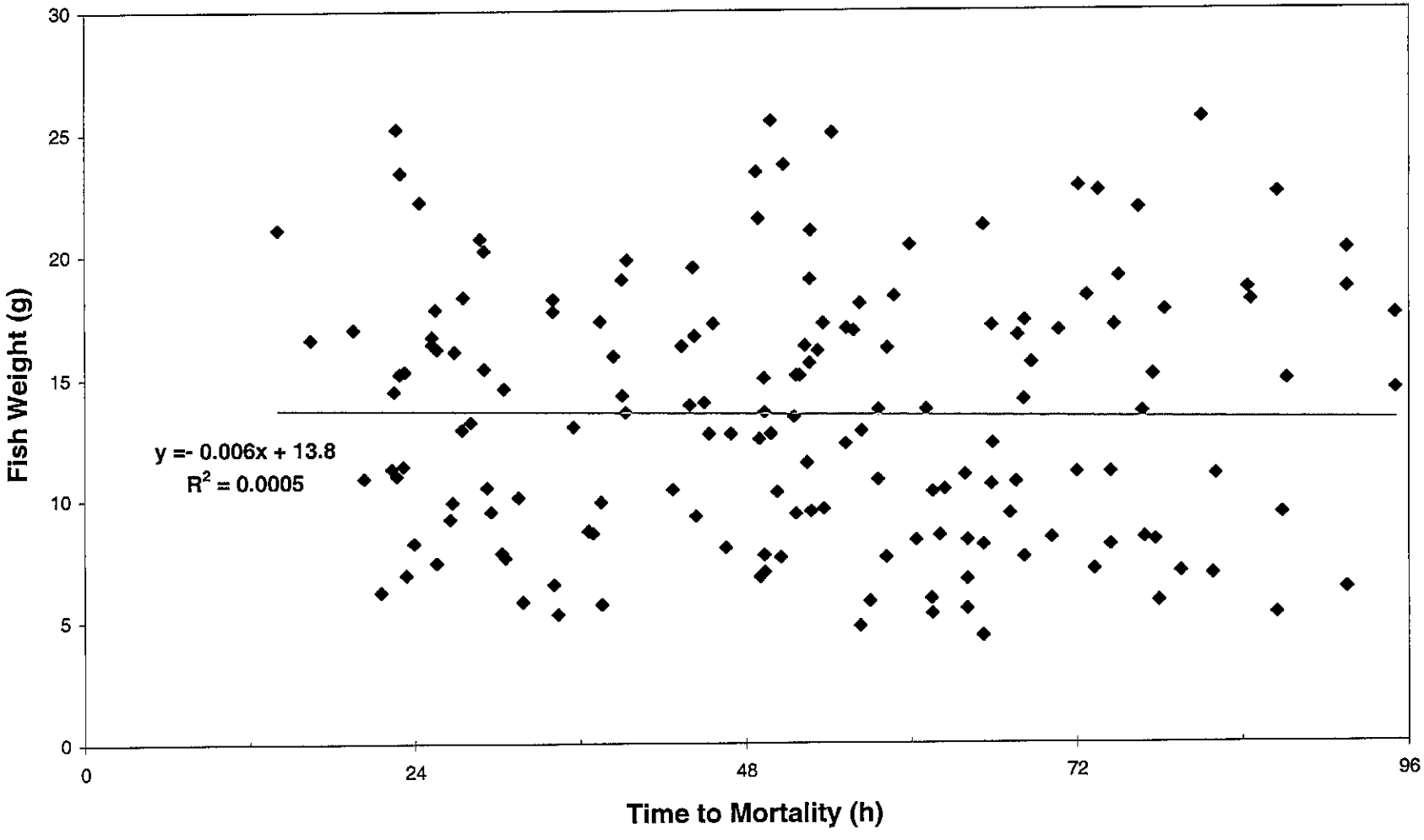

Figure 2. Time to mortality as a function of fish weight for the static exposure of juvenile rainbow trout to 122% TGP at 10°C and 0.25 m water depth.

