Gas Bubble Trauma Monitoring and Research of Juvenile Salmonids

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Gas Bubble Trauma Monitoring and Research of Juvenile Salmonids

1995 Annual Report

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EXECUTIVE SUMMARY

In 1995, the Columbia River Research Laboratory (CRRL) began research and monitoring of gas bubble trauma (GBT) in migrating juvenile salmonids in the Snake and Columbia rivers. The following report describes our first year of laboratory and field studies which are covered under Objective 6, Tasks 6.1 and 6.2. of the 1995 Statement of Work for Assessment of Smolt Condition project. A separate report will be issued describing our 1995 activities on the other tasks in the project . In 1996, Bonneville Power Administration created a new project entitled “Gas Bubble Disease Monitoring and Research of Juvenile Salmonids” (BPA No. 9602100). Future CRRL reports on the topic of GBD research and monitoring will be submitted under that project.

This report is composed of two chapters. The first chapter describes laboratory studies designed to chart the progression of GBT signs leading to mortality and the use of those signs for non-lethal assessment of GBT. First, we assessed the progression and quantified the severity of signs of GBT in juvenile salmonids exposed to different levels of total dissolved gas (TDG) and temperatures. Next, we evaluated prevalence, severity, and individual variation of GBT signs in an attempt to relate them to the likelihood of mortality. Finally, we developed and evaluated methods for a non-lethal examination of gills in fish exposed to high TDG (reported in Chapter 1, Appendix A). These studies are continuing and additional experiments using different temperatures and species will be conducted. Primary findings from the 1995 laboratory studies were:

- No single sign of GBT that we investigated was clearly correlated with mortality; but many signs of GBT become progressively worse over time.
- Understanding both prevalence and severity of GBT signs in several tissues is necessary to account for exposure history, individual variation, and possible mortality.
- Bubbles in the lateral line were the earliest sign of GBT, showed a progressive worsening over time and had low inter-individual variation; however, bubbles in lateral line may develop poorly during chronic exposures to high TDG.
- Bubbles in the fins had high prevalence, showed a progressive worsening over time,

and may be a relatively persistent sign of GBT; however, we lack a truly quantitative method for evaluating severity of fin bubbles, and they may not develop during acute exposures to high TDG.

- Bubbles in the gills appear to be the proximate cause of death in fish, and therefore, are extremely relevant; however, these bubbles may only be relevant at high TDG levels, show little progressive change over time, had a high degree of inter-individual variation, may collapse easily, and are difficult to examine and count.

The second chapter describes the results of monitoring juvenile salmonids (chinook salmon and steelhead) for signs of GBT. Emigrating fish were collected at three dams on the Snake River and three dams on the lower Columbia River. The majority of the fish were examined non-lethally for bubbles in their fins and lateral lines; however, a sub-sample of steelhead was killed and their gills were examined. Primary findings from the 1995 GBT monitoring were:

- Few fish had any signs of GBT, but it appeared that prevalence and severity increased as fish migrated downstream.

- There was no apparent correlation between GBT signs in the fins, lateral line or gills.

- Prevalence and severity of GBT in migrating fish was suggestive of long term, non-lethal exposure to relatively low level gas supersaturated water (112%), as seen in the laboratory studies.

- It appeared that GBT was not a threat to migrating juvenile salmonids in 1995.

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CHAPTER 1

Progression and Severity of Gas Bubble Trauma in Juvenile Chinook Salmon and Development of Non-lethal Methods for Trauma Assessment

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Introduction

Until recently, dissolved gas supersaturation (DGS) and its effects on salmonids in the Columbia River system were considered problems that had been solved, largely because an extensive research effort during the mid 1960's-1970's (Ebel et al. 1975; Ebel 1979; Weitkamp and Katz 1980) led to modifications in the physical structure and operation of most dams. However, because of the listing of several Snake River salmonid stocks under the Endangered Species Act and the use of increasing amounts of spill for fish passage, there is now renewed concern about the effects of DGS, particularly sublethal or indirect effects. Advocates for the use of spill argue that it provides a quick and safe journey past dams and thus increases overall survival relative to, for example, turbine passage. However, high spills may also increase levels of DGS to the point where mortality due to gas bubble trauma (GBT) in outmigrating juvenile salmonids may negate any presumed benefits associated with spill.

To help assess the efficacy of spill as a management tool, a program was initiated in 1994 to monitor juvenile salmonids for signs of GBT as they traveled to the ocean. Basically, the program consisted of examining fish collected at dams on the Columbia and Snake rivers for signs of GBT. It was thought that such monitoring would allow continuous assessment of the prevalence and severity of GBT during the outmigration and such information could serve as a basis for management decisions concerning spill. The signs of GBT monitored included bubbles in the lateral line, fins, external body surface, and gills.

One of the problems inherent in such a monitoring program is trying to quantify and ascribe some ecological significance to the severity of GBT signs observed in fish.

Although there are numerous descriptions of GBT signs in salmonids and other fishes (e.g., Dawley and Ebel 1975; Nebeker and Brett 1976; Nebeker et al. 1980; Weitkamp and Katz 1980; Lutz 1995), most such accounts describe signs in moribund or dead fish. Such descriptions, though useful, are really ecologically "too late" when attempting to evaluate signs at a sub-lethal level. There are some ancillary descriptions of the progression of GBT which do indicate the order in which signs usually appear (Meekin and Turner 1974; Dawley and Ebel 1975; Schiewe and Weber 1975). For example, at certain gas levels, it is well established that bubbles first appear in the lateral line, followed by subcutaneous blisters on the body surface or fins. Unfortunately, these accounts often lack explicit detail, do not attempt to quantify the severity of signs, or are at a histological level. Although the histological descriptions of GBT (Machado et al. 1987; Smith 1988; Machado et al. 1989) are quite detailed, they are of little practical use to a monitoring program where the emphasis is on a rapid, non-lethal assessment of GBT. Despite the large amount of research on GBT in fishes, which has primarily examined acute mortality, the development of methods to provide a rapid, quantitative description of the signs of GBT is still lacking. In addition, and perhaps more importantly, the relation of sub-lethal signs to potential mortality is necessary for a full understanding of the effects of GBT on fishes.

Our overall goal in this work was to determine an optimal method for assessing GBT in juvenile salmonids, one that is rapid, non-lethal, and examines relevant signs at a sub-lethal level. By implementing such a method into the GBT monitoring program, we hoped to place the program on a solid biological foundation and make it highly efficacious. To achieve this goal, our objectives were several-fold. First, we assessed the progression and quantified the severity of signs of GBT in juvenile salmonids exposed to different levels of total dissolved gas (TDG) and temperatures. Next, we evaluated prevalence, severity, and individual variation of GBT signs in an attempt to relate them to the likelihood of mortality. Finally, we developed and evaluated methods for a non-lethal examination of gills in fish exposed to high TDG.

Materials and Methods

Spring chinook salmon (Oncorhynchus tshawytscha; age 1+) were used for all trials (average fork length ± SD = 134 ± 12 mm, average mass ± SD = 25 ± 7 g; N = 212) except one at 130% TDG where we used age 2+ fall chinook salmon (average fork length ± SD = 152 ± 15 mm, average mass ± SD = 39 ± 12 g; N = 64). All fish were from the Little White Salmon National Fish Hatchery, Cook, Washington. The fish were transferred to our laboratory and reared in 1400-L, flow-through circular fiberglass tanks receiving well water heated to 12°C. Excess dissolved gas generated by heating the water was dissipated by a packed column. Fish were fed *ad libitum* once daily with commercial feed and held under natural photoperiod.

Experimental System

Supersaturated water was generated by a combination of heating and pumping well water under pressure and injecting atmospheric air. Water at 7°C flowed into a

114-L, circular fiberglass tank where it was then pumped under 38 psi into a single-pass 50-kW heater. A 1-HP air compressor injected atmospheric air at 60 psi directly into the water line entering the pump; a flow meter controlled the rate of air injected and hence the level of TDG we achieved. After leaving the pump, water was heated to 12°C before flowing into a 23-m-long coil of 1.3-cm-diameter garden hose to allow some time under pressure and to minimize turbulence before water entered a 111-L PVC retention tank. The retention tank vented excess bubbles and maintained a constant head pressure as supersaturated water flowed by gravity (7.0 L/min) into three 228-L flow-through circular holding tanks.

Experimental procedure

We assessed the progression of gas bubble trauma in juvenile salmonids at TDG levels of 130%, 120% and 112% in separate experiments. We stocked 75 juvenile salmon into each of the three tanks receiving supersaturated water. The water volume in each tank was 113-L and was 28 cm deep to minimize depth compensation. We used fish in two tanks to monitor the progression of GBT and fish in the third tank to monitor mortality. A fourth group of fish was held in a tank receiving normally saturated water and served as controls. During a trial, we used a TDG meter (Common Sensing, Inc., Clark Fork, ID) to record water quality variables in treatment and control tanks. We monitored barometric pressure, water temperature, total dissolved gas (P_{tot}), partial pressures of oxygen (pO_2) and nitrogen (pN_2), barometric pressure minus P_{tot} (delta P), percent total saturation, and percent saturation of oxygen.

Sampling and Examination

After stocking fish, we sampled 4 fish from each treatment tank at selected time intervals to record the progression of GBT. We sampled fish every hour at 130% and every 24 h at 112%. At 120%, we sampled fish every 12 h during the first day, every 6 h during the second day, and every 2 h up through 60 h. We completed this trial with a final sample at 80 h. Sample periods were based on preliminary experiments and published information on GBT signs and times to mortality. At the beginning and end of each trial, we sampled 10 control fish.

Fish were sampled by rapidly netting them from their tank and placing them in a lethal dose of MS-222 (200 mg/L) buffered to a pH of 7 with an equal amount of sodium bicarbonate. Anaesthetic was prepared in normally saturated water for control fish and supersaturated water for treatment fish. Fish were serially removed from the anaesthetic, weighed and measured, and placed left side up on a moist paper towel. The examination of fish for progression and severity of GBT was divided into two parts, a macro- and microscopic part, and proceeded as follows. First, we scanned for gas bubbles within the lateral line using dissecting scopes (Leica Wild M3 Z) with 8-40x zoom magnification and fiber optic illumination (Leica Lux 1000). We measured the percent of the length of the lateral that was occluded with bubbles using a hand-held micrometer. The micrometer was divided into units of about 0.5 mm and was used to measure the length of the lateral line and the total length of gas bubbles within the lateral line, thus providing the data necessary to derive percent occlusion. In the trial using fall chinook at 130% TDG, gas bubbles in the fins were recorded as present or absent. For all subsequent trials, the assessment of GBT in the fins was changed to include a measure of severity. We estimated the percent surface area of each unpaired fin covered by bubbles and ranked severity as: 0 = no bubbles present; 1 = 1-25% covered; 2 = 26-50% covered; and 3 = > 50% covered. The macroscopic examination of GBT was completed by recording bubbles as present or absent in the eye, opercle, body surface, and paired fins.

For the microscopic examination, the opercle was removed and the first gill arch was excised and placed on a glass slide. The entire gill arch was then covered with a few drops of anaesthetic solution and examined under a compound microscope at 40-100x. We counted the number of gill filaments with intravascular gas emboli in two ways. First, we made a count with filaments still attached to the bony arch. Next, we used a single-edged razor blade and blunt probe to remove the filaments from the arch, spread them in a single layer over the slide, and made a second count. Immediately after excision of the gill arch, we severed the caudal peduncle and collected blood in microcapillary tubes to measure hematocrit and extract plasma. Plasma was stored at -80°C for future analysis. Several personnel were used to conduct the examinations, which usually required about 20 minutes to complete a sample of 8 fish. Experimental trials ended when virtually all fish had been sampled from the two sample tanks.

