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ARTICLE

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Abstract

Chum Salmon *Oncorhynchus keta* alevins developing in gravel downstream of Bonneville Dam on the Columbia River are exposed to elevated total dissolved gas (TDG) when water is spilled to move migrating salmon smolts to the ocean. We studied whether alevins that were exposed to six levels of dissolved gas ranging from 100% to 130% TDG at three development periods between hatch and emergence (hereafter, early, middle, and late stages) experienced differential mortality, growth, gas bubble disease, or seawater tolerance. Each life stage was exposed for 49 d (early stage), 28 d (middle stage), or 15 d (late stage) beginning at 13, 34, and 47 d posthatch, respectively, through emergence. Mortality for all stages was estimated to be 8% (95% confidence interval [CI] = 4–12%) when dissolved gas levels were less than 117% TDG. Mortality increased as dissolved gas levels rose above 117% TDG; the lethal concentration producing 50% mortality was 128.7% TDG (95% CI = 127.2–130.3% TDG) in the early and middle stages. There was no evidence that gas exposure affected growth. The proportion of fish with gas bubble disease increased with increasing gas concentrations, and bubbles occurred most commonly in the nares and gastrointestinal tract. Early stage fish exhibited higher ratios of filamental to lamellar gill chloride cells than late-stage fish, and these ratios increased and decreased for early and late-stage fish, respectively, as gas levels increased; however, there were no significant differences in mortality between life stages after 96 h in seawater. The study results suggest that water quality guidelines for dissolved gas (≤105% TDG) offer a conservative level of protection to Chum Salmon alevins incubating in gravel habitat downstream of Bonneville Dam.

Gas supersaturation generated by spill from hydroelectric dams on the Columbia River was first acknowledged as an environmental concern in the 1960s, when total dissolved gas (TDG) levels in surface waters were found to be as high as 143% of air saturation (Beiningen and Ebel 1970). Symptoms of gas bubble disease in dead or moribund juvenile and adult salmonids *Oncorhynchus* spp. were documented, including exophthalmia (i.e., popeye), emboli in the gill blood vessels, and bubbles or blisters under the skin on the head, in the lining of the mouth, along the lateral line, and between the fin rays (Ebel 1969, 1971; Beiningen and Ebel 1970; Raymond 1970). These findings prompted considerable research on the effects of gas

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supersaturation on salmonids, with a focus on migrating salmonid smolts (Coutant 1970; Ebel 1971; Cramer and McIntyre 1975; Dawley and Ebel 1975; Blahm et al. 1976; Ebel and Raymond 1976; Nebeker and Brett 1976; Ebel et al. 1979); today, we have a relatively good understanding that salmonid smolts are minimally affected by dissolved gas levels between 110% and 120% TDG (reviewed by NOAA 1995, 2000). Consequently, the U.S. Environmental Protection Agency adopted a nationwide standard of 110% TDG for the protection of aquatic life (USEPA 1973). However, during the spring smolt out-migration season in the Columbia River, the U.S. Environmental Protection Agency grants waivers that allow up to 115% or 120% TDG in hydroelectric dam tailraces, depending on mixing of spillway and powerhouse discharge (NOAA 1995).

Salmonids spawning in the tailraces of hydroelectric dams represent a special case of gas exposure wherein developing embryos (i.e., alevins) in the gravel environment are potentially exposed to elevated dissolved gas at a sensitive life stage (McGrath et al. 2006; Arntzen et al. 2009a). Unlike migrating smolts, which can move within the water column to avoid or mitigate the effects of elevated dissolved gas (Meekin and Turner 1974; Dawley et al. 1976; Stevens et al. 1980), alevins that are developing within the gravel are not able to easily move to avoid elevated levels of dissolved gas. In addition, gas bubble disease manifests itself differently in developing alevins than in smolt-size fish, and sensitivity to supersaturation varies among salmonid species (reviewed by Weitkamp and Katz 1980). For example, Sockeye Salmon *O. nerka* alevins experienced gas bubble disease and mortality at concentrations of 108–110% TDG (Harvey and Cooper 1962), whereas Sockeye Salmon smolts were able to tolerate gas levels of 110% TDG with no mortality (Nebeker and Brett 1976). Rucker (1975) observed that fingerling-size Coho Salmon *O. kisutch* were more susceptible to gas bubble disease than smaller fish of approximately the same age. Wood (1979) recommended that Pacific salmon (species not indicated) in hatcheries should not be exposed to dissolved gas levels greater than 104% TDG at the alevin stage but that gas exposure of fingerlings could go as high as 112% TDG. Krise and Herman (1989) found intracranial hemorrhaging and subcutaneous bubbles in Lake Trout *Salvelinus namaycush* alevins after a 15-d exposure to 101% TDG; visible bubbles (intra-orbital, head, and abdomen) were observed after a 40-d exposure to 105% TDG. Montgomery and Becker (1980) noted gas bubbles and mortality of Rainbow Trout *O. mykiss* alevins at 113% TDG in hatchery troughs. Collectively, these studies would suggest that salmonid alevins are more susceptible to dissolved gas than are smolt-size fish.

The Chum Salmon *O. keta* is one Pacific salmon species for which little information exists on the tolerance of alevins to gas exposure. Columbia River adult Chum Salmon spawn in dam tailrace habitat that is affected by elevated dissolved gas caused by spilling water at Bonneville Dam, a large hydroelectric dam at river kilometer 235 on the lower Columbia River. These fish collectively represent one of two remaining populations of the lower Columbia River evolutionarily significant unit of Chum Salmon listed under the Endangered Species Act of 1973 (NOAA 2004). Spill operations at Bonneville Dam are designed to move migrating salmon smolts downstream to the Pacific Ocean during a time when pre-emergent Chum Salmon alevins are still present in the gravel in spawning areas downstream of the dam. To provide protection for pre-emergent Chum Salmon alevins, managers use a guideline of limiting dissolved gas levels downstream of Bonneville Dam to 105% TDG after allowing for depth compensation; water depth is part of the management criterion because with each approximately 1-m increase in depth, gas solubility increases by approximately 10% (Colt 1984). In other words, the dissolved gas level of the river within the spawning areas could be as high as 115% TDG at the surface and still provide protection to Chum Salmon alevins, assuming 1 m of water depth over the redds. During periods of low river flow, however, depth compensation can occur in only the deeper areas of the riverbed, and dissolved gas concentrations at the locations of pre-emergent Chum Salmon alevins in shallower spawning habitat may be higher than 105% TDG (Arntzen et al. 2007, 2008, 2009b).

Whether current management guidelines for dissolved gas are protective of Columbia River Chum Salmon alevins is unknown because very little research on the effects of elevated dissolved gas has been conducted with this species (but see Birtwell et al. 2001). In one of the few studies on Chum Salmon alevins, symptoms of gas bubble disease were noted at around 119–121% TDG, and mortality occurred at around 125% TDG (Hand et al. 2008, 2009). However, sample sizes in that study were small and the results were equivocal. Chum Salmon alevins that were exposed to 113% TDG emerged earlier and were smaller than untreated fish, but differences were small and were not consistently observed in other groups exposed to the same gas levels (Hand et al. 2008, 2009). Subtle differences in the size of Chum Salmon alevins at emergence could be important to their survival because small fish may be more prone to predation in estuarine habitat or may be less able to transition to exogenous feeding, which could reduce growth. Dissolved gas concentrations of around 108% TDG also appeared to cause epithelial injury (i.e., cell hypertrophy and separation) to the gills of developing Chum Salmon alevins (Hand et al. 2008); this could be particularly problematic if it leads to impaired respiratory gas transfer because Chum Salmon often spawn in groundwater areas where dissolved oxygen concentrations are less than fully saturated (Arntzen et al. 2009a). It is not known whether epithelial injury to gill tissue as observed by Hand et al. (2008) would affect osmoregulation; the TDG concentration causing epithelial injury could directly or indirectly affect osmoregulation by altering the abundance and distribution of both lamellar and filamental chloride cells, which function as sites of ion uptake for Chum Salmon in freshwater (Uchida et al. 1996; Shikano and Fujio 1998). Moreover, epithelial cell injury from elevated dissolved gas exposure occurring at or near emergence may cause impaired respiration, leading to compensatory responses

that may indirectly affect preparatory changes for seawater entry and increase the likelihood of delay in the onset of seawater tolerance. If this leads to delay in the onset of seawater tolerance.