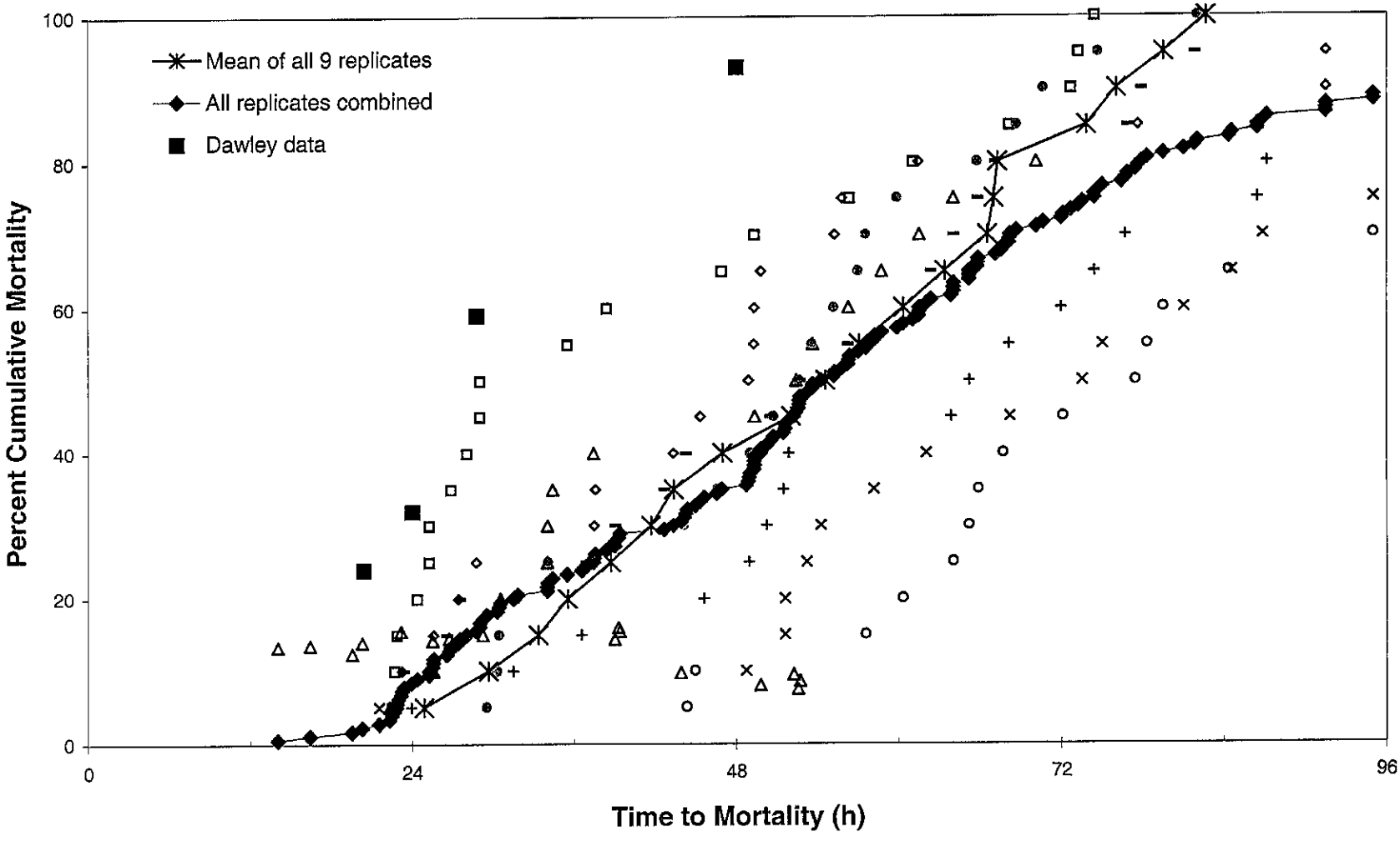

Figure 3. Cumulative mortality in juvenile rainbow trout as a function of time after static exposure to 122% TGP at 10°C and 0.25 m water depth for individual replicates (represented by different symbols), the mean of all replicates, and all replicates combined. See text for description of Dawley *et al.* (1976a) data.

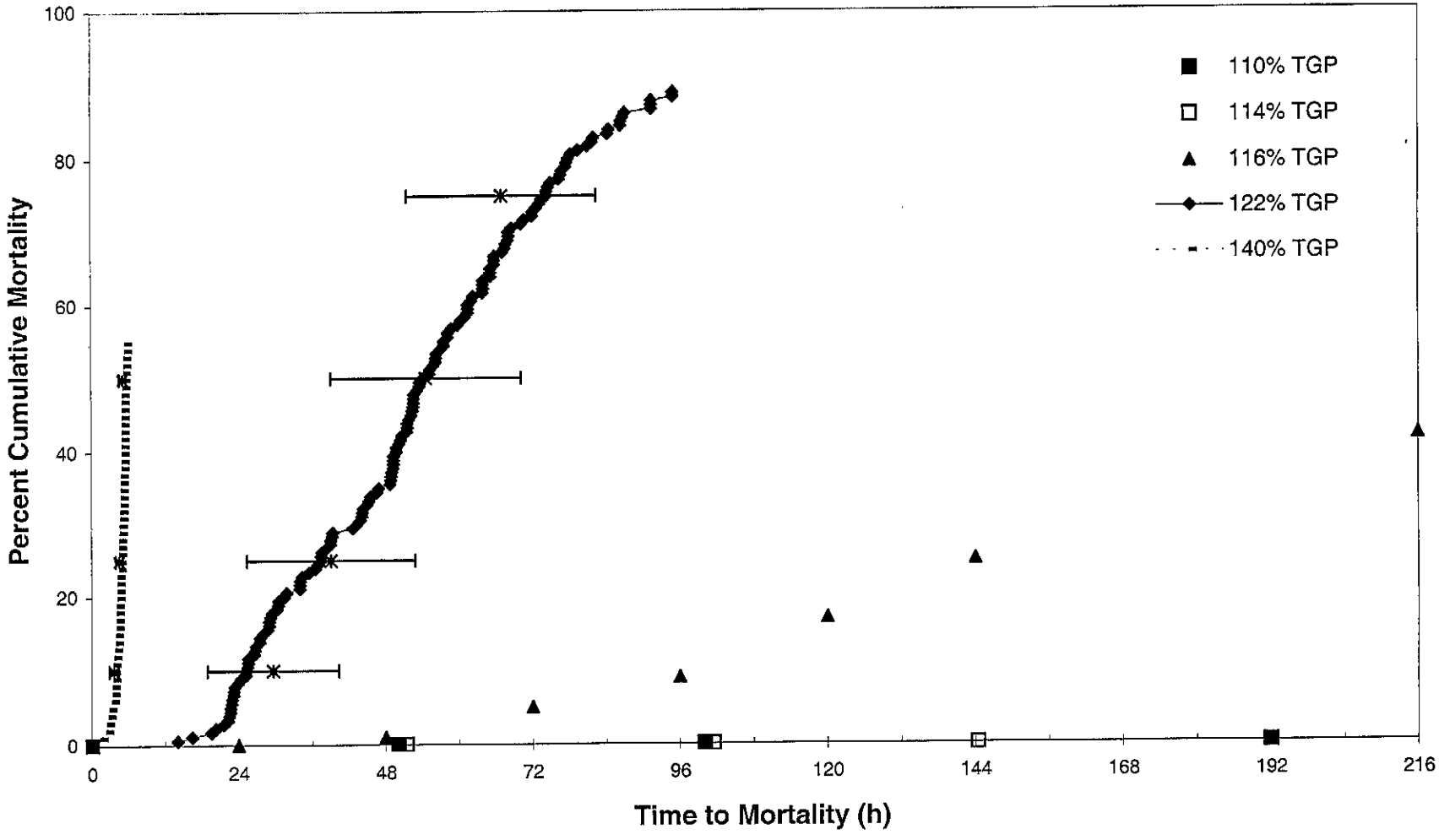

Figure 4. Cumulative mortality in juvenile rainbow trout as a function of time after static exposure to 110%, 114%, 116%,122% and 140% TGP, at 10°C and 0.25 m, for all replicates combined. Asterisk equals the mean (± SD) LT10, LT25, LT50, or LT75 calculated from data for replicates using a logistic model.