Data Analysis

Mortality was plotted as a cumulative percentage over time. We fitted a curve through the points by eye and estimated the time to 50% mortality (i.e., the LT_{50}) by extrapolation. Within each time interval, we averaged lateral line and gill data, determined their prevalence and plotted the data over time. For the fins, we plotted average and maximum severity rankings and prevalence over time using data from all fins combined or data from selected fins.

Results

130% TDG

At 130% TDG, we examined a total of 128 fish during two trials. Each trial lasted about 9 h, with spring chinook salmon showing a faster rate of cumulative mortality (Fig. 1). Mortality increased sigmoidally before peaking at about 80% at the end of the trials. By extrapolation, we estimated the time to 50% mortality to be about 6 h. The progression of bubbles in the lateral line differed slightly between the two trials (Fig. 2). For fall chinook, lateral line occlusion increased in a linear fashion, reaching a mean of about 20% after 4.5 h and peaking at about 40% after 8-9 h. For spring chinook, lateral line occlusion increased linearly through 6 h, also averaging about 20% half-way through the trial, but then peaked at about 60% toward the end. Lateral line bubbles were typically rod shaped and often coalesced into long chains. In both trials, the prevalence of lateral line bubbles was 100% for all sample periods. Although inter-individual variation in lateral line occlusion was relatively low, as evidenced by our standard errors, such variability did tend to increase with time.

In the trial using spring chinook, average severity of bubbles in the fins increased gradually during the first 5 h and then rose to a fairly stable peak from 6-8 h (Fig. 3). Typically, severity of bubbles grew progressively worse in all fins except the pectorals. The number of fish with no bubbles in their fins (i.e., a rating of 0) decreased during the

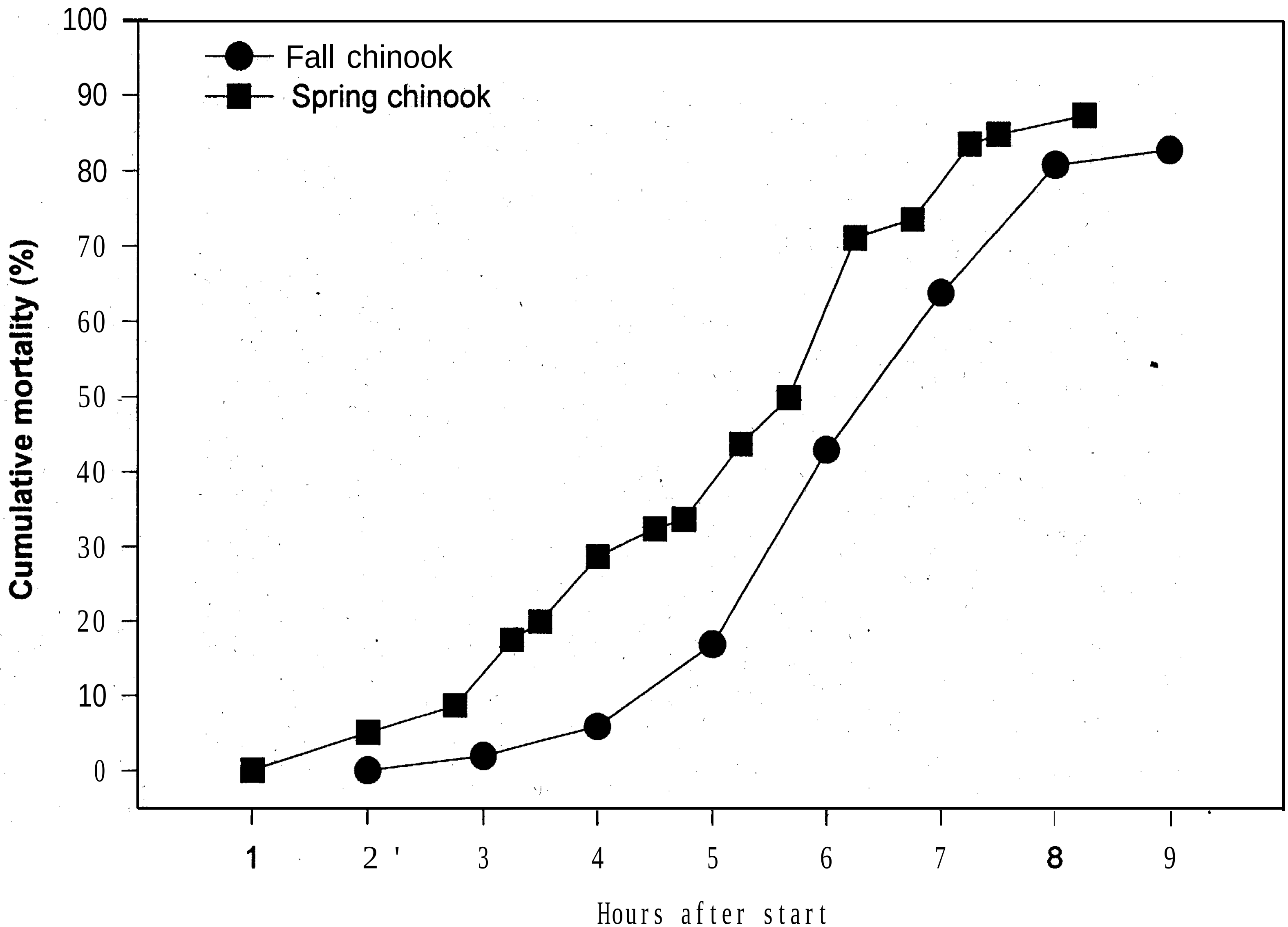


Figure I.-Cumulative mortality of juvenile chinook salmon during exposure to 130% TDG at 12°C. Total number of fish exposed was 75 in each group.

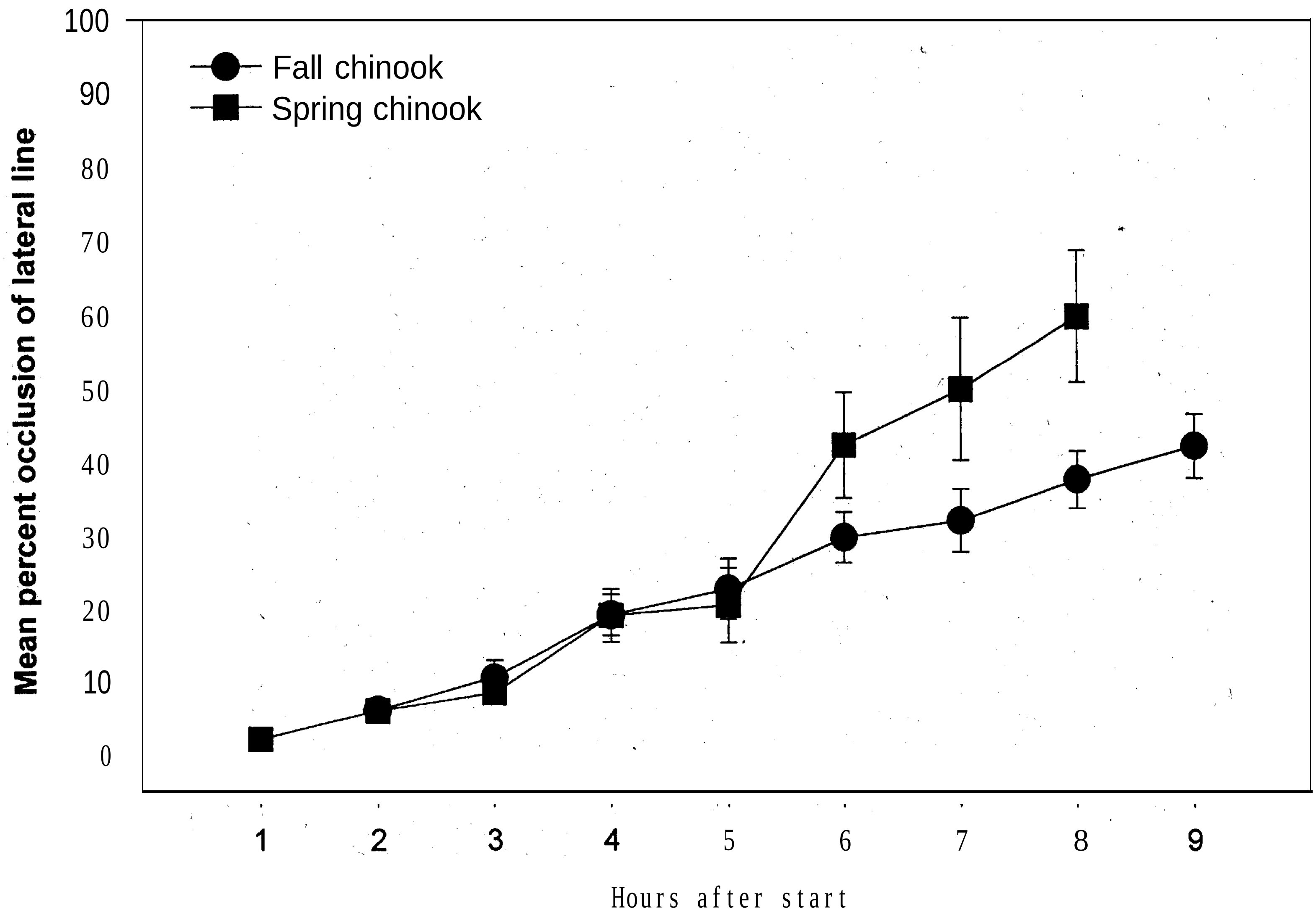


Figure 2.-Mean ± SE (N = 8 per point) lateral line occlusion in juvenile chinook salmon during exposure to 130% TDG at 12°C.

first 3 h of the trial and were rare thereafter (Fig. 4). The most common maximum fin severity rating was 1, with severity ratings of 2 and 3 showing up only during the last 4 h of the trials. Collectively, fin bubbles were common, maintaining 85-100% prevalence from about 4 h on (Fig. 5).

The number of gill filaments with bubbles was highly variable in both trials (Fig. 6). In addition, the progression of gill bubbles differed between trials, but these trends are difficult to compare because of the extreme inter-individual variation. In both trials, bubble counts using the intact arch were typically lower than counts made with the gill filaments removed (Fig. 6). In the trial using fall chinook, mean counts of bubbles in the gills remained low during the first 3 h and showed an erratic trend thereafter. In the trial using spring chinook, counts were low during the first 2 h but then increased and remained elevated for the duration of the trial. However, variation in this trial was more extreme than that observed with fall chinook. The prevalence of gill bubbles within a sample also differed somewhat between trials. For fall chinook, prevalence was generally moderate and steady for the first 5 h and then increased during hours 7-9 (Fig. 7). Small, irregular-shaped bubbles in the tips of gill filaments accounted for most of the bubbles we observed during hours 2-7. For spring chinook, prevalence was high initially (due to the presence of small bubbles) but then decreased to an average of about 50% for the rest of the trial (Fig. 7).