The objective of this study was to document the occurrence of gas bubble disease in Chum Salmon alevins of varying developmental stages at dissolved gas levels ranging up to 130% TDG. We studied whether Chum Salmon alevins exposed to increasing dissolved gas levels at three development periods between hatch and emergence suffered differential mortality, growth, gas bubble disease, or seawater tolerance. Three developmental stages were studied to determine whether susceptibility to increased dissolved gas levels was influenced by early ontogeny. Organ structure and function differ notably between posthatch alevins and pre-emergent fry, particularly for gas exchange (Wells and Pinder 1996a, 1996b), and such differences could potentially influence the form and degree of response to elevated dissolved gas. The goal of this study was to determine whether existing water quality criteria are protective of Chum Salmon alevins developing in gravel habitat downstream of Bonneville Dam during the period when water is spilled to promote downstream migration of salmonid smolts.

METHODS

Incubation of eggs and embryos.—Eyed eggs ($n = 9{,}000$) of Chum Salmon were obtained from the Minter Creek Hatchery (Washington Department of Fish and Wildlife, Gig Harbor) and transferred to the Pacific Northwest National Laboratory's (PNNL) Aquatic Research Laboratory on January 7, 2011. The eggs had been fertilized on November 29, 2010, and incubated at a mean water temperature of 9.7°C. The accumulated thermal units (ATU) at the time of transfer equaled 378. Prior to transport, eggs were wrapped in damp burlap and were placed in wooden mesh trays inside a cooler with ice packs to maintain a temperature of 7–10°C. Upon arrival at the laboratory, the eggs were disinfected in 100-μL/L iodophor for 30 min and were then divided into four vertical-flow incubator trays (MariSource, Fife, Washington) that were supplied with a mix of river water and well water at a temperature of 10°C.

Four days after transfer, the eggs were distributed into 72 egg cups. The egg cups were constructed from polyvinyl chloride (PVC) pipe (diameter = 76 mm; height = 50 mm), with nylon screen attached (by use of silicone caulk) to the bottom of the cup and to the top of the removable lid. Each cup was assigned to a dissolved gas treatment level (100, 105, 115, 120, 125, or 130% TDG), a life stage (early, middle, or late), and a replicate that either was used to determine survival from hatch to emergence (cups 1, 2, or 3) or was used for sampling to measure growth from 50% hatch to 50% emergence (cup 4). Fifty eggs were counted and placed in each cup used for survival analysis, whereas 90 eggs were counted and placed in the cups used for growth sampling. After eggs were distributed among the cups, the egg cups were placed into two vertical-flow incubators (50-L round tanks) and were held at an initial temperature of 10°C and a flow rate of 20 L/min until placement in the supersaturated gas system.

Supersaturated gas system and treatment design.—Chum Salmon alevins were exposed to the six dissolved gas levels by using a treatment system that consisted of two water supplies (i.e., saturated water and supersaturated water), six 50-L vertical-flow incubators, and an integrated dissolved gas sensing and regulating platform. A dissolved gas level of approximately 140% TDG was generated with a gas supersaturation column (Specific Mechanical Systems Ltd., Victoria, British Columbia) that consisted of a 0.25-m^3 stainless-steel column with inflow lines for both compressed air and water. Water and compressed air were injected at the top of the column, while a water-level float switch and a gate valve at the bottom of the tank were used to control the air-to-water volume inside the tank, the flow rate, and the internal tank pressure.

Saturated river water (~100% TDG) was mixed with the gassed water at each incubator to achieve the desired dissolved gas treatment level. The incubators consisted of 50-L, circular, cone-bottom tanks with a perforated aluminum plate near the bottom to hold the egg cups and to allow for uniform flow through the outlet. Mixed water was introduced just below the surface by using solenoid valves (Irritrol Ultra Flow 700-1; Irritrol Systems, Riverside, California) to control the flow of supersaturated water and saturated water. The dissolved gas levels in each incubator were monitored by a sensor (Model T507; In-Situ, Inc., Fort Collins, Colorado) and were maintained by a barometric pressure sensor (Model CS100; Campbell Scientific, Inc., Logan, Utah), data logger (Model CR1000; Campbell Scientific), and controller (Model SDM-CD16AC; Campbell Scientific) to within a range of ±4.8 mm Hg, which represents an SD less than or equal to 0.25% of the mean depth-compensated target TDG. The dissolved gas sensors were calibrated (Fluke 719-30G; Fluke Corporation, Everett, Washington) to an accuracy of ±2 mm Hg over the range of 400–1,400 mm Hg. A computer program (written in CRBasic and implemented via LoggerNet; Campbell Scientific) operated two solenoid valves for each incubator. Temperature for the incubators was controlled by mixing ambient and chilled river water with well water. Over the study period, the mean water temperature ± SD was 7.7 ± 0.6°C (temperature range = 6.3–9.2°C).

Each of the three development stages of Chum Salmon alevins was exposed to the six treatment levels of dissolved gas over a period of 49 d (early stage), 28 d (middle stage), or 15 d (late stage). Exposure of the early stage alevins began at 13 d posthatch (50% hatch of all alevins occurred on January 19, 2011), whereas exposure for the middle- and late-stage groups was initiated at 34 and 47 d posthatch, respectively. Each life stage was held under its assigned treatment conditions until the predicted date of emergence. The predicted date of emergence was determined from previous research (Hand et al. 2008, 2009) showing that volitional emergence of Minter Creek Chum

Salmon fry from egg tubes occurred at approximately 932 ATU. Based on a mean incubation temperature ± SD of 7.7 ± 0.84°C during the study, the predicted date for 50% emergence was March 22, 2011 (62 d posthatch). At that time, the dissolved gas level in each test incubator was returned to 100% TDG, and the remaining alevins were either euthanized or held at saturation for an additional 3 d prior to the seawater challenge.

Survival and growth.—During the period of exposure, the egg cups were checked daily for mortality. Dead eggs and alevins were removed, recorded, and externally examined for signs of gas bubble formation. The mortality from initial exposure to the estimated date of emergence (March 22, 2011) was totaled and expressed as a proportion (cups 1–3 only). A mild outbreak of the fungus *Saprolegnia* occurred in the exposure system, which affected mortality primarily in the fish from the middle and late stages. Therefore, individual fish that were noted to be affected by fungus were removed and recorded as mortalities separate from the mortalities due to gas bubble disease and are not reported here. A segmented regression of two simple linear models connected by a join point (spline model) was fitted to the proportional mortality of each development stage as a function of dissolved gas. The slope of the first linear model was constrained to be zero. The spline model was

$$y = \begin{cases} \hat{\alpha} & \text{for } x < x_0 \\ \hat{\alpha} + \hat{\beta}\,(x - x_0) & \text{for } x \geq x_0 \end{cases},$$

where $\hat{\alpha}$ is the intercept, x_0 is the join point, and $\hat{\beta}$ is the slope of the second linear model. The spline models were used to estimate the lethal concentration of dissolved gas that produced 50% mortality (i.e., LC50) for each life stage.

Alevins were sampled for growth at three points during the course of the study: on the date of 50% hatch, immediately prior to exposure, and on the predicted date of 50% emergence. Alevins were removed ($n = 15$ from each life stage and treatment), euthanized in tricaine methanesulfonate (MS-222), blotted dry, weighed, measured for FL, and then preserved in a 10% solution of neutral buffered formalin (NBF). After at least 80 d in the NBF (Heming and Preston 1981), the preserved samples were dissected to measure the dry weight of body tissue separated from the yolk. The dissected body tissues were pooled into separate aluminum weigh boats (4.5-cm diameter), were dried in an oven at 60°C for 48 h, and were weighed (±1 mg). To determine differences in growth rates among treatments, the differences between wet weights and FLs within each life stage at initial exposure and at emergence were regressed against dissolved gas level. A similar analysis of the change in dry tissue weight between hatch and emergence was performed.

Gas bubble disease.—Alevins from each life stage and treatment ($n = 10$) were sampled at 48 h postexposure and on the predicted date of 50% emergence for gross histopathology to determine the incidence of gas bubble disease. Alevins euthanized with MS-222 were examined under stereomicroscopes by four observers who looked for external signs of gas bubble disease (bubbles in the nares, mouth, gills, fins, yolk sac, and eyes); the alevins were then necropsied to identify internal signs of gas bubble formation (gastrointestinal tract and kidney). Bubbles in each of the examined areas were recorded as either mild (<50% of surface area), severe (>50% of surface area), or not present. For each treatment, we calculated the binomial probability (P) of observing at least as many of the tested fish (x out of n exposed fish) that showed bubbles in any of the combined 11 body locations examined:

$$P\,(X \geq x) = \sum_{k=x}^{n} \binom{n}{k} p^k\,(1 - p)^{n-k}.$$

The estimated "natural" probability of observing bubbles in the body (p) was either the control proportion showing bubbles for each test group or the minimum of all the observed control proportions greater than zero. Significance was determined with a Bonferroni correction for the number of comparisons conducted in each test group (i.e., $P < \alpha/5$, where $\alpha = 0.05$ and 5 is the number of treatments [$n = 6$] minus the control). The binomial probability tests the null hypothesis that the number of exposed fish with observed bubbles is not greater than would be expected by chance alone.