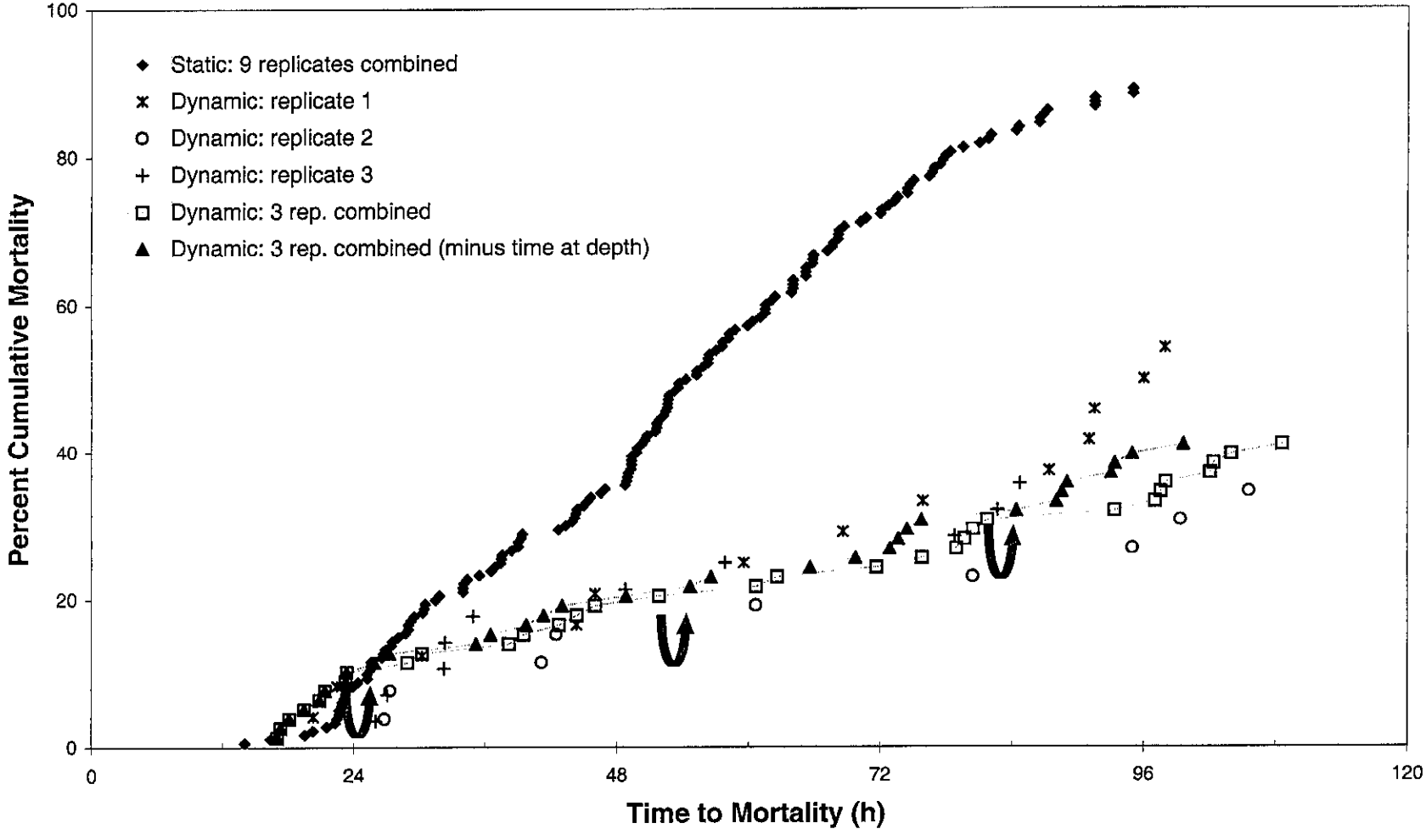

Figure 5. Cumulative mortality for the static (0.25 m depth) and dynamic (0.25 m depth with 3 h intervals below the compensation depth) exposure of juvenile rainbow trout to 122% TGP at 10°C. Arrows show the 3 h depth intervals.