We did occasionally observe other signs of GBT in fish, but these were generally of minor significance relative to those just described. Bubbles in the gill filaments rarely occurred in isolation--that is, there were almost always other signs present. Fish that

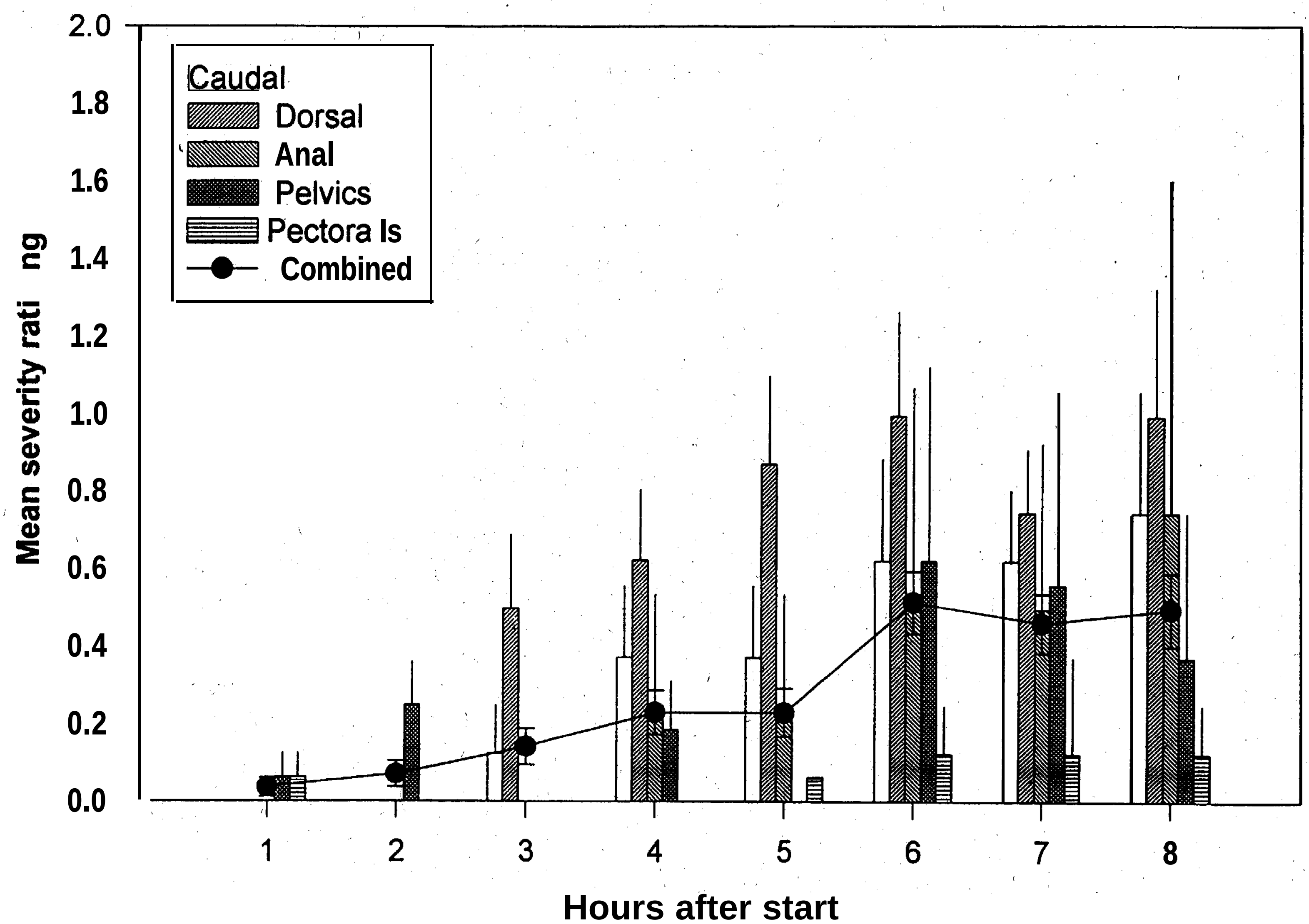


Figure 3.-Mean (and SE) severity ratings of bubbles in the fins of juvenile chinook salmon during exposure to 130% TDG at 12°C. Bars represent averages derived from fins on 8 fish; points are the average of the bars at each time interval.

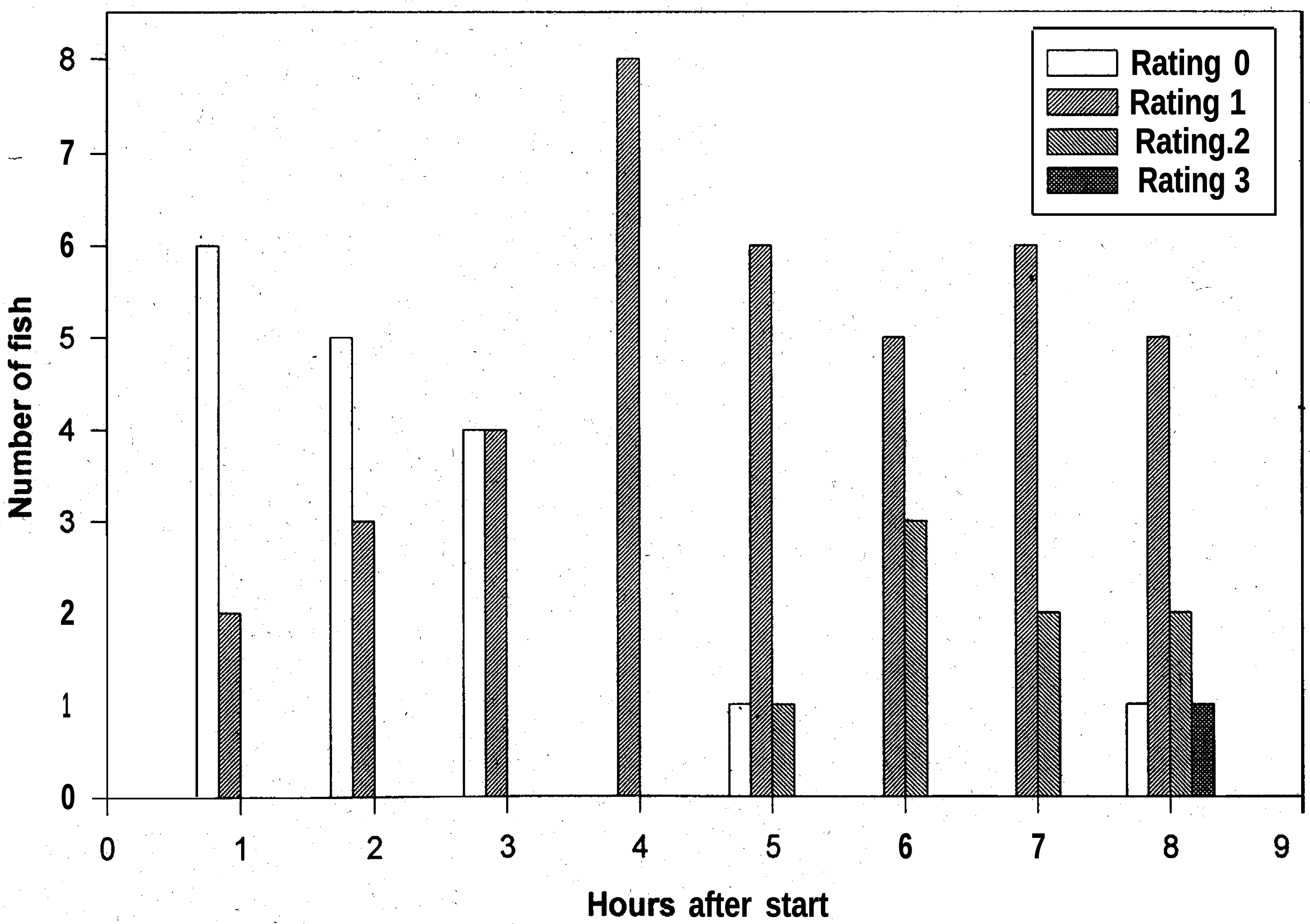


Figure 4.--Maximum severity ratings of bubbles in fins of juvenile spring chinook salmon during exposure to 130% TDG at 12°C. Ratings derived from all fins combined.

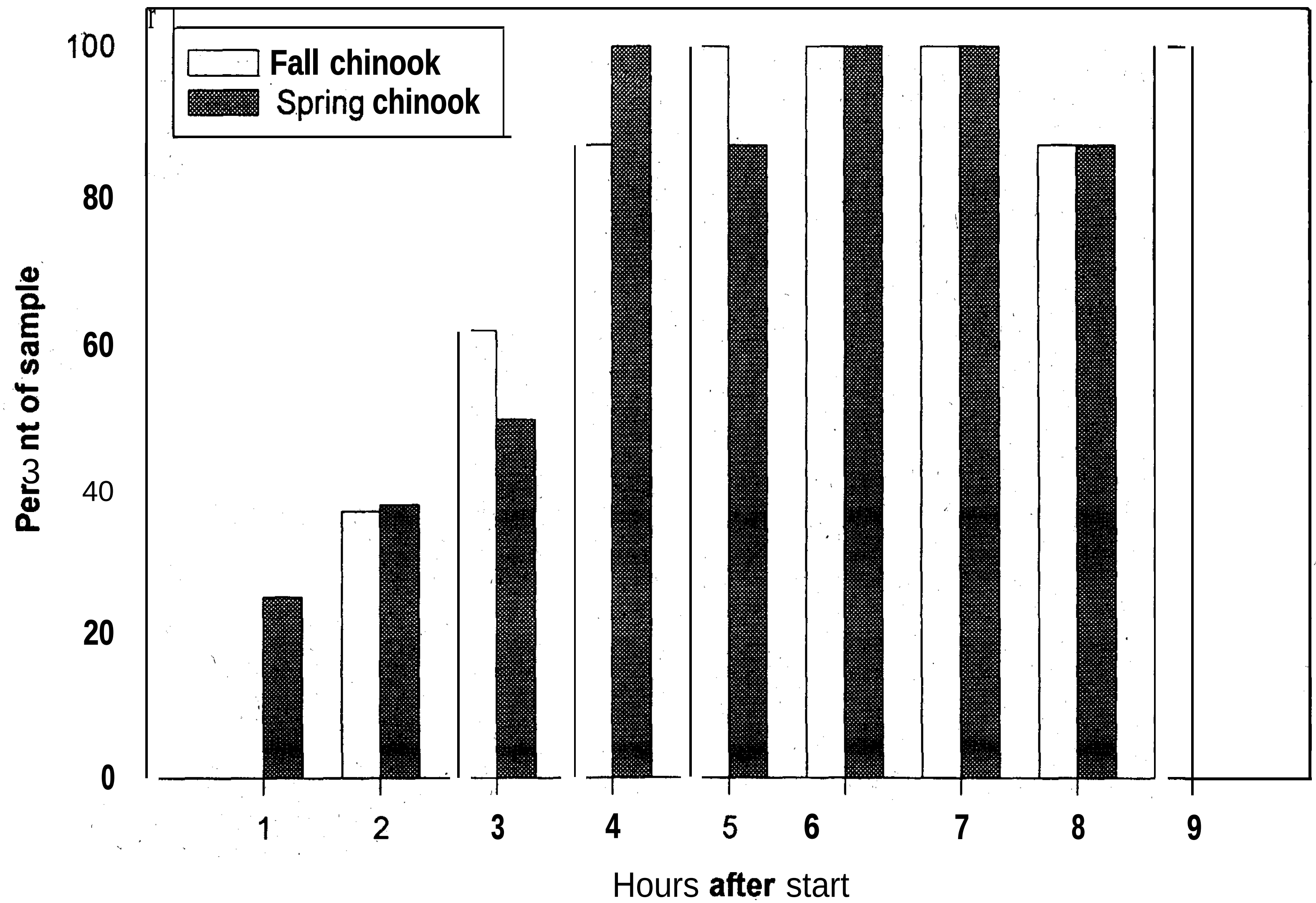


Figure 5.–Prevalence (N = 8/bar) of bubbles in the fins of juvenile chinook salmon during exposure to 130% TDG at 12°C. Fall chinook were sampled only during hours 2-9; spring chinook were sampled during' hours l-8.