Five fish sampled from each treatment group on the date of 50% emergence were x-rayed in an effort to quantify internal gas bubble formation. Images were acquired by using an x-ray tube with a molybdenum anode (Comet MCD100H-1x) and a detector (RadIcon Shad-o-box 4k) placed approximately 2 m from the source. Sampled fish were mounted vertically on a 50-cm^2 Plexiglas plate by simple adhesion and were placed 2–4 cm from the detector. The x-ray images were analyzed by three individuals separately using an image processing program (ImageJ; National Institutes of Health) to determine the ratio of total bubble area to fish body area. The surface area of each fish was calculated by using ImageJ to trace the perimeter of the fish. The surface area for each bubble was measured by tracing each observable bubble separately and then summing all of the areas to obtain a total bubble area for that fish. Bubbles in the fish were identified as light-gray or white circular shapes in the x-ray image. The swim bladder and other internal organs were also identified in all images and were not included in the bubble measurements. Any fish with major discrepancies (measurements with an SD > 0.1) either in bubble area or total fish body area among the three observers were then remeasured by all three observers.

The consistency in measuring the total bubble area for each fish was tested with a general linear model that included the main effects of life stage, dissolved gas level, and observer and the interaction of life stage and dissolved gas level. The average total bubble area measured by an observer was then divided by the given average fish surface area to produce the bubble area : body area ratio. For each life stage, the bubble area : body area

ratio was fitted with a sigmoidal dose–response model using $\log_{10}$(TDG). We used *F*-tests and residual plots to compare models with separate and common parameter values.

Seawater tolerance.—Chloride cell distribution and abundance on gill tissue were determined for a subsample of alevins that were sampled prior to the seawater challenge. Ten alevins from each life stage and treatment were euthanized in MS-222, fixed for 2 h in a modified Bouin's solution (24 mL of ethanol, 12 mL of formalin, 3 mL of glacial acetic acid, and 6 mL of picric acid), and then stored in 90% ethanol. These samples were dissected to remove the second gill arch, which was dehydrated and embedded in paraffin. Two gills representing two fish per treatment were embedded in each block and were oriented to allow sectioning parallel to the gill surface. Sections of 5–8-mm thickness were cut through the whole gill, resulting in four to six slides with 8–16 sections/slide. Sections were arranged on the slides so that the entire thickness of the gill was represented on each slide. One slide for each life stage and treatment was stained with hematoxylin and eosin. Immunocytochemical localization of Na^+,K^+-ATPase in gill chloride cells was performed as described by Nearing et al. (2002). The anti-Na^+,K^+-ATPase antibody α5, which was used to localize immune-positive gill chloride cells, was obtained from the Developmental Studies Hybridoma Bank (Department of Biology, University of Iowa, Iowa City). The relative abundance of gill chloride cells on sagittal gill sections that were stained for Na^+,K^+-ATPase was determined for a minimum of 8 fish (range = 8–10 fish) from two life stages (early and late) and three dissolved gas treatment levels (100, 115, and 120% TDG). Counts were made for up to three gill sections from each fish. Each section was examined to determine the number of primary (filamental) and secondary (lamellar) chloride cells that were present on and between 10 lamellae on each side of a filament. The cell types were distinguished by size and location following Uchida et al. (1996) and Shikano and Fujio (1998). Thus, two counts were made for each gill section. The number of primary cells and secondary cells in each count was then expressed as a ratio, and the mean ± SD of these ratios was determined for each fish. A generalized linear model of the main effects and interaction of life stage and dissolved gas treatment level was used to test the significance of the interaction effect on the chloride cell ratio. For each life stage, we conducted (1) a Dunnett's test comparing the mean cell ratio from dissolved gas treatment levels with the mean control response and (2) a simple linear regression of the $\log_e$(chloride cell ratio) on the dissolved gas level.

After the gill histology sampling, a 96-h seawater challenge was conducted using a recirculating seawater bath, which was created by mixing evaporated sea salt (Instant Ocean; Spectrum Brands, Madison, Wisconsin) with river water in a 300-L fiberglass trough to achieve a salinity of 30‰. The final salinity was determined by using a refractometer (Model ATC-S/Mill-E; ATAGO U.S.A., Inc., Bellevue, Washington). A pressurized heat exchange system consisting of polyethylene tubing laid in a trough of 5°C river water was used to maintain a constant temperature of approximately 8.5°C in the exposure system. Dissolved oxygen at the outlet (pump) end of the seawater trough was checked daily.

The seawater challenge was initiated on March 25, 2011, which was 3 d after the estimated date of 50% emergence. Fifty fish (25 fish/cup; two replicates from each life stage and treatment) were transferred into horizontally perforated PVC pipe (diameter = 76 mm; height = 150 mm) with a screened bottom and were placed randomly throughout the seawater trough. Mortalities were recorded and removed daily. The seawater challenge was terminated on March 29, 2011 (96 h after the fish were transferred to seawater). Percentage mortality data from the two replicates were arcsine transformed to reduce the heterogeneity of the within-class variances. Regression analysis was performed on the transformed data to determine whether mortality in seawater increased with increasing dissolved gas level. Finally, in the analysis of all data, transformations were made (when appropriate) based on the pattern of residuals, nonparametric analyses (Kruskal–Wallis test followed by Dunn's multiple comparison test) were conducted when an assumption could not be met, and Minitab version 16 (Minitab, Inc., State College, Pennsylvania) and Prism version 4 (GraphPad Software, La Jolla, California) were used to conduct the analyses.

RESULTS

Survival and Growth

The three life stages (early, middle, and late) showed a mortality response to increasing dissolved gas levels, with mortality increasing as dissolved gas increased above 115% TDG 1 (Table 1). The baseline mortality between 100% and 117% TDG was estimated to be 8% (95% confidence interval [CI] = 4–12%). The individual spline mortality models for the early (mean LC50 ± 95% CI = 129.7 ± 1.9% TDG) and middle (127.9 ± 1.9% TDG) life stages were not significantly different (P = 0.37) from one another and were thus combined and used to estimate the LC50 for these two groups, even though the maximum proportion mortality in the early life stage was only 0.48. For the early life stage, the mean proportion mortality values for all but the 100% TDG treatment group increased monotonically (Figure 1). Using the common model for the early and middle life stages, the estimated LC50 was 128.7% TDG (95% CI = 127.2–130.3% TDG). The maximum proportion mortality at the highest dissolved gas treatment in the late life stage was only 0.35, and the mean proportion mortality did not monotonically increase with dissolved gas level (Figure 1); therefore, an LC50 was not estimated.

Approximately 20% to nearly 50% of the fish from the early stage died in dissolved gas levels of 120–130% TDG by the end of the study. However, these fish were able to tolerate 120–130% TDG for several days before suffering any mortality and were able to tolerate these levels for 14–44 d before experiencing at least 10% mortality (Figure 2). In contrast, middle- and late-stage fish that were exposed to the three highest gas levels began

TABLE 1. Absolute number of fish that died (n) and the mean (±SE) percentage mortality (%) through 50% emergence (March 22, 2011) for Chum Salmon fry in all subreplicates (i.e., cups 1–3). The absolute number of deaths and the percentage mortality do not include fungus-related mortality and culls. Exposure to total dissolved gas (TDG) began on February 1, 2011, for the early stage fish; February 22, 2011, for the middle-stage fish; and March 7, 2011, for the late-stage fish.