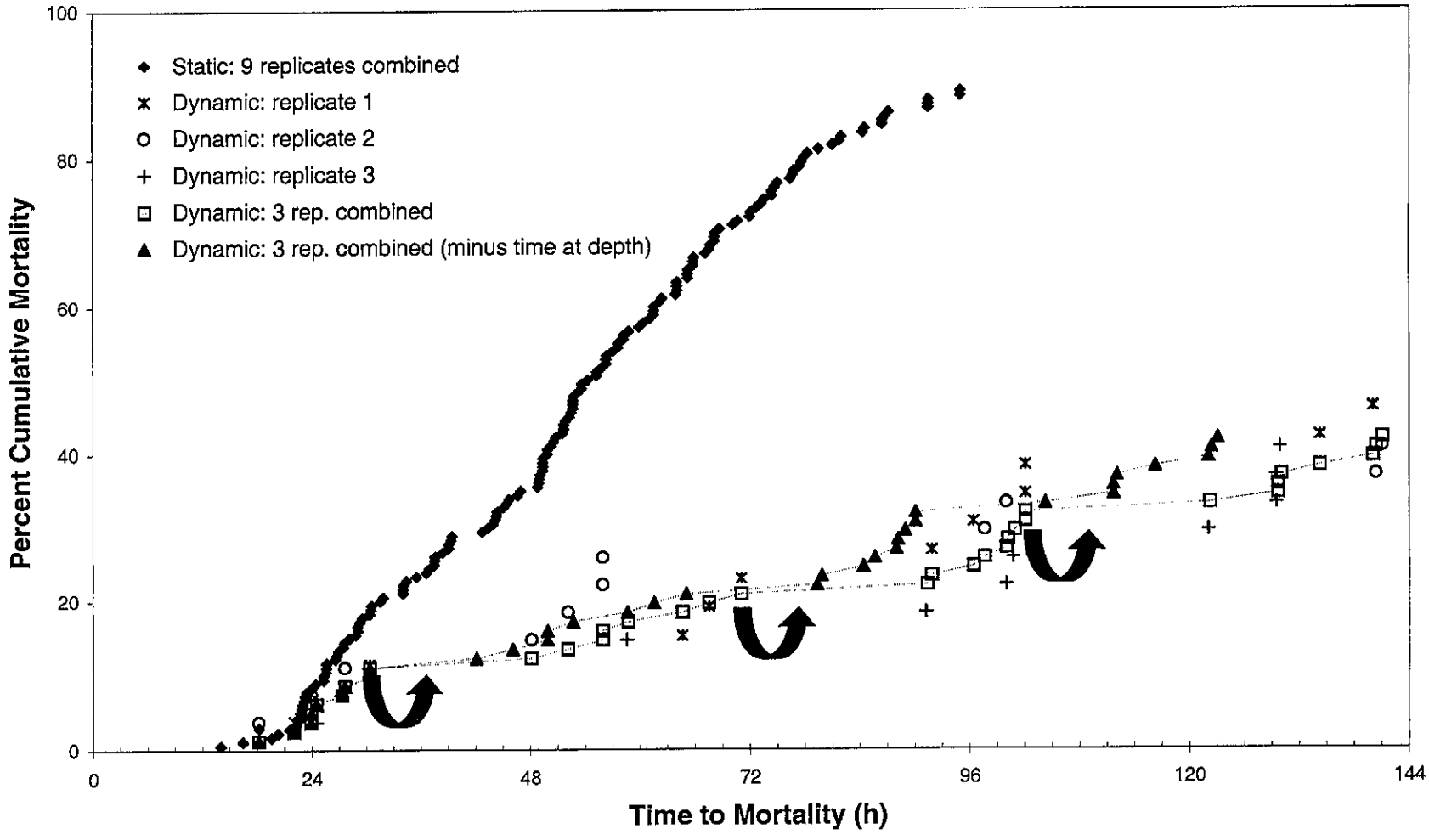

Figure 6. Cumulative mortality for the static (0.25 m depth) and dynamic (0.25 m depth with 6 h intervals below the compensation depth) exposure of juvenile rainbow trout to 122% TGP at 10°C. Arrows show the 6 h depth intervals.

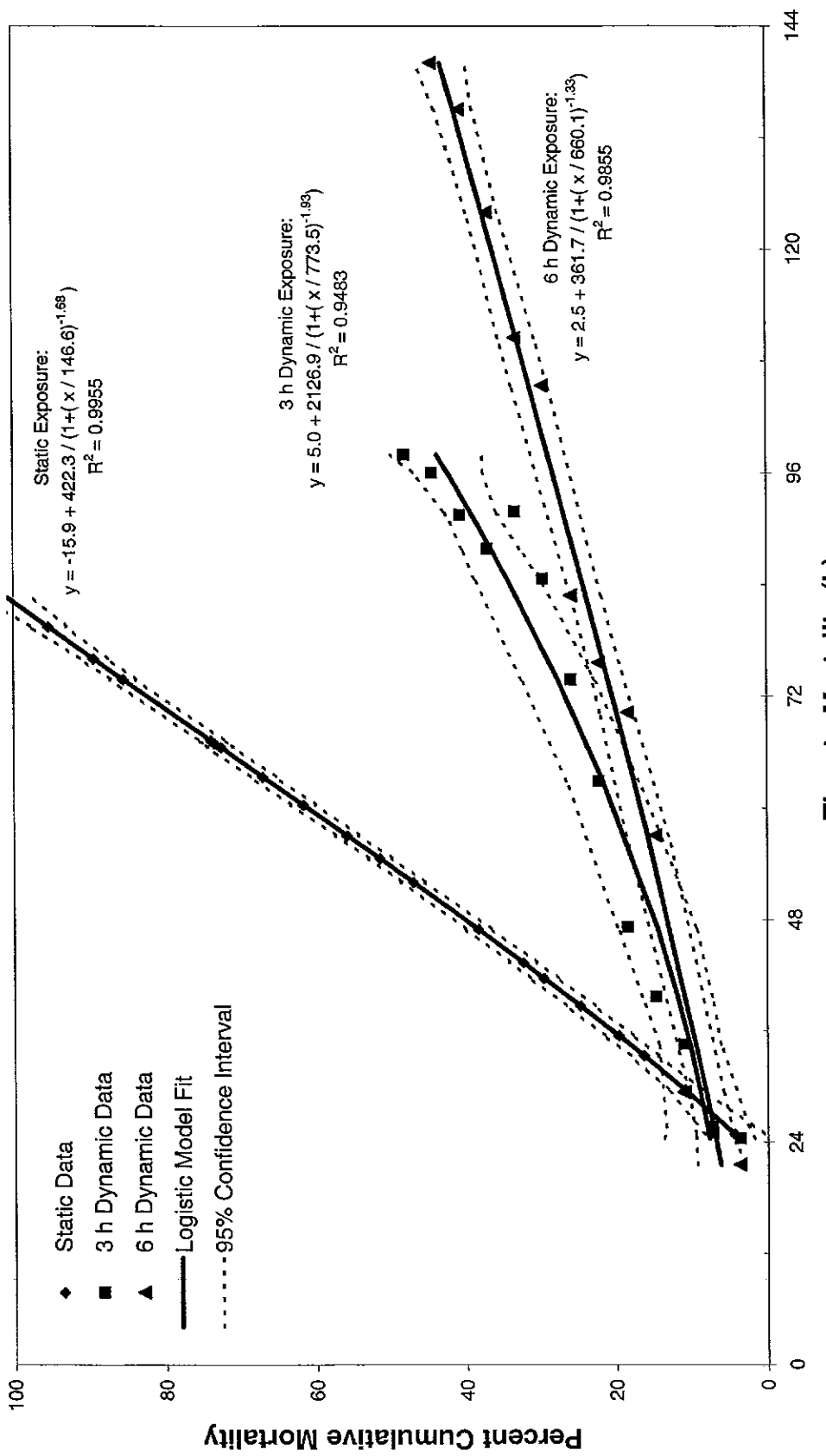

Figure 7. Logistic model fit to the replicate data for the 122% TGP static and dynamic exposures (3 and 6 h intervals at depth); 10°C. Each data point represents the average time to mortality among replicates for a given percent cumulative mortality.