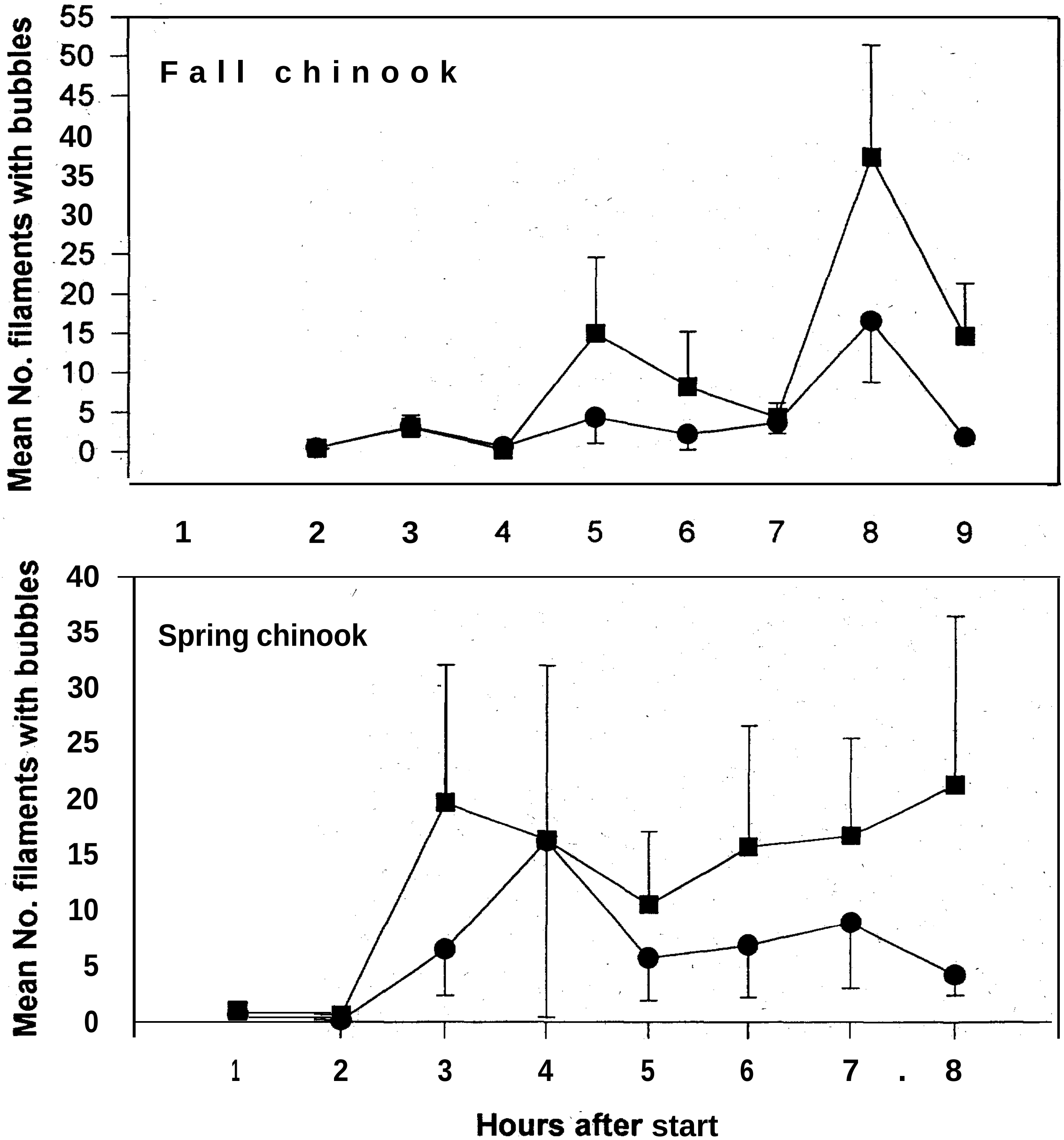


Figure 6.-Mean (and SE) number of gill filaments with bubbles in juvenile chinook salmon during exposure to 130% TDG at 12°C. Circles are data from a single, intact gill arch; squares are data from gill filaments cut off the arch.

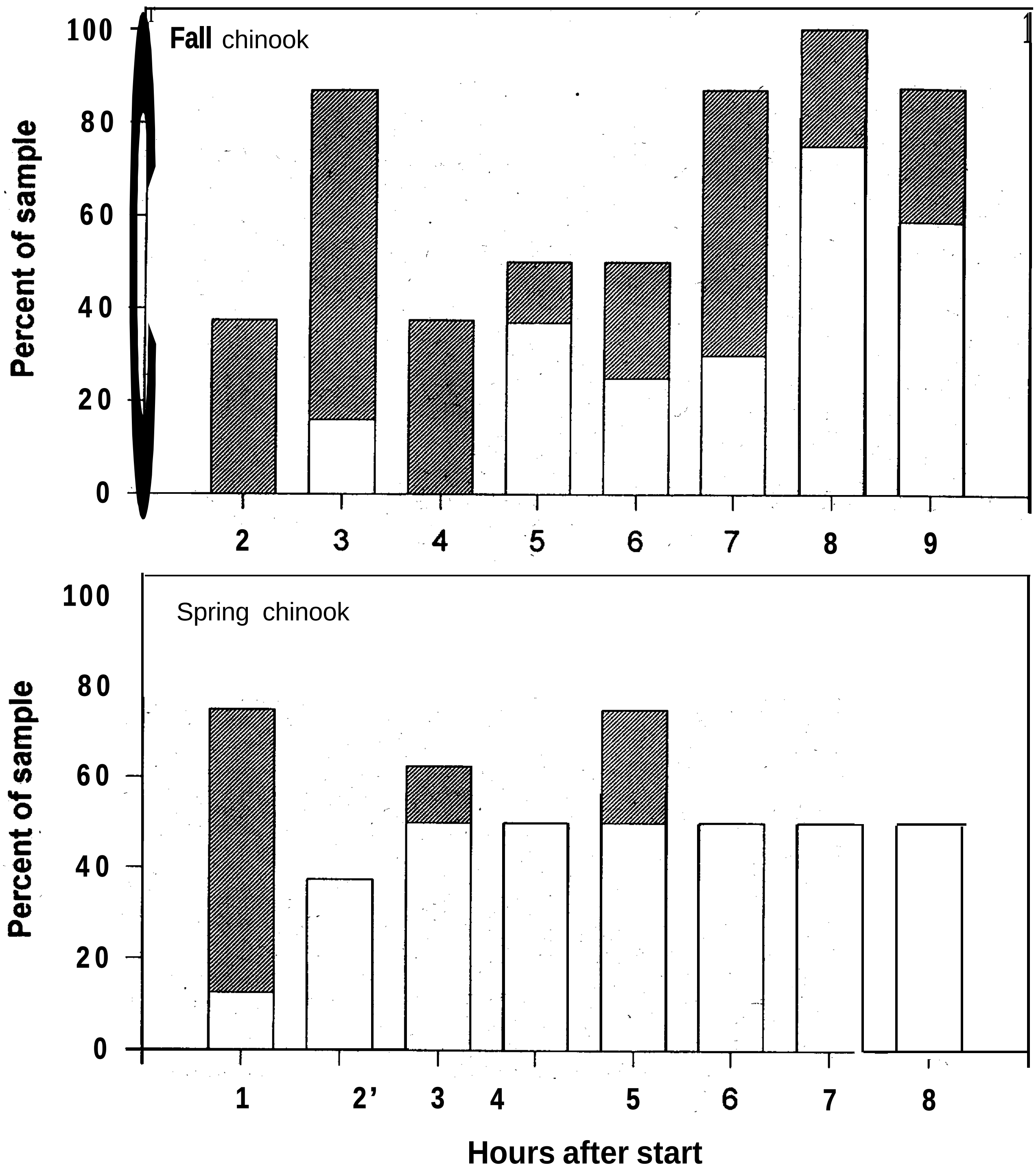


Figure 7.--Prevalence (N = 8/bar) of bubbles in the gill filaments of juvenile chinook salmon during exposure to 130% TDG at 12°C. Open areas denote large, well-developed bubbles; hatched areas denote very small, irregular-shaped bubbles.

died during the trials had virtually all their gill filaments occluded with bubbles but, as we observed with live fish, also consistently had other signs of GBT.

120% TDG

We conducted one trial at 120% TDG, examining a total of 104 live fish; the trial lasted 80 h (we monitored mortality through 95 h). Mortality increased sigmoidally during the first 60 h, with about 40% mortality occurring after 52 h (Fig. 8). After 60 h, mortality peaked at about 50% and changed little during the remainder of the trial. Average lateral line occlusion increased steadily during the first 60 h, but then reached a plateau of 50% occlusion thereafter (Fig. 9). Bubbles in the lateral line were common, with a prevalence of 100% for all sample periods except the first. Inter-individual variability in lateral line occlusion was relatively low throughout the trial.

Average severity of bubbles in the fins increased progressively to a peak of about 0.5 at 52 h and remained close to this level for the remainder of the trial (Fig. 10). Although average severity showed no evident trends in selected fins, the dorsal, caudal, and anal fins generally had the highest severity ratings (Fig. 10). Maximum severity ratings in fins indicated that fish with no bubbles (i.e., a rank of 0) were common only during the first 24 h (Fig. 11). The most common maximum fin severity rating was 1. Maximum ranks of 2 and 3 appeared after 30 h and made up 40-80% of a sample during hours 54 to 80 of the trial. The prevalence of fin bubbles increased rapidly, maintaining at least 80% from about 30 h on (Fig. 12).