	100% TDG		105% TDG		115% TDG		120% TDG		125% TDG		130% TDG	
Life stage	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Early	8	6.1 (5.1)	2	1.3 (0.7)	11	7.6 (0.4)	29	19.2 (2.8)	68	45.6 (1.5)	69	46.0 (1.5)
Middle	7	5.1 (0.9)	12	10.8 (8.5)	7	4.9 (2.8)	21	14.9 (5.7)	50	33.8 (13.0)	77	60.8 (9.3)
Late	20	17.6 (6.1)	9	7.9 (4.4)	10	7.2 (3.2)	16	11.2 (1.6)	43	30.9 (9.0)	37	26.0 (6.4)

to die within the first day of exposure, and a cumulative mortality of 10% was reached between days 7 and 22 in the middle stage and between days 4 and 14 in the late stage (Figure 2). These results show that 90% of the pre-emergent Chum Salmon fry from the early stage were able to tolerate 120% TDG for about twice as long as fish from the middle stage and almost three times as long as fish from the late stage. A simple linear model with a zero intercept was fitted to the cumulative mortality for each life stage in the 130% TDG treatment divided by the maximum mortality (i.e., 61.1% for the middle stage at 130% TDG). The resulting slopes were significantly different among the early (slope ± SE = 0.017 ± 0.0002), middle (slope = 0.036 ± 0.0004), and late (slope = 0.036 ± 0.0006) life stages (F-test of common slope: $F_{2,92} = 967$; $P < 0.001$) but not between

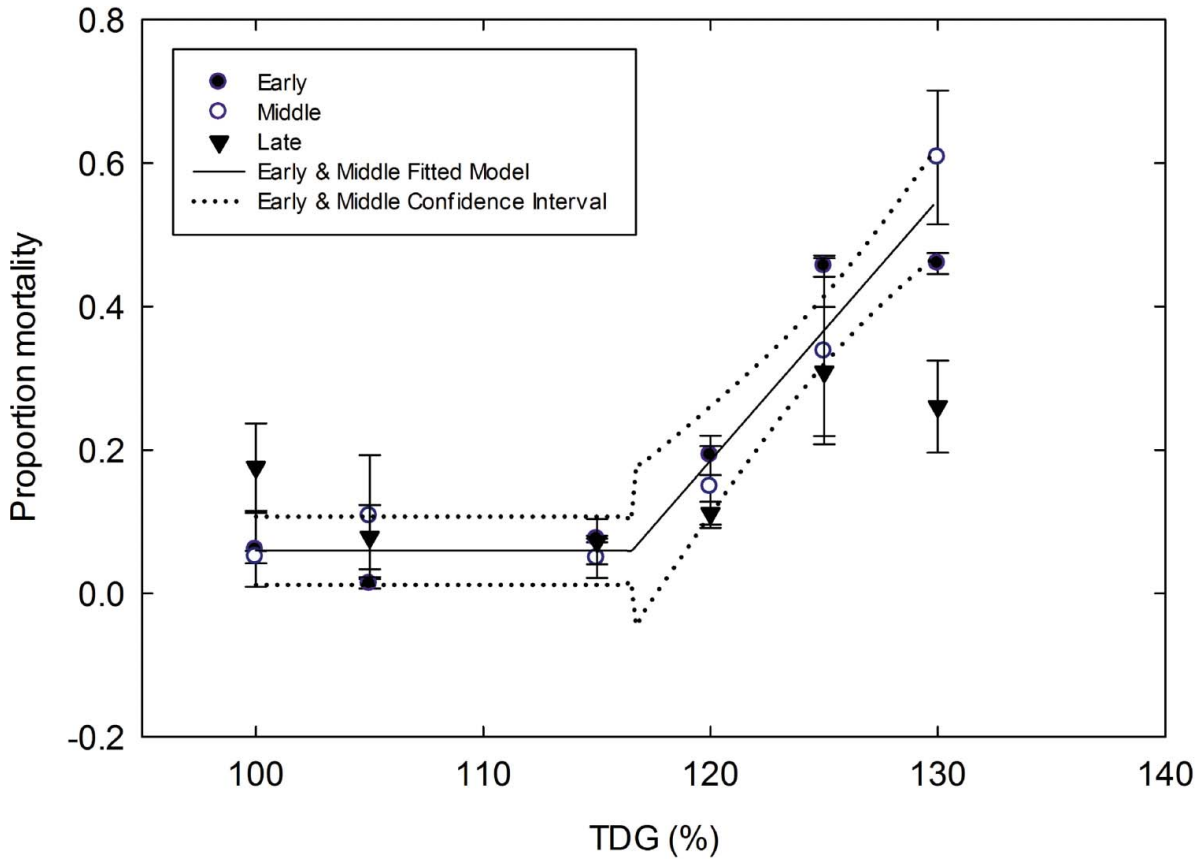

FIGURE 1. Proportional mortality of Chum Salmon fry as a function of the level of total dissolved gas (TDG). Each point represents the mean of the three subreplicates, with the SE indicated by the vertical line. A combined fitted mortality model (solid line) with 95% confidence interval (dotted lines) is shown for the early and middle life stages. [Figure available in color online.]

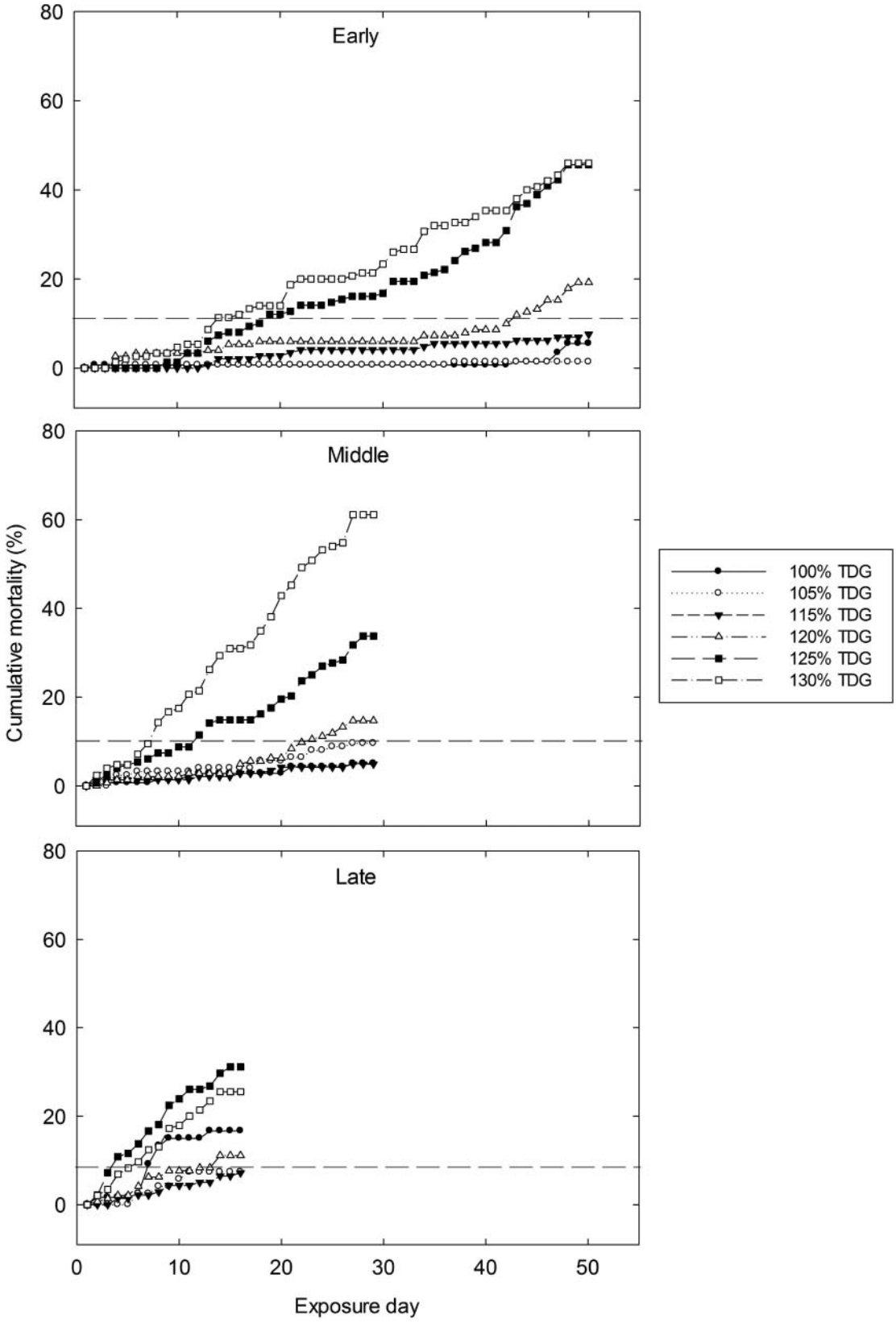

FIGURE 2. Cumulative mortality by exposure day for three Chum Salmon life stages exposed to six total dissolved gas (TDG) treatments. Exposure was initiated on day 1 of the treatment, which varied by life stage (day 1 was February 1, 2011, for the early stage; February 22, 2011, for the middle stage; and March 7, 2011, for the late stage), and continued through emergence (March 22, 2011, for all three stages). For reference, the horizontal dashed line in each panel represents 10% cumulative mortality.