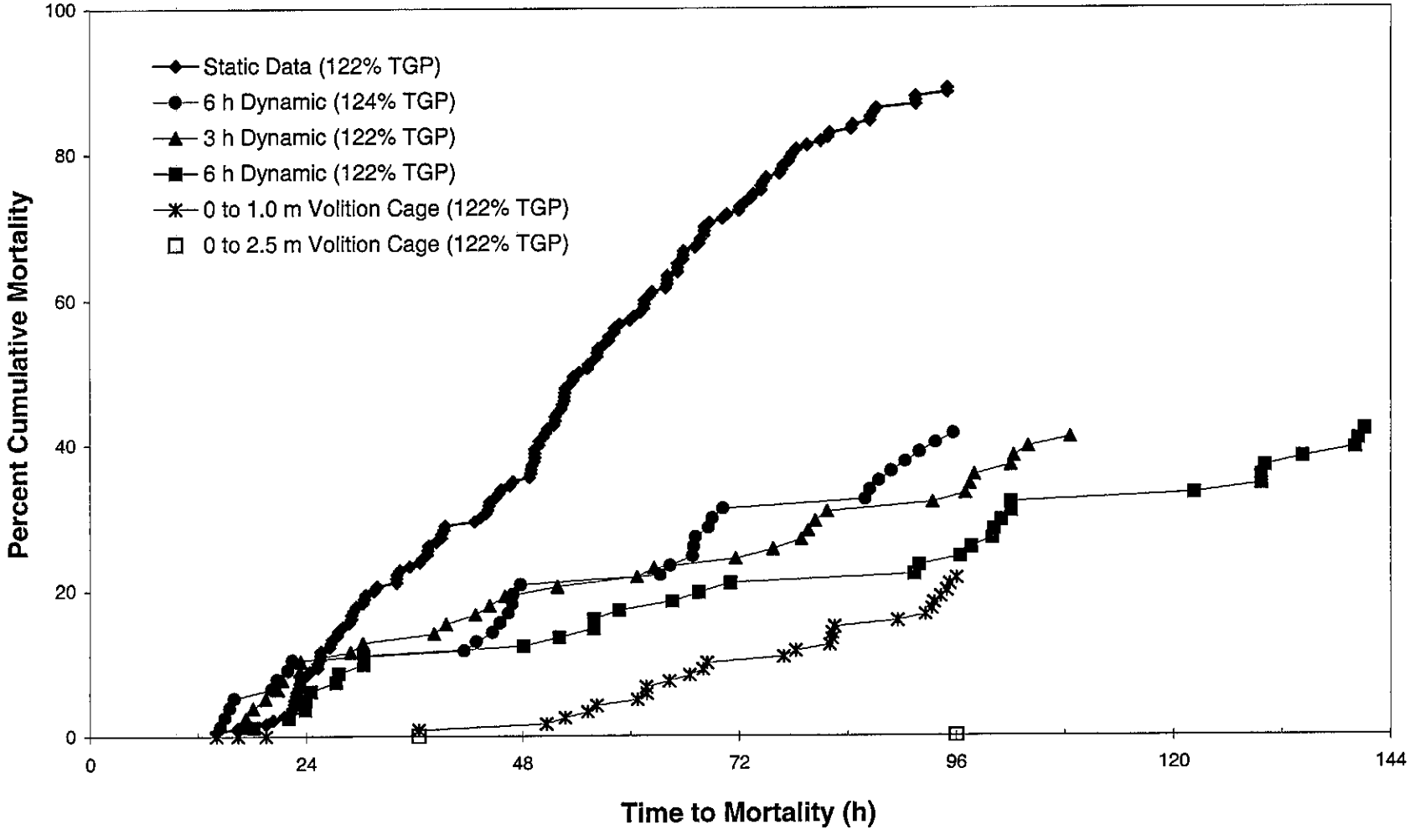

Figure 8. Cumulative mortality as a function of time to mortality for all static and dynamic exposures of juvenile rainbow trout to 122% or 124% TGP; 10°C. Data for all replicates combined.

APPENDICES

Appendix 1: Nomenclature, Definitions, and Biophysical Concepts

Nomenclature

DGS = dissolved gas supersaturation
TGP = total gas pressure: mm Hg = P_T
GBT = gas bubble trauma
mm Hg = millimetres of mercury
pO_2 = partial pressure of oxygen – gaseous or dissolved: mm Hg
pN_2 = partial pressure of nitrogen plus trace gases – gaseous or dissolved: mm Hg
pH_2O = vapor pressure of water: mm Hg
ρ = density of water
g = gravitational constant
h = depth in water column: metres
σ = surface tension of water: converted to mm Hg•cm
pAtm = atmospheric pressure: mm Hg
p_S = blood pressure in cardiovascular system where GBT bubbles are present
r = radius of spherical GBT bubble
r_0 = radius of nucleation site from which GBT bubble grows
Δp = pressure differential between dissolved gas pressure and interfacial gas phase pressure
ΔP = water surface Δp
ΔP_p = physical Δp across a macroscopic bubble in water at depth h
ΔP_F = physiological Δp across a macroscopic bubble in fish cardiovascular system at depth h
ΔP_{FN} = physiological Δp across a microscopic bubble in fish cardiovascular system at depth h
h_P = physical compensation depth: metres
h_F = physiological compensation depth for macroscopic bubbles in fish cardiovascular system: metres
h_{FN} = physiological compensation depth for microscopic bubbles in fish cardiovascular system: metres
ΔP_{SB} = threshold ΔP at water surface required to initiate overinflation of the swim bladder in rainbow trout
ΔP_{EW} = threshold ΔP at water surface required to initiate sub-dermal emphysema and extra-corporeal bubble growth in water
ΔP_{CV} = threshold ΔP at water surface required to initiate cardiovascular bubble growth in rainbow trout
TGP_{SB} = threshold TGP to initiate swim bladder overinflation in rainbow trout
TGP_{EW} = threshold TGP required to initiate sub-dermal emphysema and extra-corporeal bubble growth in water
TGP_{CV} = threshold TGP required to initiate cardiovascular bubble growth in rainbow trout at depth h

$TGP\%_{SB}$ = threshold TGP% required to initiate overinflation of the swim bladder in rainbow trout at depth h

$TGP\%_{EW}$ = threshold TGP% required to initiate sub-dermal emphysema and extra-corporeal bubble growth in water at depth h

$TGP\%_{CV}$ = threshold TGP% required to initiate cardiovascular bubble growth in rainbow trout

Definitions

$TGP = pO_2 + pN_2 + pH_2O$

$TGP\% = 100 \cdot (pN_2 + pO_2 + pH_2O)/pAtm$

$\Delta P = TGP - pAtm$

$\Delta P_P = TGP - pAtm - \rho \bullet g \bullet h$

$\Delta P_F = TGP - pAtm - \rho \bullet g \bullet h - p_S$

$\Delta P_{FN} = TGP - pAtm - \rho \bullet g \bullet h - (2 \bullet \sigma / r) - p_S$