The mean number of gill filaments with bubbles was variable and showed no obvious trend over time (Fig. 13). The mean number of filaments affected did increase

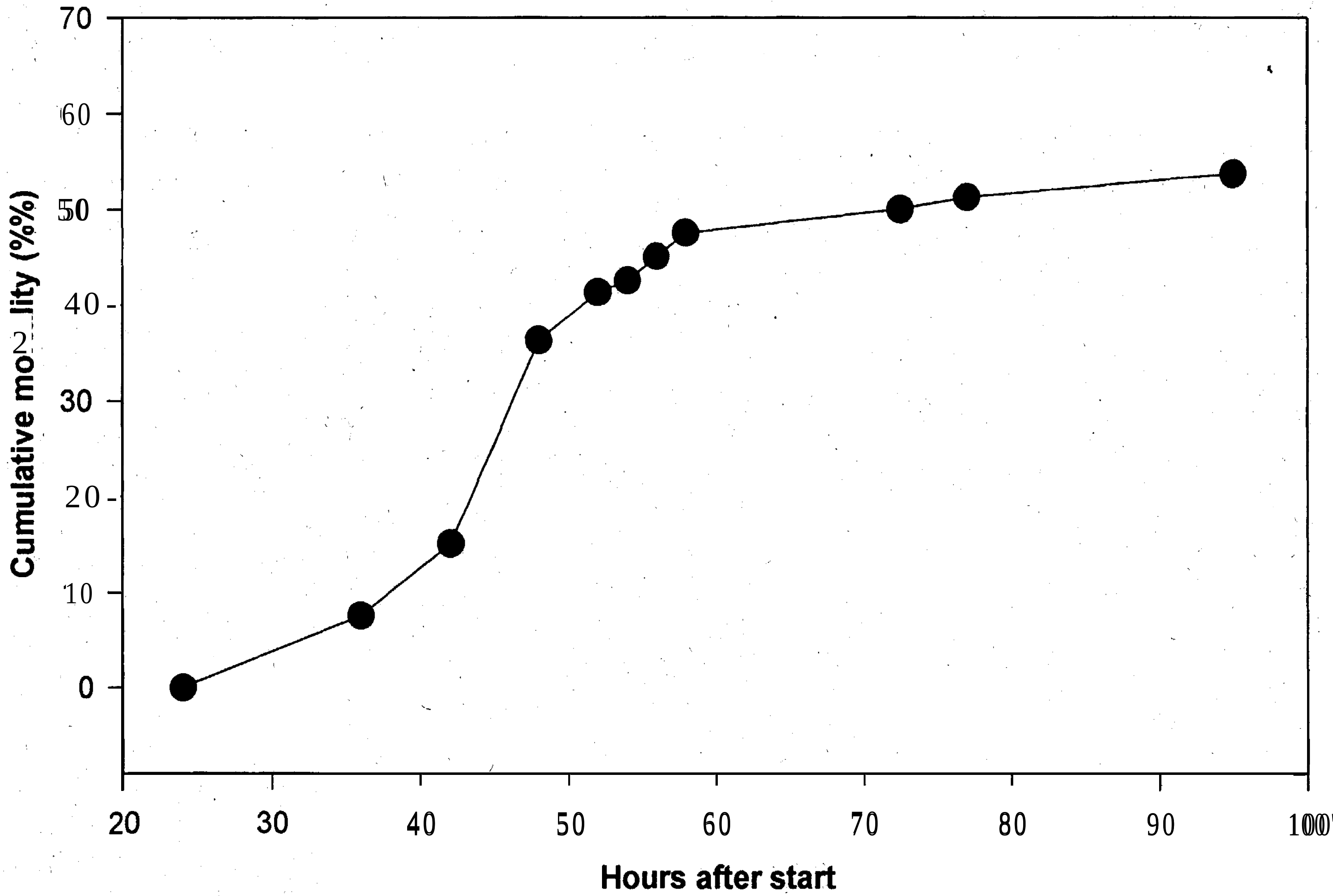


Figure 8.-Cumulative mortality of juvenile chinook salmon during exposyre to 120% TDG at 12°C. Total number of fish exposed was 75.

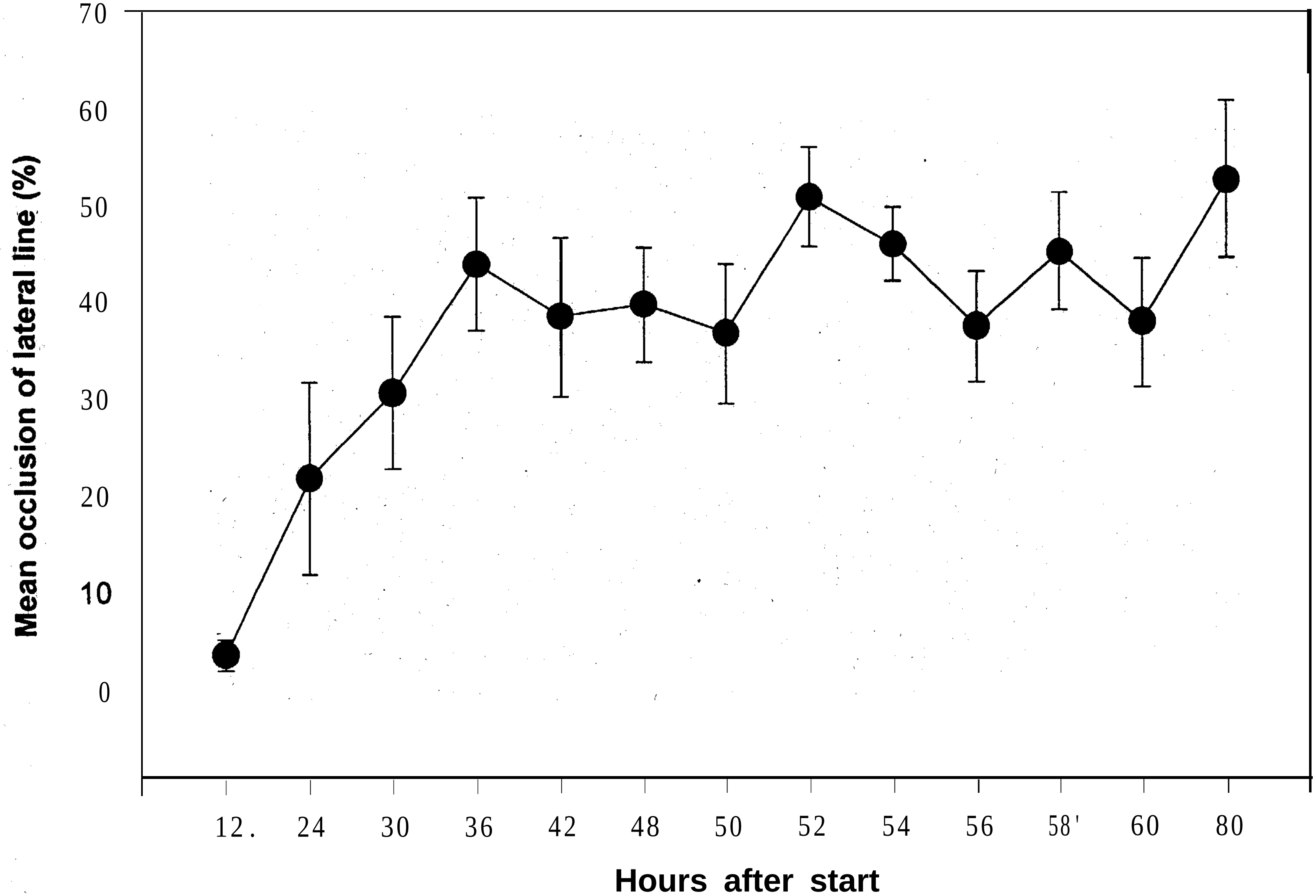


Figure 9.-Mean ± SE (N = 8 per point) lateral line occlusion in juvenile chinook salmon during exposure to 120% TDG at 12°C.

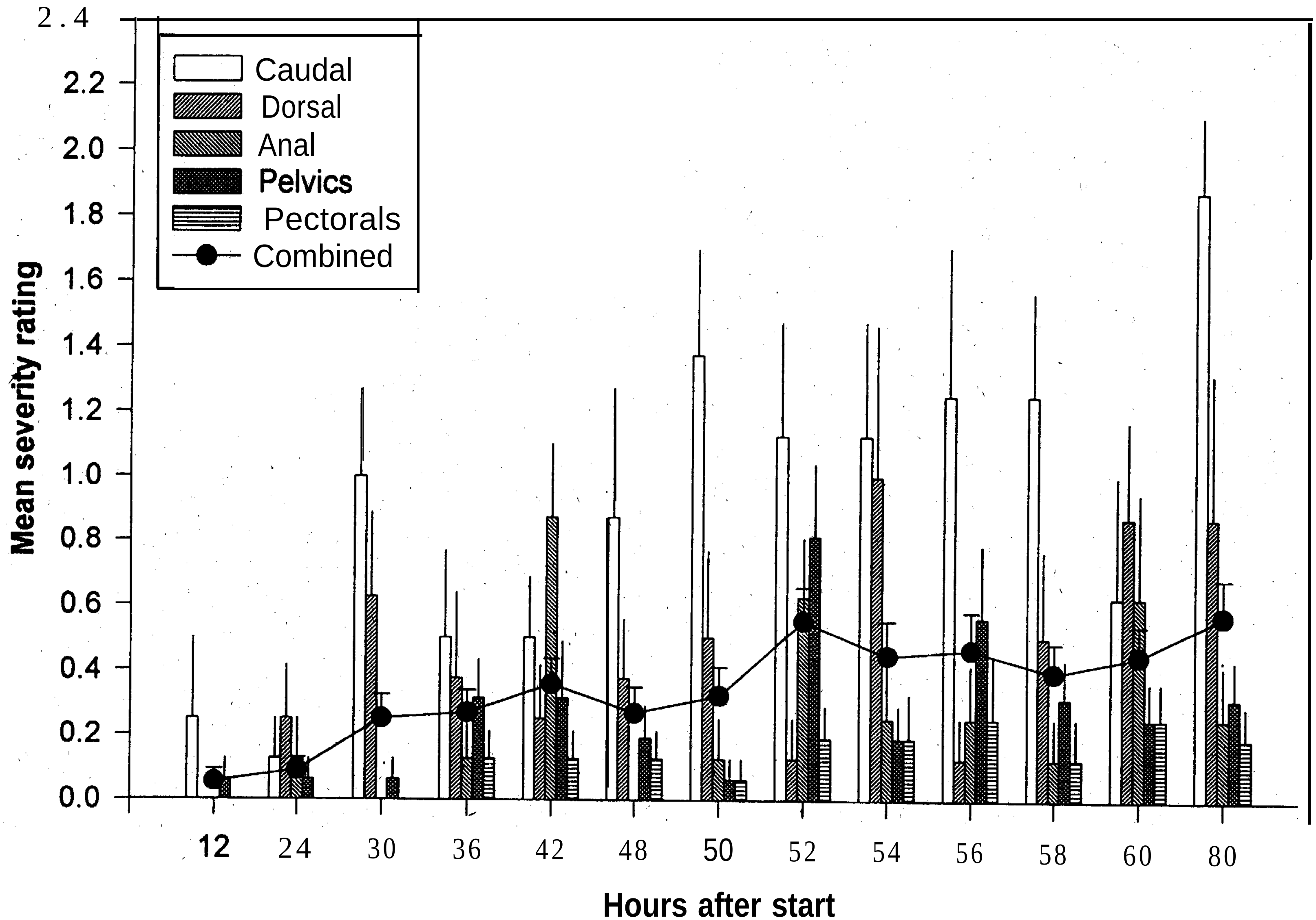


Figure 10.–Mean (and SE) severity ratings of bubbles'in fins on juvenile chinook salmon during exposure to 120% TDG at 12°C. Bars represent averages from fins on 8 fish; points are the average of the bars at each time interval.