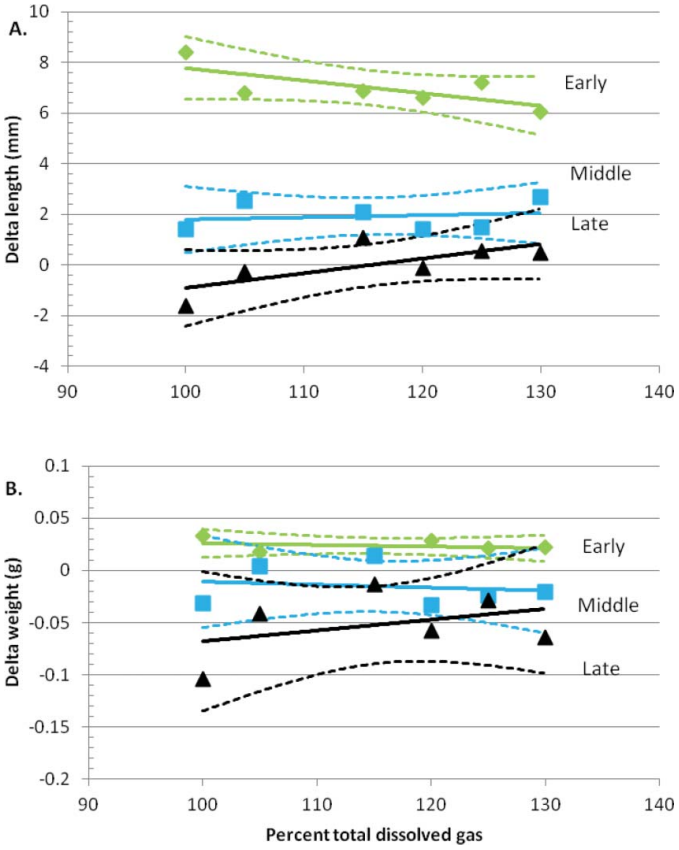

FIGURE 3. (**A**) Change in fork length (delta length) and (**B**) change in wet weight (delta weight) of Chum Salmon alevins sampled immediately prior to dissolved gas exposure and at 50% emergence. Three life stages (early stage = diamonds; middle stage = squares; late stage = triangles) were exposed to six levels of total dissolved gas (100, 105, 115, 120, 125, and 130%). Each point represents a mean of the samples (in most cases, $n = 15$); the solid line represents the linear regression, and the dotted lines represent the 95% confidence interval around the regression. [Figure available in color online.]

the middle and late stages (F-test of common slope: $F_{1,43} = 0.0778$; $P = 0.78$). These results suggest that the middle and late life stages succumb to gas bubble disease at a faster rate than the early life stage.

The growth rate based on the change in FL between initiation of exposure and emergence (delta length, Figure 3A) did not change significantly at any dissolved gas level among the life stages (early stage: mean slope $\pm$ SE $= -0.05 \pm 0.02$, $P = 0.102$; middle stage: slope $= 0.01 \pm 0.02$, $P = 0.748$; late stage: slope $= 0.06 \pm 0.03$, $P = 0.111$). Mean FL ranged from approximately 28 to 37 mm at the initiation of exposure and from 35 to 38 mm at emergence (Table 2 shows values at emergence). Mean wet weight ranged from around 240 to 380 mg at initiation of exposure and from around 260 to 350 mg at emergence (Table 2), and there was no significant change in wet weight (delta weight, Figure 3B) during exposure at any gas level for the early stage (slope $\pm$ SE $= -0.0002 \pm 0.0003$; $P = 0.581$), middle stage (slope $= -0.0003 \pm 0.0008$; $P = 0.747$), or late stage (slope $= 0.0010 \pm 0.0013$; $P = 0.457$). The change in dry tissue weight from hatch to emergence was also not a function of dissolved gas concentration, with values decreasing from approximately 69–83 mg at hatch to 24–54 mg at emergence (Table 2); the slope of the regression line (early stage: slope $\pm$ SE $= 0.35 \pm 0.32$; middle stage: slope $= 0.15 \pm 0.16$; late stage: slope $= -0.20 \pm 0.13$) was not statistically different from zero ($P > 0.21$ for all three life stages).

Gas Bubble Disease

Significantly more bubbles were found in the bodies of fish exposed to increased dissolved gas than in control fish (binomial test with a Bonferroni correction: $P < 0.01$), and the proportion of fish affected with bubbles increased with dissolved gas concentration (Figure 4). The bubble area : body area ratio appeared to increase noticeably when concentrations were at or above 120% TDG (Figure 5). Fish were found to have half the maximum ratio of bubble area to body area at 118% TDG (95% CI = 116–120% TDG) in the middle and late stages and at 120% TDG (95% CI = 119–121%) in the early stage. Gas bubbles were observed in the nares and gastrointestinal tracts of fish immediately after exposure was initiated and also after long-term exposure. Within 48 h of exposure to dissolved gas levels of 115% TDG or higher, 20–40% of the fish examined were found to contain bubbles in the nares; similar results were observed at emergence. Bubbles in the gastrointestinal tract were not obvious in the early stage fish at 48 h postexposure, but the other life stages showed a significant dose–response between gas levels and bubbles in the gastrointestinal tract both at 48 h postexposure and at emergence. More than half of the fish that were exposed to dissolved gas levels of 120% TDG or greater were observed to have gas bubbles in their gastrointestinal tracts. In addition to gas bubbles in the nares and gastrointestinal tract, 50–70% of the early stage fish exposed to gas levels of 115% TDG or higher were found to have gas bubbles in their yolk sacs at 48 h postexposure; none of the fish that were exposed to 100% or 105% TDG were found with gas bubbles in their yolks. By the time these early stage fish reached emergence, however, the bubbles in the yolk sacs had disappeared, and bubbles were not present in the yolk sacs of fish from the other life stages. Only a few fish had bubbles in the gill filaments and eyes, and no fish were found to have bubbles in the kidneys.

Seawater Tolerance

The interaction term between life stage and dissolved gas treatment level in the generalized linear model was significant ($P = 0.005$), so the analysis was conducted by life stage to assess treatment effects on chloride cell ratios. In the early stage fish, the ratio of primary to secondary chloride cells increased significantly with increasing dissolved gas level from 100% to 120% TDG (Figure 6; regression of $\log_{10}$ transformed ratios: error df = 24, $P = 0.006$). Further, the median ratio of primary to secondary chloride cells in fish that were exposed to 120% TDG was significantly greater than the median ratio in fish that were exposed to 100% TDG (Kruskal–Wallis test: $P = 0.031$; Dunn's multiple comparison test: $P < 0.05$). Within the late-stage fish, there was a significant decrease in the ratio of primary to

TABLE 2. Mean (±SD) wet weight (mg) and fork length (FL, mm) of Chum Salmon fry sampled at 50% emergence (March 22, 2011) after exposure to six levels of total dissolved gas (TDG). Also shown are mean dry weights for Chum Salmon fry at emergence (total weight divided by the sample size of 15 fish); variances could not be calculated because the dry weights of individual fish were not determined.

			Wet weight (mg)		FL (mm)		
Life stage	TDG (%)	n[a]	Mean	SD	Mean	SD	Mean dry weight (mg)
Early	100	15	308.3	52.9	36.7	1.7	43.1
	105	15	281.1	38.2	35.7	1.8	40.4
	115	15	281.7	45.1	35.8	1.7	41.6
	120	15	291.7	37.9	35.9	1.7	38.5
	125	15	281.1	27.5	36.1	0.9	39.1
	130	10	259.2	38.2	34.7	1.6	24.4
Middle	100	15	312.3	40.1	37.1	1.3	46.5
	105	15	310.1	53.4	37.0	1.3	44.4
	115	15	258.9	36.6	34.5	1.7	36.5
	120	15	306.1	37.5	37.0	1.5	44.9
	125	15	300.4	40.7	36.8	1.2	44.8
	130	15	273.6	34.4	36.1	1.3	40.4
Late	100	14	272.5	39.3	35.4	1.2	34.8
	105	15	317.3	45.9	36.9	1.2	44.4
	115	15	354.4	44.4	38.2	1.5	53.6
	120	15	299.3	40.8	36.7	1.2	42.9
	125	15	313.7	35.0	37.4	1.5	47.3
	130	15	292.8	35.9	37.1	1.3	43.2

[a]Sample size (n) is listed for wet weight and FL; sample size for dry weights was 15 for all treatments and life stages.

secondary cells in the gills with increasing exposure to dissolved gas levels (regression of $\log_{10}$ transformed ratios: error df = 26, $P = 0.043$; Figure 6). The Kruskal–Wallis comparison of the median responses between treatment levels, however, was only nearly significant ($P = 0.067$). Mortality after the 96-h exposure to 30‰ seawater for each life stage and gas level ranged from 0% to 18% (Table 3), and there was no relationship between dissolved gas level and mortality at any life stage (P-values ranged from 0.213 to 0.828).