$\Delta P = pAtm \bullet [(TGP\%/100) - 1]$

$\Delta P_P = pAtm \bullet [(TGP\%/100) - 1] - \rho \bullet g \bullet h$

$\Delta P_F = pAtm \bullet [(TGP\%/100) - 1] - \rho \bullet g \bullet h - p_S$

$\Delta P_{FN} = pAtm \bullet [(TGP\%/100) - 1] - \rho \bullet g \bullet h - (2 \bullet \sigma / r) - p_S$

$h_P = (TGP - pAtm)/\rho \bullet g$

$h_F = [TGP - pAtm - p_S]/\rho \bullet g$

$h_{FN} = [TGP - pAtm - (2 \bullet \sigma / r) - p_S]/\rho \bullet g$

$h_P = [(TGP\% \bullet pAtm/100) - pAtm)]/\rho \bullet g$

$h_F = [(TGP\% \bullet pAtm/100) - pAtm - p_S]/\rho \bullet g$

$h_{FN} = [(TGP\% \bullet pAtm/100) - pAtm - (2 \bullet \sigma / r) - p_S]/\rho \bullet g$

$\Delta P_{SB} = 73.89 \cdot h + 0.15 \cdot pO_2$

$\Delta P_{EW} = 73.89 \cdot h + 83.0$

$\Delta P_{CV} = 73.89 \cdot h + 0.21 \cdot pO_2 + 83.0$

$TGP_{SB} = pAtm + 73.89 \cdot h + 0.15 \cdot pO_2$

$TGP_{EW} = pAtm + 73.89 \cdot h + 83.0$

$TGP_{CV} = pAtm + 73.89 \cdot h + 0.21 \cdot pO_2 + 83.0$

$TGP\%_{SB} = 100 \bullet (pAtm + 73.89 \cdot h + 0.15 \cdot pO_2)/pAtm$

$TGP\%_{EW} = 100 \bullet (pAtm + 73.89 \cdot h + 83.0)/pAtm$

$TGP\%_{CV} = 100 \bullet (pAtm + 73.89 \cdot h + 0.21 \cdot pO_2 + 83.0)/pAtm$

Biophysical Concepts in DGS and GBT

a) In well-mixed water columns, TGP and TGP% do not vary with depth.
b) Only Δp varies with depth (see above definitions of Δp, ΔP, ΔP_p, ΔP_F, ΔP_{FN}, ΔP_{SB}, ΔP_{EW}, ΔP_{CV}).
c) Δp also varies with p_S and r.
d) TGP alone controls the rate at which a fish takes up or depurates dissolved gases. The higher the TGP the more rapid the uptake and the higher the equilibrium TGP reached in the fish.
e) The time for TGP to reach bubble growth thresholds (i.e. TGP_{SW}, TGP_{EW}, TGP_{CV}) introduces a time lag in the initiation of bubble growth and contributes (independent of Δp), to a portion of the exposure time required to cause mortality.
f) Δp controls the rate of bubble growth once the internal TGP has exceeded the respective TGP threshold (i.e. TGP_{SW}, TGP_{EW}, TGP_{CV}).
g) For fish that have had no previous exposure to supersaturated water, GBT bubbles must grow from microscopic nucleation sites ($r \approx 10 - 15$ μm) in the water, sub-dermal tissue, and cardiovascular system.
h) Once bubble growth has proceeded to macroscopic size (i.e., r = 50μm), surface tension effects on bubble internal gas pressure and growth are negligible.
i) Macroscopic bubbles above their respective compensation depths (i.e., h_P, h_F, h_{FN}) will continue to grow, with the rate of growth increasing with distance above compensation depth.
j) Macroscopic bubbles below their respective compensation depths (i.e., h_P, h_F, h_{FN}) will collapse, with the rate of collapse increasing with increasing depth.
k) Macroscopic bubbles at their respective compensation depths (i.e., h_P, h_F, h_{FN}) will neither grow nor collapse.
l) Bubble growth in water and the cardiovascular systems of fish is initially controlled by surface tension (i.e., small r_0). Once r > 50 μm, surface tension effects are negligible.
m) Bubble growth in the cardiovascular system is slowed by lower Δp (i.e., ΔP_F) and the higher system pressure (p_S).
n) Sub-dermal bubble growth (emphysema) is controlled (and slowed significantly) by the tensile strength of the tissue in which the bubble is growing.
o) At low TGP (i.e., < 120%) and shallow depths (i.e., < .2 m) subdermal emphysema bubbles generally grow more rapidly than cardiovascular system bubbles.
p) At high TGP (i.e., > 120%) and shallow depths (i.e., < .2 m) subdermal emphysema bubbles generally grow more slowly than cardiovascular system bubbles.
q) For fish that range up and down in a supersaturated water column, GBT bubbles can undergo a wide range of growth, stabilization, and collapse stages and corresponding wide range of growth or collapse rates.
r) Cardiovascular bubble growth appears to be initiated in the atrium of the heart, most likely in the valve between the sinus venosus and the atrium.
s) Once these bubbles reach a certain size, blood flow strips them from the epithelial surface and they are swept through the heart, bulbous arteriosis, ventral aorta, and into the branchial arteries. From there, they move into the afferent arteries of the gill filaments or

reduce blood pressure to where tubular bubble growth can begin in the afferent filamental arteries.

t) Once the afferent filamental arteries are approximately 50% blocked, bubble growth begins in the efferent filamental arteries (for juvenile fish).

u) Once afferent filamental arteries are approximately 80% blocked, fish death occurs in juvenile fish.

v) In small fish (e.g., less than approximately 50 mm), bubble growth in the branchial arteries alone (and not in the filamental arteries) can cause mortality.

Appendix 2: TGP and Temperature Data for TGP Bioassays

All temperature data were from temperature loggers, except where indicated. Where only mean Barometric Pressure (BP) was presented, TGP was calculated from ΔP and mean BP. All other TGP values were calculated from individual ΔP and BP readings, taken at the same time (DL meter = TBO-DL meter, S meter = TBO-L meter).