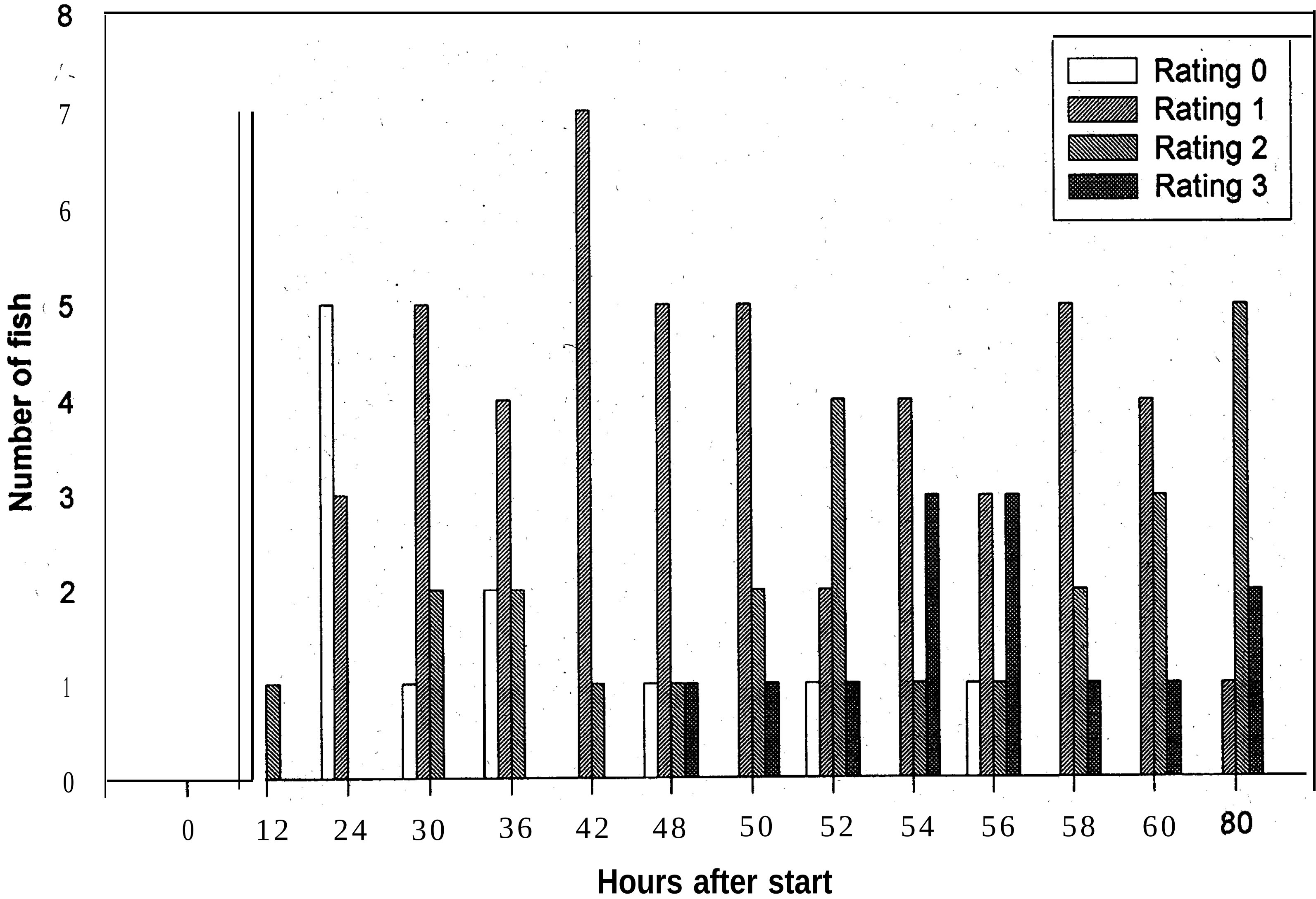


Figure 11 .-Maximum severity ratings of bubbles in fins of juvenile chinook salmon during exposure to 120% TDG at 12°C. Ratings derived from all fins combined.

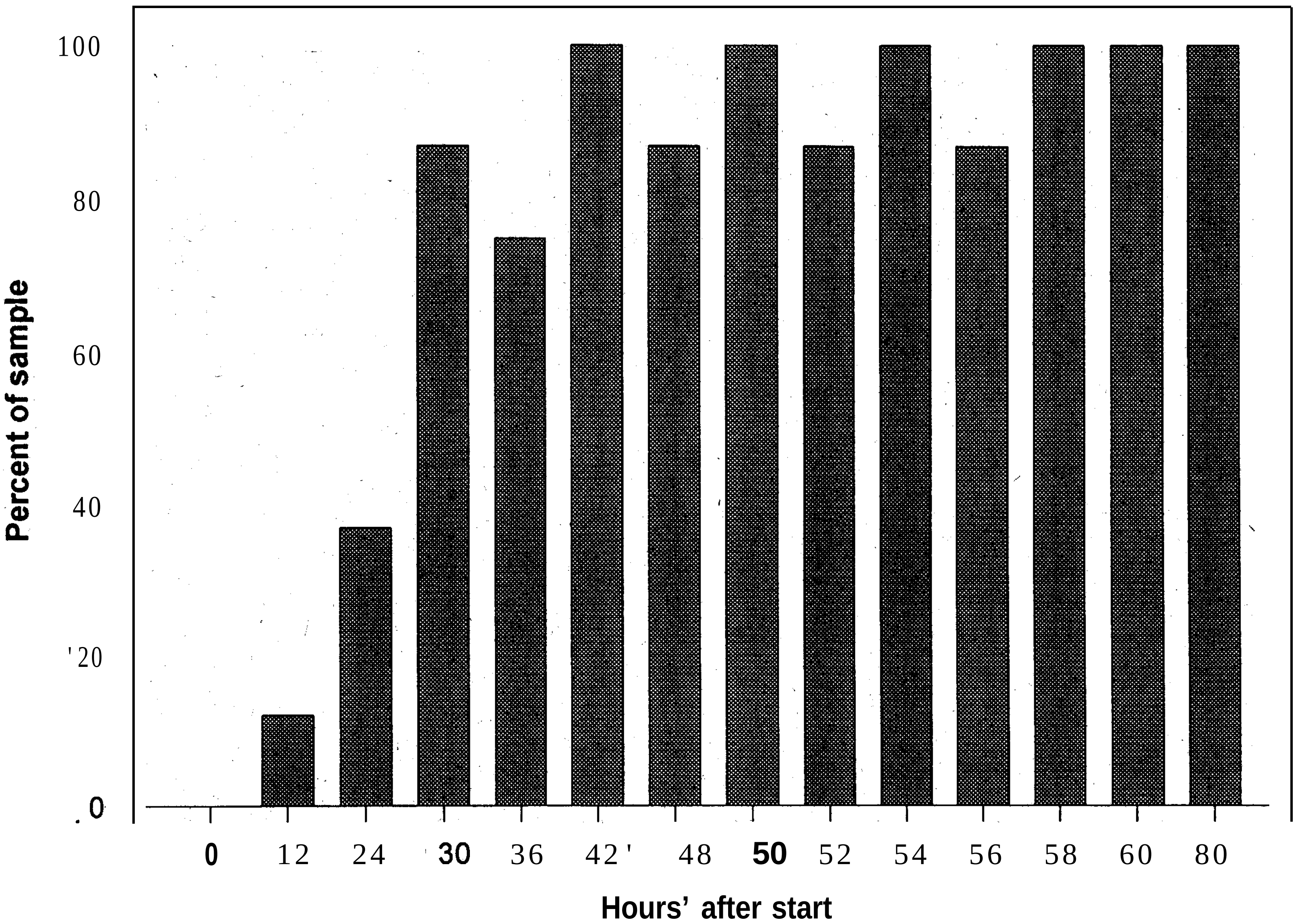
from 0 (at 12 h) to about 10 (at 36 h), but was erratic and, on average, never increased significantly above 10 after 36 h. Prevalence of gill bubbles increased up through 52 h and, except for the sample at 54 h, remained high for the remainder of the trial (Fig. 14). The presence of small bubbles in the gill filament tips was erratic. Again, gill bubbles were consistently associated with other signs.

112% TDG

We examined 144 live fish during one 22 d trial. There were no mortalities. Lateral line occlusion increased only slightly during the trial, never exceeding 5% on average (Fig. 15). Prevalence of bubbles in the lateral line was variable and only exceeded 50% a few times, particularly toward the end (Fig. 16).

Fin bubbles showed more definite trends. Average severity of bubbles in the fins increased gradually throughout the trial (Fig. 17). Only the caudal fin showed any obvious trend in average severity over time (Fig. 17). Fish with maximum severity ranks of 0 in the fins were common early but became infrequent after day 12 (Fig. 18). The number of fish with a maximum severity rating of 1 increased steadily during the first 13 days and remained relatively constant thereafter (Fig. 18). Although a few fish with a maximum severity ranking of 2 or 3 were observed during the first 10 d, such fish became more common after day 12. The prevalence of fin bubbles increased steadily during the first 13 d and maintained levels of at least 80% thereafter (Fig. 19).

The occurrence of gill bubbles was infrequent, rarely affected more than 1 or 2 filaments, and varied little among individuals (Fig. 20). Among other signs observed



Figure, 12.--Prevalence (N = 8/bar) of fin bubbles in juvenile chinook salmon during exposure to 120% TDG at 12°C.