DISCUSSION

Chum Salmon fry that were exposed to gas concentrations between 100% and 115% TDG at various posthatch developmental stages survived equally well to emergence, averaging about 92% survival. However, survival rates for all developmental stages decreased significantly when the gas concentration was about 117% TDG or above. In previous studies with this same stock of Chum Salmon, we showed that dissolved gas levels up to 113% TDG resulted in very little mortality, whereas mortality increased once gas levels exceeded 120% TDG (Hand et al. 2008, 2009). Our findings are consistent with observations from studies of smolt-size juveniles of other salmonid species (e.g., Chinook Salmon *O. tshawytscha*, Steelhead [anadromous Rainbow Trout], Coho Salmon, and Sockeye Salmon), which have shown that mortality increases rapidly once dissolved gas levels rise above 115% TDG (Dawley and Ebel 1975; Nebeker and Brett 1976; Nebeker et al. 1979). Although Chum Salmon alevins were as tolerant of elevated dissolved gas levels as

TABLE 3. Absolute number of fish that died (n) and the mean (±SE) percentage mortality (%) after a 96-h exposure to 30‰ seawater for two subreplicates (i.e., cups 1–2) of three life stages of Chum Salmon fry that had been exposed to six total dissolved gas (TDG) treatment levels through emergence. Initial sample size was 25 fry/cup, for a total of 50 fry per dissolved gas level.

	100% TDG		105% TDG		115% TDG		120% TDG		125% TDG		130% TDG	
Life stage	n	%	n	%	n	%	n	%	n	%	n	%
Early	4	8	3	6	4	8	9	18	3	6	3	6
		(0)		(2)		(4)		(2)		(6)		(2)
Middle	0	0	3	6	1	2	3	6	2	4	3	6
		(0)		(6)		(2)		(2)		(0)		(6)
Late	3	6	2	4	0	0	1	2	0	0	4	8
		(2)		(0)		(0)		(2)		(0)		(4)

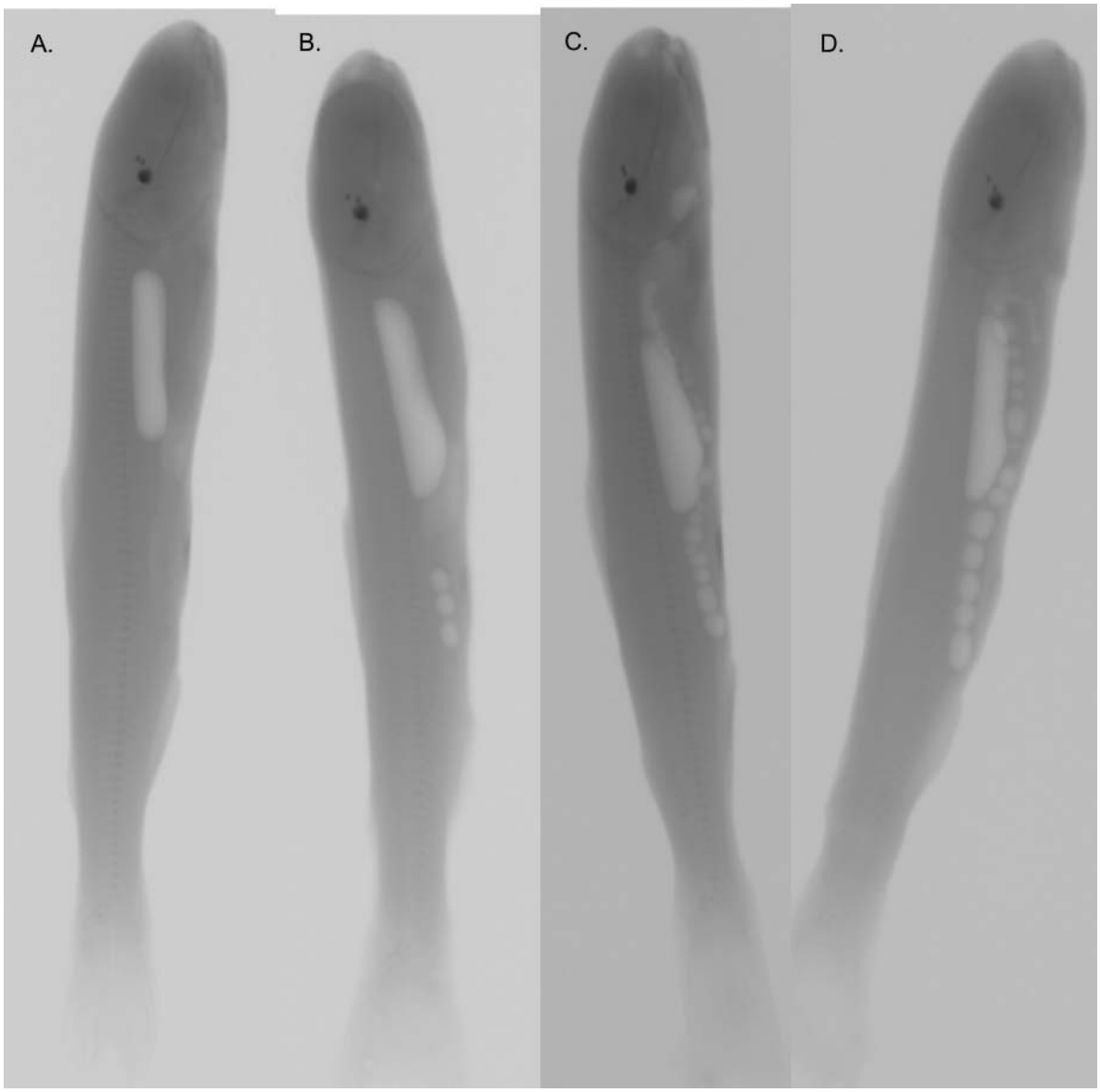

FIGURE 4. Four x-ray images collected from early stage Chum Salmon at the time of emergence. The images are arranged from left to right in order of increasing total dissolved gas (TDG) exposure level: **(A)** control fish (exposed to < 100% TDG), **(B)** fish exposed to 115% TDG, **(C)** fish exposed to 120% TDG, and **(D)** fish exposed to 130% TDG. The large, elongated, light-colored area in the anterior half of the fish is the swim bladder. Gas bubbles in the gastrointestinal tract, gills, and nares appear as smaller, light-colored circles.

smolt-size fish of similar species, we did find that the earliest developmental stage of fish in our study was more tolerant to elevated dissolved gas levels than the middle or late stage; 90% of pre-emergent Chum Salmon fry from the early stage were able to tolerate 120% TDG twice as long as the middle stage and three times as long as the late stage. This finding was consistent with results of other studies on salmonid alevins, which have shown that mortality increased as the fish developed toward emergence (Shirahata 1966; Meekin and Turner 1974; Rucker 1975; Nebeker et al. 1978; Jensen et al. 1986).

One explanation for why the tolerance to elevated gas is greater for posthatch fry than for fry nearing emergence may relate to the physiological development of the alevin respiratory system. As the gills of posthatch alevins develop, the young fish rely on passive diffusion of oxygen through the skin, yolk sac, and other membranous tissue for respiration (Wells and Pinder 1996a, 1996b). This diffusive respiratory process continues until the gills are more developed (i.e., occupy a larger surface area relative to the skin and yolk sac) and able to carry blood and respiratory gases to and from body tissue (Rombough 1999). Once the gills become the primary organ for respiration, the fish may be more susceptible to bubbles from supersaturated gas, which block small capillaries of the gill tissue, thus leading to death by asphyxiation. Counihan et al. (1998) found that White

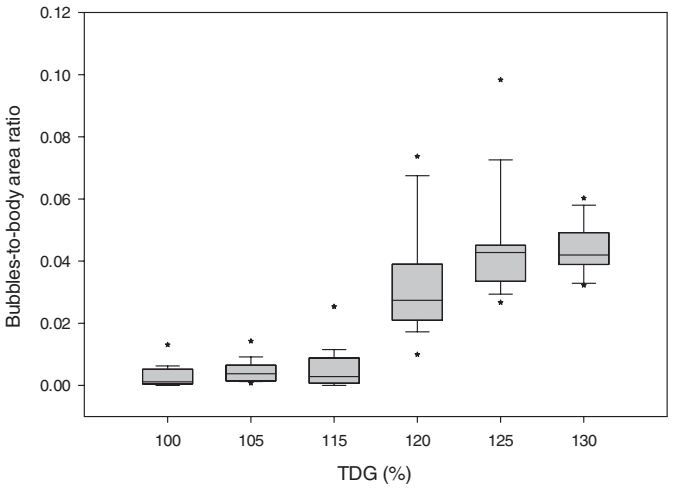

FIGURE 5. Ratio of bubble area to fish body area for five Chum Salmon from each life stage (data pooled for early, middle, and late stages) exposed to six levels of total dissolved gas (TDG) and then sampled at emergence (i.e., termination of exposure). The horizontal line through the middle of each box represents the median; the lower and upper boundaries of the box represent the 25th and 75th percentiles, respectively; the whiskers represent the 10th and 90th percentiles; and asterisks represent the outliers.