Experiment	Treatment	Statistic	ΔP (mm Hg)	BP (mm Hg)	TGP %	Temperature (°C)
Static:	Control	Mean	11	758	101.5	9.9
122% TGP	(n=75)	SD	± 2		± 1	± 0.1
		Min.	8		101.1	9.6
		Max.	14		101.8	10.1
	TGP	Mean	170	758	122.4	10.0
	(DL	SD	± 1.0	± 3	± 0.2	± 0.1
	Meter)	Min.	163	754	121.6	9.6
	(n=1167)	Max.	175	762	123.1	10.2
	TGP	Mean	171	763	122.0	
	(S Meter)	SD	± 1.6	± 3	± 0.1	
	(n=90)	Min.	166	758	121.8	
		Max.	175	767	123.0	
Static:	Control	Mean	9	761	101.2	9.9
122% TGP,	(n=29)	SD	± 3		± 1	± 0.1
no acclim.		Min.	4		100.5	9.8
		Max.	16		102.1	9.9
	TGP	Mean	167	762	122.0	9.8
	(DL	SD	± 0.7	± 3	± 0.1	± 0.1
	Meter)	Min.	162	758	121.3	9.6
	(n=290)	Max.	169	769	122.2	10.2
	TGP	Mean	171	761	122.4	
	(S Meter)	SD	± 1.6	± 3	± 0.2	
	(n=33)	Min.	167	758	121.9	
		Max.	176	769	123.1	

a) Data were from DL meter.

b) Data were from temperature logger.

Appendix 2: Continued

Experiment	Treatment	Statistic	ΔP (mm Hg)	BP (mm Hg)	TGP %	Temperature (°C)
Static: 110% TGP	Control (n=53)	Mean	16	760	102.1	10.2
		SD	± 2.4		± 1.0	± 0.2
		Min.	11		101.5	9.9
		Max.	19		102.5	10.4
	TGP (DL Meter) (n=56)	Mean	77	760	110.1	9.9
		SD	± 2.4		± 1.0	± 0.1
		Min.	69		109.1	9.5
		Max.	81		110.7	10.2
Static: 114% TGP	Control (n=32)	Mean	16	755	102.2	9.9
		SD	± 2.1		± 1.0	± 0.1
		Min.	13		101.7	9.8
		Max.	22		102.9	10.2
	TGP (DL Meter) (n=48)	Mean	105	755	113.9	9.9
		SD	± 3.7		± 1.0	± 0.1
		Min.	97		113	9.5
		Max.	111		114.7	10.2
Static: 116% TGP	Control (n=32)	Mean	18	762	102.3	9.8
		SD	± 2.0		± 1	± 0.1
		Min.	12		101.5	9.5
		Max.	22		102.9	10.2
	TGP (DL Meter) (n=283)	Mean	123	762	116.2	9.8
		SD	± 1.5		± 0.2	± 0.1[a]
		Min.	120		115.8	9.6
		Max.	127		116.7	10.0
	TGP (S Meter) (n=29)	Mean	123	764	116.1	10.0
		SD	± 2.3		± 0.3	± 0.2[b]
		Min.	119		115.5	9.7
		Max.	128		116.7	10.5

a) Data were from DL meter.
b) Data were from temperature logger.

Appendix 2: Continued

Experiment	Treatment	Statistic	ΔP (mm Hg)	BP (mm Hg)	TGP %	Temperature (°C)
Static:	Control	Mean	14	762	101.8	10.1
140% TGP	(n=13)	SD	± 3		± 1	± 0.1
no acclim.		Min.	8		101.1	9.9
		Max.	16		102.1	10.2
	TGP	Mean	307	764	140.2	10.0
	(S Meter)	SD	± 1.6		± 0.2	± 0.1
	(n=13)	Min.	304		139.9	10
		Max.	309		140.5	10.1
Dynamic:	Control	Mean	12	761	101.6	9.8
122% TGP	(n=51)	SD	± 4.7		± 1.0	± 0.1
0 to 1 m		Min.	3		100.4	9.7
		Max.	18		102.4	10.1
	TGP	Mean	169	761	122.2	10.0
	(DL	SD	± 1.5	± 3	± 0.2	± 0.1
	Meter)	Min.	166	756	121.7	9.8
	(n=384)	Max.	171	766	122.5	10.5
	TGP	Mean	169	761	122.1	
	(S Meter)	SD	± 1.3	± 3	± 0.2	
	(n=65)	Min.	166	758	121.7	
		Max.	172	767	122.6	
Dynamic:	Control	Mean	15	761	102	9.9
122% TGP	(n=13)	SD	± 2		± 1	± 0.2
0 to 2.5 m		Min.	9		101.6	9.6
		Max.	18		102.3	10.2
	TGP	Mean	171	767	122.3	10.0
	(DL	SD	± 1.6	± 5	± 0.2	± 0.2
	Meter)	Min.	168	759	121.8	9.8
	(n=190)	Max.	175	776	122.9	10.4

a) Data were from DL meter.
b) Data were from temperature logger.

Appendix 2: Continued

Experiment	Treatment	Statistic	ΔP (mm Hg)	BP (mm Hg)	TGP %	Temperature (°C)
Dynamic:	TGP	Mean	174	777	122.3	
122% TGP	(S Meter)	SD	± 0.6		± 0.1	
0 to 2.5 m	(n=4)	Min.	173	777	122.2	
		Max.	174	777	122.4	
Dynamic:	Control	Mean	11	760	101.4	9.8
122% TGP	(n=32)	SD	± 2.1	± 3	± 0.3	± 0.1
3 h depth		Min.	6	754	100.8	9.6
Intervals		Max.	14	763	101.8	10.0
	TGP	Mean	170	758	122.4	9.8
	(DL	SD	± 3	± 3	± 0.4	± 0.1[a]
	Meter)	Min.	148	753	119.6	9.5
	(n=522)	Max.	173	766	122.8	10.0
	TGP	Mean	168	759	122.1	10
	(S Meter)	SD	± 3.6	± 3	± 0.5	± 0.1[b]
	(n=74)	Min.	144	751	118.9	9.8
		Max.	171	763	122.6	10.1
Dynamic:	Control	Mean	16	755	102.2	9.8
122% TGP	(n=38)	SD	± 2.1		± 1.0	± 0.1
3 h depth		Min.	13		101.7	9.7
Intervals		Max.	22		102.9	10.1
	TGP	Mean	169	756	122.3	9.9
	(DL	± SD	± 1.5	± 2	± 0.2	± 0.1
	Meter)	Min.	161	748	121.5	9.8
	(n=564)	Max.	177	759	123.7	10.2
	TGP	Mean	168	755	122.2	
	(S Meter)	SD	± 1.8	± 4	± 0.3	
	(n=101)	Min.	163	742	121.6	
		Max.	171	760	122.6	

a) Data were from DL meter.
b) Data were from temperature logger.