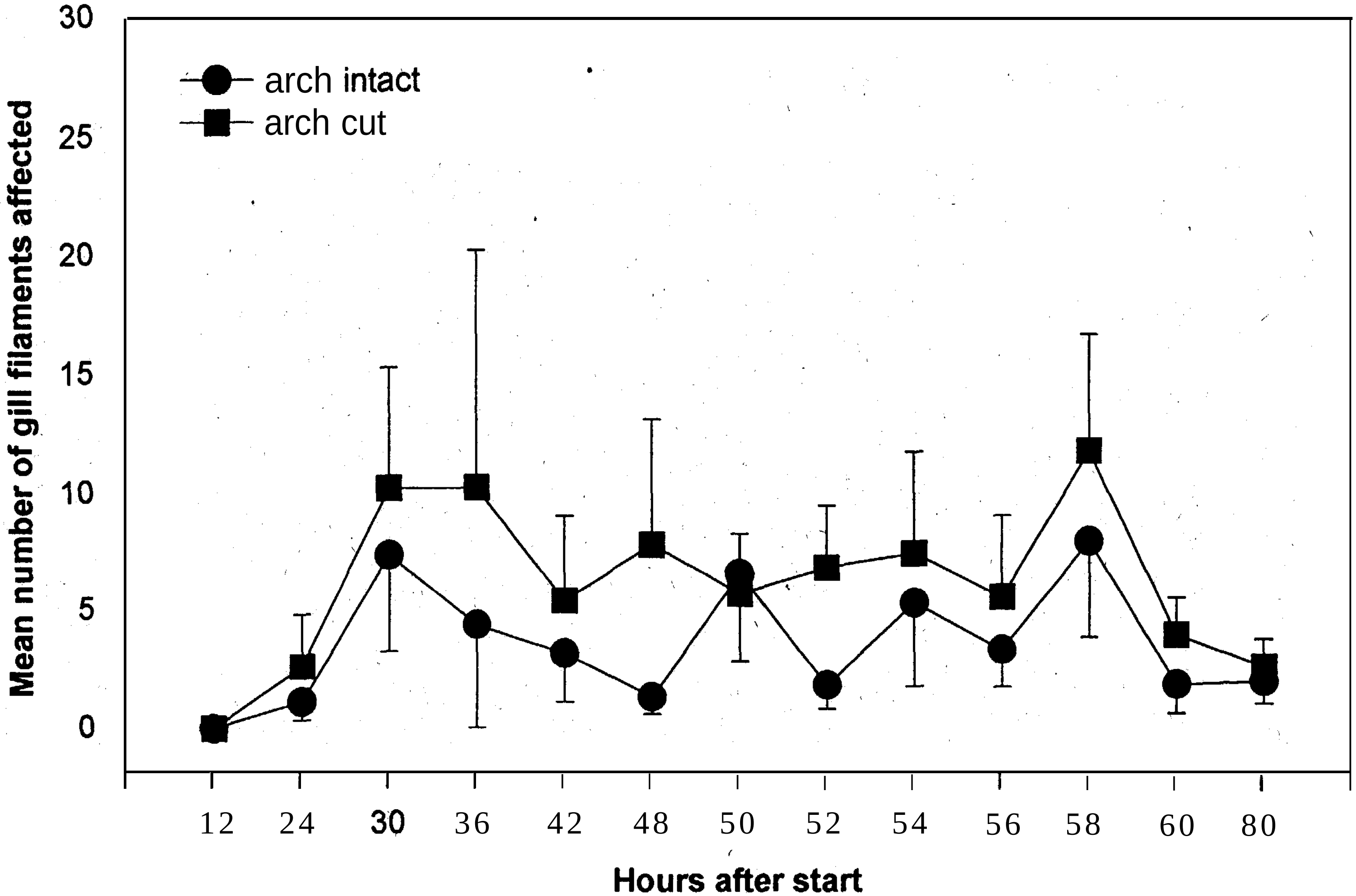


Figure 13.--Mean (and SE) number of gill filaments with bubbles in juvenile chinook salmon during exposure to 120% TDG at 12°C. Circles are data from a single, intact gill arch; squares are data from gill filaments cut off-the arch.

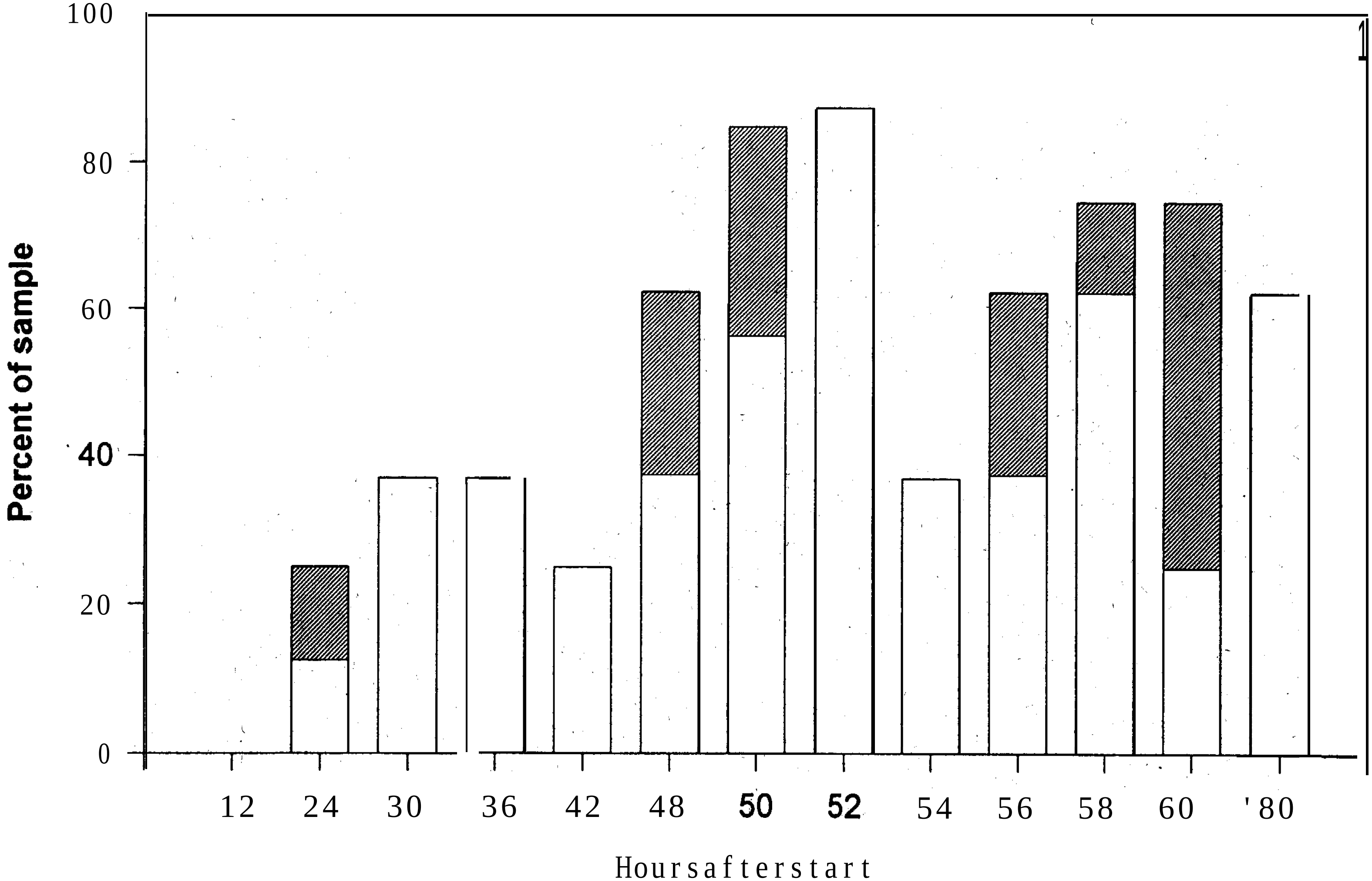


Figure 14.-Prevalence (N = 8/bar) of bubbles in the gills of juvenile chinook salmon during exposure to 120% TDG at 12°C. Open areas denote large, well-developed bubbles; hatched areas denote very small, irregular-shaped bubbles.

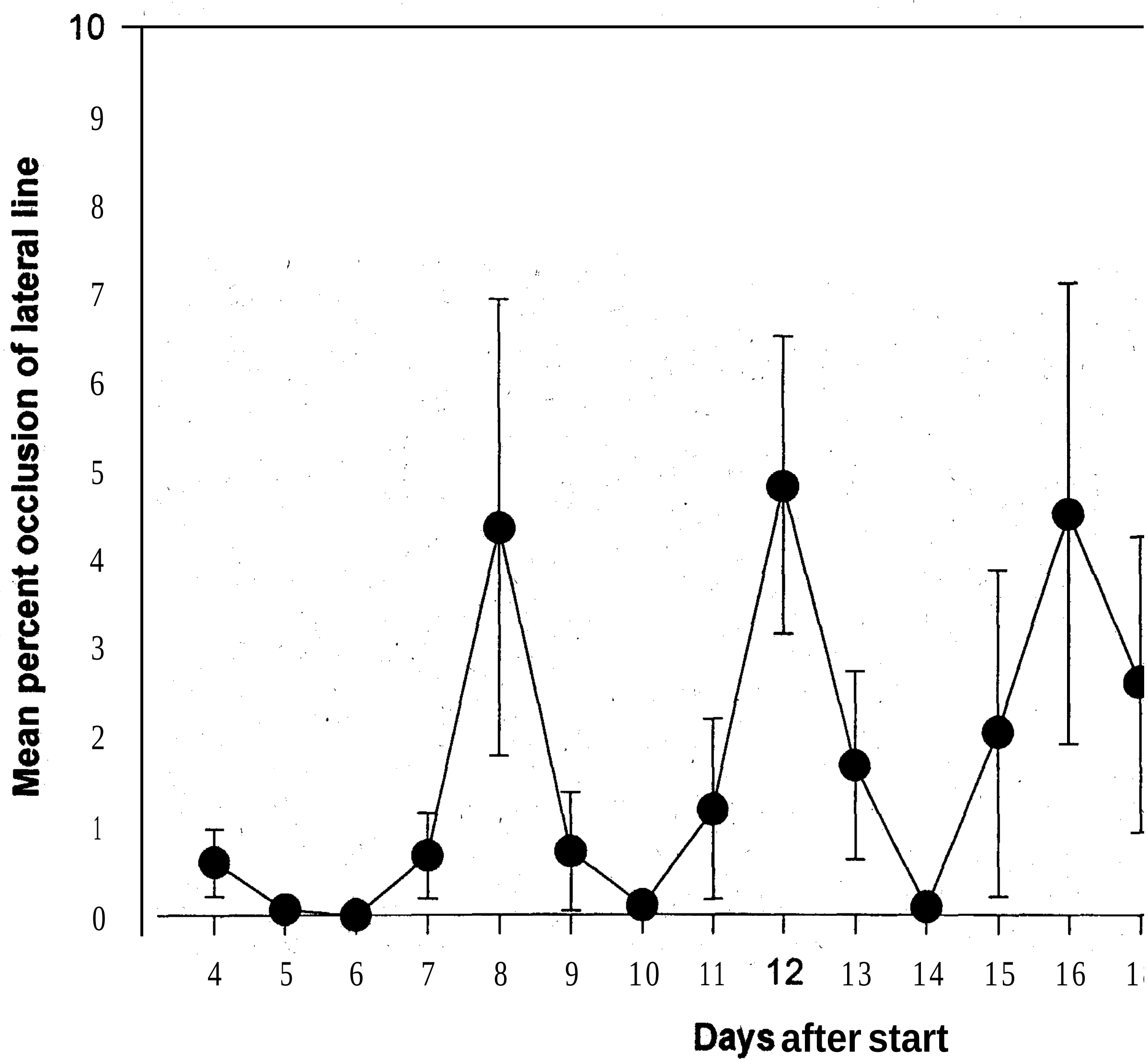


Figure 15.–Mean ± SE (N = 8 per point) lateral line occlusion in juvenile chinook salmon c

TDG at 12°C.