Sturgeon *Acipenser transmontanus* larvae became susceptible to gas bubble disease soon after they began gill respiration; those authors proposed that the opening of the buccal cavity might provide sites where gas bubbles could form, followed by rapid diffusion into the branchial system. Egusa (1959) suggested that the elasticity and tenacity of the body wall tissues explained why

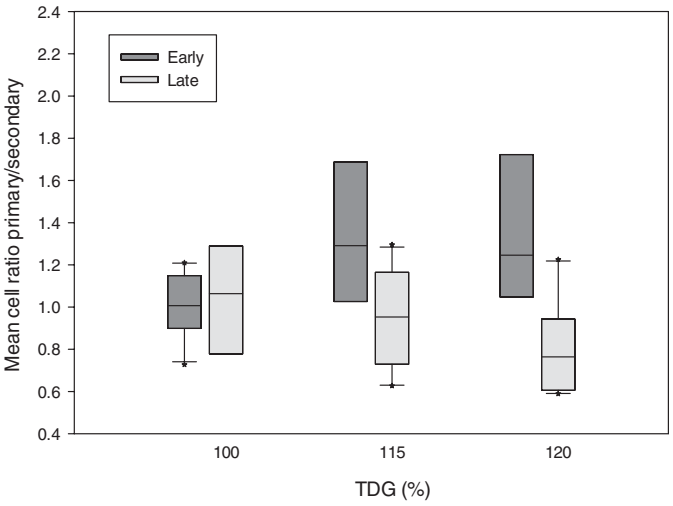

FIGURE 6. Distribution of the mean ratio of primary to secondary chloride cells in gill sections from Chum Salmon fry (early and late stages) exposed to three levels of total dissolved gas (TDG) and then sampled at emergence. The horizontal line through the middle of each box represents the median; the lower and upper boundaries of the box are the 25th and 75th percentiles, respectively; the whiskers represent the 10th and 90th percentiles (for sample sizes > 8); and asterisks represent the outliers.

Japanese Medaka *Oryzias latipes* fry immediately after hatching were more resistant to elevated dissolved gas levels than older fry. Collectively, these results suggest that the time it takes for fish to convert from a passive diffusion process to gill respiration will depend on the species and environmental conditions.

Whether the impermeability of skin to water and ions protects young fish from dissolved nitrogen is unknown, but the skin of young Sea Trout (anadromous Brown Trout *Salmo trutta*) and Atlantic Salmon *Salmo salar* fry immediately after hatch was suspected to be highly impermeable to water and ions, allowing the fry to survive in seawater concentrations that killed older trout that were closer to emergence (Talbot et al. 1982). In addition, the yolk sacs of young alevins could act as a nitrogen sink and protect the fish from bubble formation (Hand et al. 2009). Although we do not know whether this pertains to fish, body fat influences gas bubble formation in humans who have undergone rapid decompression; apparently, the lipids in the fat solubilize nitrogen faster than other tissue (Philp and Gowdey 1964). This concept is highly speculative and should be confirmed in fish.

Dissolved gas levels and exposure time did not result in significantly lower growth rates between the initiation of exposure and emergence for the treated and untreated fish. In fact, the range of FLs at emergence for our treated and untreated fry (35–38 mm) was similar to that reported for nine British Columbia stocks (33–36 mm) held at similar temperatures, although the British Columbia stocks were heavier than our fish (363–430 mg versus 260–350 mg; Beacham and Murray 1987). The development rate of Chum Salmon fry from British Columbia was dependent mostly on incubation temperature, which varied geographically from north to south, but the development rate was also under genetic control through variations in female egg size (Beacham and Murray 1987). The finding that exposure to dissolved gas did not affect growth rates in our study is similar to the results of some other studies. For example, surviving Steelhead showed no difference in growth rates after exposure to dissolved gas levels up to 126% total gas pressure (TGP) for 90 d (Nebeker et al. 1978). There were also no differences in the growth rates of juvenile Lake Trout (TL = 2.9–3.9 cm) that were exposed for 30 d to five gas levels ranging from 101.3% to 122.0% TDG (Krise and Smith 1991). However, Dawley and Ebel (1975) showed that long-term (35-d) exposure of juvenile Chinook Salmon and Steelhead to sublethal levels of dissolved nitrogen and argon (105% to 115% of saturation) resulted in mean weights and lengths that were significantly lower than those of control fish; those authors noted that after 30 d of testing at 115% saturation, the Chinook Salmon fingerlings became lethargic in their feeding response.

Gas bubble disease was more prevalent in treated versus untreated fish, and there was a general increase in gas bubble disease as the dissolved gas level increased. Fish were found to have half the maximum ratio of bubble area to body area at about 118% TDG (95% CI = 116–120% TDG) in the middle and late stages and at about 120% TDG (95% CI = 119–121% TDG) in the early stage. The gastrointestinal tract and the nares

were the most significant locations on the body where gas bubbles formed. Moreover, gas bubble disease also appeared to be developmentally dependent. In early stage fish, bubbles were also found in the yolk sac after the fish had been in the exposure system for 48 h, whereas 48-h postexposure examinations of yolk sacs from middle- and late-stage fish did not reveal significant numbers of bubbles. In extreme cases, gas bubbles have been observed in the gut, mouth, exterior surface, or yolk sac (Rucker and Kangas 1974; Wood 1979), with variable effects on behavior, physiology, and survival. Birtwell et al. (2001) and Harvey and Cooper (1962) observed that the effects of gas bubble trauma included impaired swimming performance and sensory capabilities; affected individuals floated and swam head up or abdomen up. Immediate causes of death from bubble formation in the tissues of these fish might include (1) bubbles in the buccal cavity, leading to suffocation (Fidler 1988); or (2) rupture of the perivitelline membrane (Stroud et al. 1975). Gas bubble disease, as indicated by exophthalmia and by bubbles in the mouth, nares, and the rim of the eye, was present in young Lake Trout at 117% and 122% TGP (Krise and Smith 1991). Lake Trout sac fry were tolerant of dissolved gas levels up to 120.2% TGP; the death rate was not affected through a 40-d study (Krise and Herman 1989). However, signs of gas bubble disease, including a distended abdomen or a hyperinflated swim bladder, were evident during the entire Krise and Herman (1989) study; at the end of that study, many fish were moribund due to the high gas levels but were still alive. Histological lesions "were inconsistent within and between groups, showing no particular patterns other than a general increase in lesions with increasing time of exposure and gas level. Intracranial hemorrhages were the only lesions that were potentially life threatening" (Krise and Herman 1989: p. 271). We have previously noted the inconsistent histological lesions relative to gas levels in Chum Salmon fry (Hand et al. 2008, 2009).

We examined seawater tolerance by using the abundance and distribution of chloride cells on gill tissue because a change in chloride cell ratios from gill filaments and lamellae represents the combined change that is known to occur as anadromous fish transition from freshwater to seawater (Foskett and Scheffey 1982; Richman et al. 1987; McCormick 1995). Within chloride cells, the enzyme Na^+,K^+-ATPase is the major molecule that is responsible for this function; in general, chloride cells in freshwater-adapted fish are found on both gill filaments and lamellae, whereas in seawater-adapted fish the lamellar chloride cells are either absent or greatly reduced in number (Pisam et al. 1987; Uchida et al. 1996; Shikano and Fujio 1998; Evans et al. 2005). More recently, Bystriansky et al. (2006) showed that gill Na^+,K^+-ATPase in salmonids exists as two specific isoforms (α1a and α1b) that are reciprocally expressed in freshwater and seawater; these isoforms are respectively localized to the filamental and lamellar chloride cells of fish in freshwater and are primarily found in the filamental chloride cells of fish in seawater (McCormick et al. 2009). Our findings suggest that early stage fish were potentially more tolerant to seawater than late-stage fish and that the early stage fish were potentially more tolerant to seawater as gas levels increased; an example of a sectioned gill arch from an early stage fish exposed to 115% TDG, which shows a higher number of chloride cells on the primary filament versus the secondary lamellae, is depicted in Figure 7. This trend, however, was not reflected in survival after 96 h in seawater, as we found no significant differences among life stages or treatment groups. The reason for this may relate to the fact that as Chum Salmon adapt to seawater, much of the increase in Na^+,K^+-ATPase activity is associated primarily with the increase in size of Na^+,K^+-ATPase immunopositive chloride cells on the gill filament (Uchida et al. 1996) or the specific chloride cell isoform (Bystriansky et al. 2006); however, neither the size of Na^+,K^+-ATPase immunopositive chloride cells nor the chloride cell isoform was measured in this study. Early and late-stage exposed fry may well have had similar Na^+,K^+-ATPase activity, which could have obviated any observed differences in chloride cell ratios and presumptive importance for seawater adaptation. Moreover, the salinity challenge of 30‰ may have been too low to detect a difference in survival between the two groups. Shikano and Fujio (1998) reported that an increase in the number of immunopositive chloride cells on the gill filaments and a reduction in the number of those cells on the lamellae were both significantly correlated with seawater survival but at salinities ranging from 34‰ to 46‰.