Appendix 2: Continued

Experiment	Treatment	Statistic	ΔP (mm Hg)	BP (mm Hg)	TGP %	Temperature (°C)
Dynamic:	Control	Mean	10	754	101.3	9.8
124% TGP		SD				± 0.1
6 h depth		Min.				9.6
Intervals		Max.				10.1
	TGP	Mean	180	766	123.5	10.0
	(DL	SD	± 3.2	± 3	± 0.5	± 0.1
	Meter)	Min.	143	732	118.7	10.0
		Max.	184	769	124.0	10.0
	TGP	Mean	182	765	123.7	
	(S Meter)	± SD	± 1.7	± 6	± 0.3	
		Min.	180	746	123.4	
		Max.	187	770	124.4	

a) Data were from DL meter.
b) Data were from temperature logger.

Appendix 3: LT10, LT25, LT40, LT50, and LT75 Data

The following tables provide predicted LT10, LT25, LT40, LT50, and LT75 (time to mortality for the specified percent cumulative mortality) data for the various experiments, calculated from a logistic model. Values in brackets represent the actual times to 10%, 25%, 40%, 50%, and 75% cumulative mortality (where they represented discrete data points) for comparison with predicted LT data.

LT data for the 122% TGP static exposure

Replicate number	LT10 (h)	LT25 (h)	LT40 (h)	LT50 (h)	LT75 (h)
1	17.1 (16.4)	23.6 (23.1)	32.3 (39.0)	39.1 (39.4)	58.8 (52.7)
2	24.2 (23.4)	34.7 (36.9)	45.4 (44.2)	52.3 (52.7)	68.5 (65.8)
3	24.1 (22.7)	24.3 (25.2)	26.3 (28.0)	30.9 (29.0)	56.7 (56.4)
4	38.5 (48.7)	53.4 (53.2)	66.0 (62.0)	73.6 (73.5)	90.4 (95.0)
5	26.9 (23.2)	30.4 (28.7)	37.0 (43.3)	43.2 (48.9)	64.9 (55.8)
6	49.5 (45.0)	60.8 (64.0)	70.9 (67.7)	77.1 (77.5)	NA NA
7	31.5 (31.5)	45.5 (48.9)	57.8 (51.9)	65.4 (65.2)	82.6 (86.5)
8	25.7 (25.6)	32.1 (34.0)	41.0 (37.4)	48.1 (52.4)	69.1 (64.0)
9	30.4 (30.3)	37.8 (34.1)	45.5 (49.0)	50.7 (52.6)	63.9 (59.9)
Mean	29.8 (29.6)	38.1 (38.7)	46.9 (46.9)	53.4 (54.6)	69.4 (67.0)
All Combined	25.5 (25.2)	36.3 (37.4)	49.8 (49.8)	55.6 (54.3)	75.6 (74.4)

LT data for the 122% TGP unacclimated static exposure

Replicate number	LT10 (h)	LT25 (h)	LT40 (h)	LT50 (h)
1	20.5 (20.8)	27.6 (28.9)	35.9 (33.3)	42.0 (41.3)
2	21.5 (24.8)	31.1 (28.8)	37.0 (41.1)	NA NA
3	19.9 (20.6)	33.5 (34.7)	45.4 (47.5)	NA NA
4	18.4 (22.3)	28.5 (29.2)	37.3 (35.7)	42.3 (45.1)
Mean	20.1 (22.1)	30.2 (30.4)	38.9 (39.4)	42.2 (43.2)
All Combined	20.9 (22.6)	30.6 (29.4)	40.9 (41.2)	47.9 (47.5)

Appendix 3: Continued

LT data for the 122% TGP dynamic exposure with 3 h depth intervals

Replicate number	LT10 (h)	LT25 (h)	LT40 (h)
1	25.9	60.2	86.9
2	31.6	86.5	110.1
3	29.0	62.2	88.5
Mean	28.8	69.6	95.2
All Combined	24.7	70.3	106.1

LT data for the 122% TGP dynamic exposure with 6 h depth intervals

Replicate number	LT10 (h)	LT25 (h)	LT40 (h)
1	36.2	80.0	120.7
2	28.0	69.2	143.2
3	32.5	110.5	129.6
Mean	32.2	86.6	131.2
All Combined	35.2	91.1	137.8

LT data for the 124% TGP dynamic exposure with 6 h depth intervals

Replicate Number	LT10 (h)	LT25 (h)	LT40 (h)
1	22.3	59.4 (66.8)	87.4
2	42.1	93.8	161.1
3	17.7	47.1	74.2
Mean	27.4	66.8	107.6
All Combined	29.0	64.6	93.7

Appendix 4: Results for Degassing Tests

Apparatus	Tank #	True* TGP%	Measured TGP%	Δ TGP%	Flow L/min	Probe Activity
Shallow	1B	122.2	120.7	- 1.5	2.5	
Tanks	1B	122.4	121.1	- 1.3	2.5	Gently shook probe
	2A	121.7	120.4	- 1.3	2.5	every few minutes to
	3B	121.7	120.4	- 1.3	2.5	dislodge bubbles
	3B	122.4	121.4	- 1.0	2.5	
	3B	122.8	122.0	- 0.8	2.5	
	3B	122.8	120.7	- 2.1	2.5	Shook probe
	3B	122.8	118.0	- 4.8	2.5	vigorously and
	3B	122.8	118.4	- 4.4	2.5	continuously
	5B	121.7	120.4	- 1.3	2.5	Gently shook
	5B	122.8	121.3	- 1.5	2.5	probe every few
	6B	121.7	120.4	- 1.3	2.5	minutes to
	7B	122.4	121.0	- 1.4	2.5	dislodge bubbles
Shallow	1B	122.8	122.2	- 0.6	4.0	Increased flow and
Tanks with	1B	122.4	121.4	- 1.0	5.0	held probe directly
Increased	1B	122.4	121.4	- 1.0	7.0	into flow to dislodge
Flow	1B	122.4	121.9	- 0.5	10	bubbles
	1B	122.1	120.8	- 1.3	4.0	Flow delivery nozzle moved to mid-depth
Short MC (0.25 m)	10B	122.9	121.4	- 1.5	2.5	Gently shook every few minutes
Deep	Surface	121.1	120.8	- 0.3	15	Gently shook probe
Tanks	Surface	122.2	121.6	- 0.6	15	while at surface

* In the shallow water tests, the true TGP was measured in the 3 m deep measuring column (MC). In the deep tanks, true TGP was measured at the bottom of the tank.