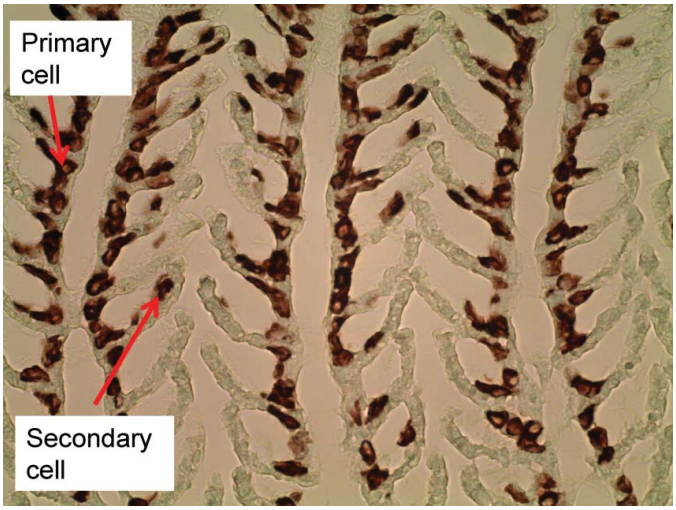

FIGURE 7. Primary chloride cells (positioned on the gill filament) and secondary chloride cells (positioned on the gill lamellae) on a sectioned gill arch from a Chum Salmon fry that was exposed to 115% total dissolved gas during the early life stage and was sampled immediately after emergence (40× magnification). [Figure available in color online.]

These contrasting results are not entirely unexpected because earlier studies of the effects of increased dissolved gas levels on the survival of salmonids transferred to seawater produced similarly equivocal results. Dawley et al. (1976) reported that fall Chinook Salmon that were transferred to 25‰ seawater after 127 d of exposure to dissolved gas levels up to 120% TDG suffered a total mortality of 98%, whereas Steelhead experienced

mortality of up to 16%. In both cases, the surviving fish were significantly larger than those that died. Dawley et al. (1976) concluded that much, if not most, of the observed mortality was more likely related to the fish not achieving the threshold size for seawater adaptation rather than to any effect from dissolved gas level. Despite the apparent size effect on seawater survival, these results are surprising given that the brackish water salinity (25‰) and time of transfer for Chinook Salmon and Steelhead (July and June, respectively) are near optimal for the direct transfer of these two species into seawater, particularly for the Steelhead, which were about 180 mm in length. However, water temperature during the tests was substantially lower than normal conditions in the Columbia River during migration of these fish groups and may have inhibited the typical physiological smoltification processes prior to seawater transition (E. Dawley, National Oceanic and Atmospheric Administration [retired], personal communication). By contrast, Bouck et al. (1976) observed no mortality in spring Chinook Salmon, Sockeye Salmon, and Steelhead smolts that were held in 30‰ seawater for 5 d after a 48-h exposure to 120% TDG. The results of Bouck et al. (1976) are similar to ours (mortality = 0–18%) and reflect the fact that exposure to high dissolved gas levels does not seem to impair osmoregulation in salmonids when they are at the appropriate size and time for seawater entry.

In general, there were few problems during our study. Dissolved gas levels remained constant throughout the study. We believe that the system we used for dissolved gas exposure of fry in this study was more representative than the system we used in previous years (e.g., Hand et al. 2008, 2009). As such, we believe that the mortality rates in the present study are more representative than our previous results. The exception was that in mid-March, we documented an outbreak of bacterial gill disease in Chum Salmon fry held as reserves in incubators that were kept separate from the exposure system. Prior to the diagnosis of bacterial gill disease, some of these reserve fish were used in the middle and late stages in our study. Although we did not test for bacterial gill disease in the fish within the exposure system, there was an increase in mortalities among the early and late-stage control fish in the exposure system during mid-March, and we cannot entirely attribute this mortality increase to gas bubble disease. It is possible that some of these fish died from undetected *Saprolegnia* infection, undetected bacterial gill disease, or both; these unexplained mortalities were included in the mortalities reported here.

Management Implications

The results from this study support the existing management guideline that limits depth-compensated dissolved gas levels to 105% TDG at Chum Salmon spawning locations. This guideline appears to provide a conservative level of protection to Chum Salmon sac fry incubating in the gravel downstream of Bonneville Dam. Our results would suggest that exposure to elevated dissolved gas levels early in the development period would be better than exposure occurring later. In general, as salmon develop from yolk sac fry to alevins, they tend to become more susceptible to mortality from asphyxiation due to gas bubbles that form in developing gill filaments; yolk sac fry at earlier stages of development may be able to tolerate higher dissolved gas levels because their respiration is diffusive, primarily through the skin. We do not know exactly when this susceptibility to asphyxiation begins to increase, but some general thresholds can be seen within the cumulative mortality of the early stage fish (Figure 2, top panel). During the period between hatch and 25 d posthatch (exposure day 12), early stage fish were generally tolerant of all gas levels (i.e., cumulative mortality was less than 10%). Between 25 and 53 d posthatch (exposure days 12–40), the relative mortality varied, as gas concentrations of 125% and 130% TDG were increasingly lethal and mortality at dissolved gas levels of 120% TDG and below was relatively low and stable. Between 53 and 61 d posthatch (exposure days 40–50), when emergence was estimated to occur, mortality rates increased substantially in the 120% TDG group but stayed low in the groups that were exposed to levels less than 120% TDG. These observations suggest that in the early stage fish, there were at least two distinct mortality thresholds that were a function of exposure time and dissolved gas concentration: one threshold occurred at about 25 d posthatch in all exposure groups (exposure day 12; February 12, 2011), and another threshold occurred at about 53 d posthatch in the 120% TDG group (exposure day 40; March 12, 2011).

Whether the thresholds in this laboratory study are transferable to the field is uncertain. Chum Salmon emergence in spawning areas of the Columbia River downstream of Bonneville Dam is spatially and temporally variable and is a function of hyporheic water temperature (Murray et al. 2011). In a case study conducted from 2009 to 2010, Murray et al. (2011) showed that emergence could begin as early as February 10 and could extend all the way to April 9 (50% emergence was estimated to be March 25). Thus, there is more than likely a range of developmental stages of Chum Salmon fry present in the spawning areas at any given time, and there are likely few windows of time during which gas levels above 117% TDG will not result in mortality for at least a portion of the alevins present. Critically, however, for alevins and fry that survive exposure to dissolved gas above this level, the latent effects on seawater tolerance and estuarine survival are likely to be negligible, irrespective of the stage of development at which exposure occurs. Early and late-stage fish that were exposed to dissolved gas up to 120% TDG survived equally well upon direct entry into 30‰ seawater, despite the variability in abundance and distribution of gill chloride cells, which suggested potential differences in seawater tolerance between the groups. After their emergence from the gravel, Chum Salmon fry are highly dependent on estuaries to forage and grow, and environmental effects that delay entry or extend estuarine residence time have the potential to reduce subsequent survival. Our results indicate that the current depth-compensated guideline of 105% TDG for Chum Salmon spawning areas provides sufficient protection to limit such losses.

Monitoring of dissolved gas in the Chum Salmon spawning areas downstream of Bonneville Dam revealed that depth-compensated dissolved gas levels exceeded the 105% TDG management guideline when water levels were low (up to 7% of the incubation period in 2007 and up to 4% of the incubation period in 2008; Arntzen et al. 2007, 2008, 2009b). The relative risk to the Chum Salmon population in these spawning areas can be determined through knowledge of Chum Salmon redd elevations, the hyporheic dissolved gas levels, and the river surface elevation. The elevation of Chum Salmon redds within the tailwater spawning area can be variable, and shallow redds are not always protected from elevated dissolved gas because water depths cannot provide adequate compensation. Further, due to groundwater interactions within the hyporheic zone, the surface water concentrations of dissolved gas are not representative of the dissolved gas level experienced by the alevins incubating in the gravel. These findings suggest that Chum Salmon redd location, surface water depth within the spawning areas, and dissolved gas at the depth of an egg pocket should be monitored during the period before Chum Salmon emergence in order to more fully understand the impacts of dissolved gas on Chum Salmon alevins incubating in the gravel downstream of Bonneville Dam.

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