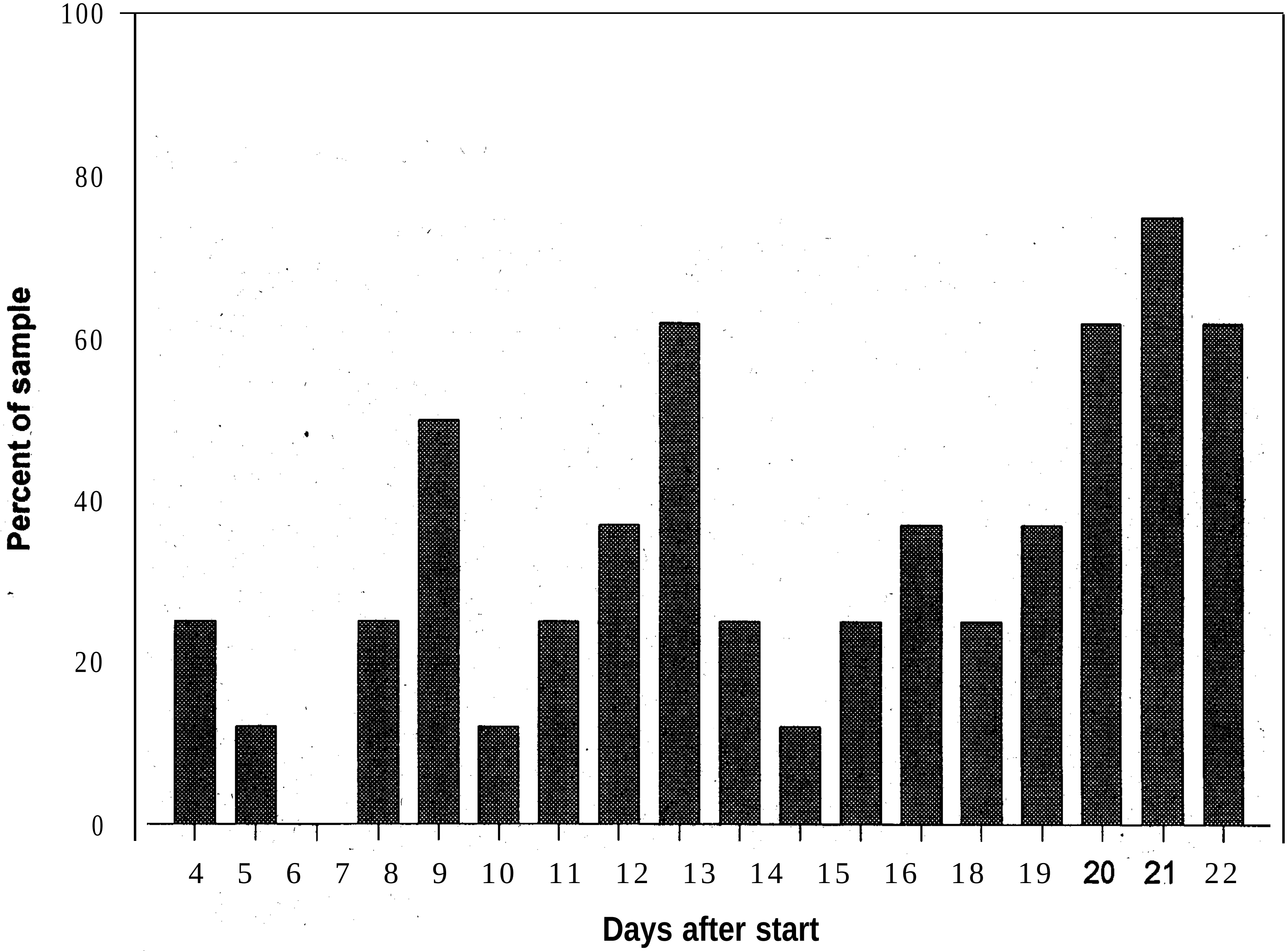


Figure 16.-Prevalence (N = 8/bar) of lateral line bubbles in juvenile chinook salmon during exposure to 112% at 12°C.

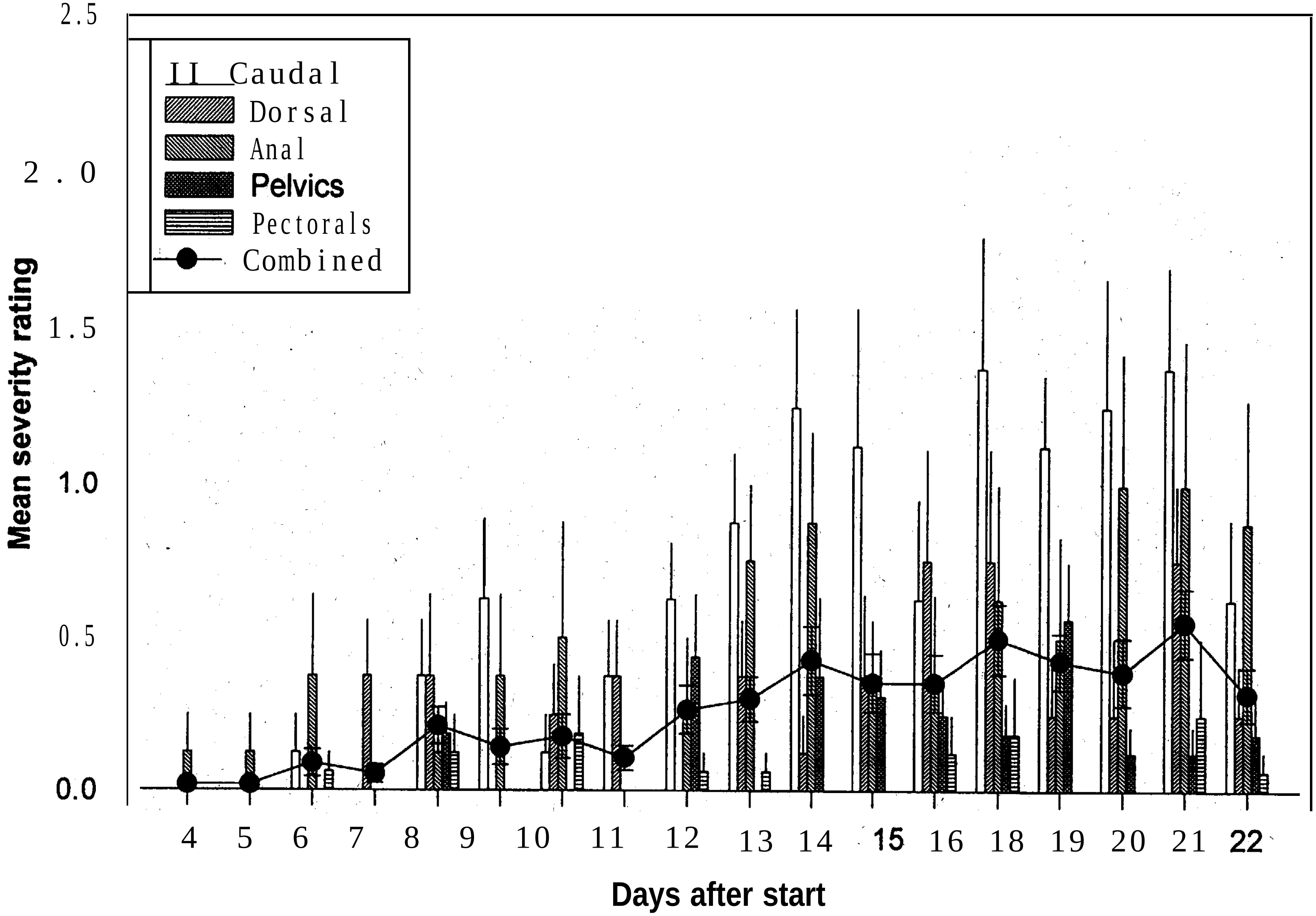


Figure 17.-Mean (and SE) severity ratings of'bubbles in fins on juvenile chinook salmon during exposure to 112% TDG at 12°C. Bars represent averages from fins on 8 fish; points are the average of the bars at each time interval.

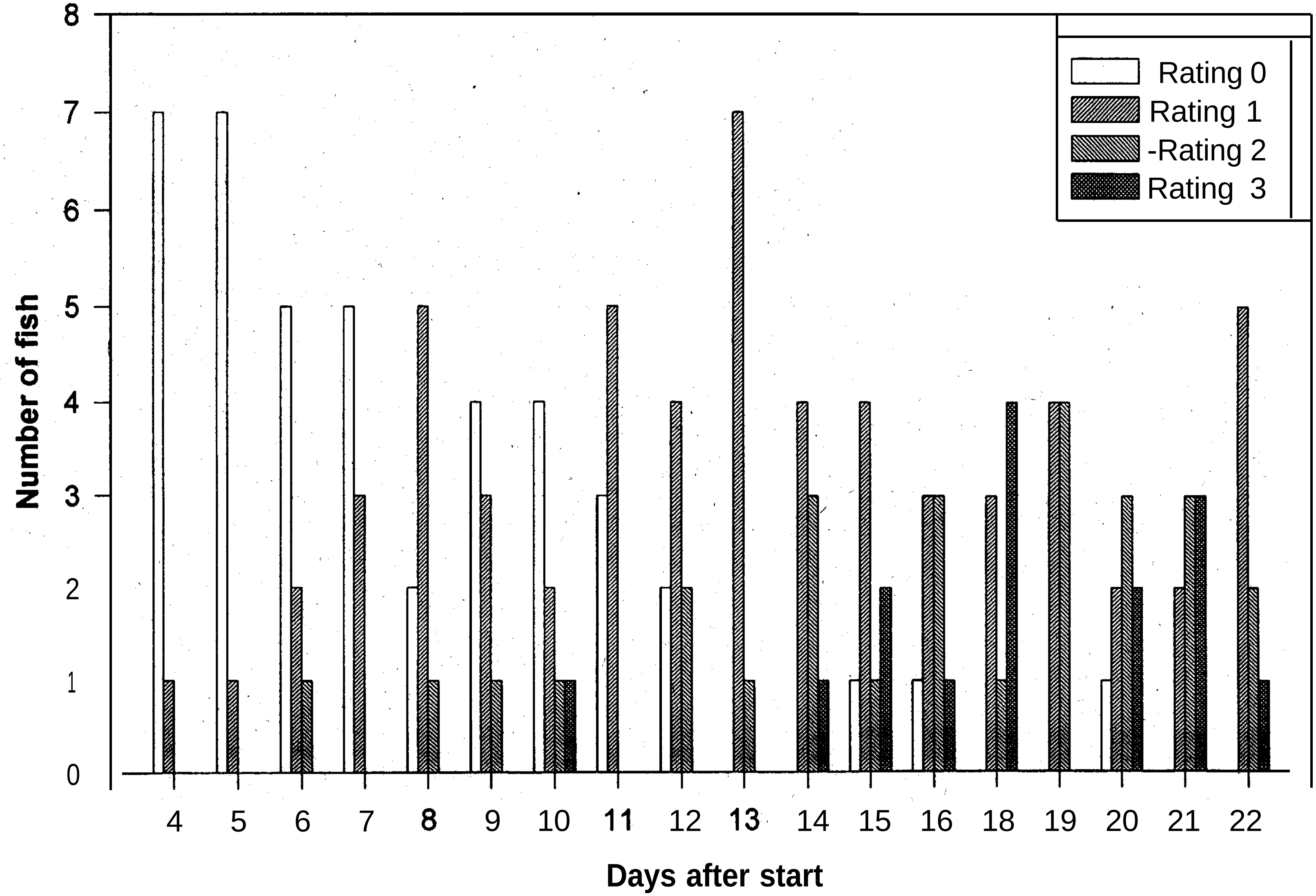


Figure 18.-Maximum severity ratings of bubbles in the fins of juvenile chinook salmon during exposure to 112% TDG at 12°C. Ratings derived from all fins combined.

during this trial, exopthalmia was relatively common, particularly during the last several days. Overall, exopthalmia occurred in 14% of the fish we sampled.

Discussion

Although there have been numerous descriptions in the literature of GBT signs in juvenile salmonids, ours is the first study to monitor in detail the progression of GBT signs, ascribe measures of relative severity to such signs, and attempt to relate signs of sub-lethal GBT to potential mortality. Our goal was to provide managers with methods that could be used in a system-wide monitoring program that examines outmigrating smolts for signs of GBT. To be most efficacious, the monitoring program should provide an assessment of the general well-being of the population, serve as an early-warning of the possibility of mortality due to GBT, and use methods that are non-lethal, easy and relatively quick. We believe the methods and progression of GBT we described in this paper make substantial contributions to a biologically sound monitoring program but, as we discuss below, there are several unanswered questions that need to be addressed before we obtain a sufficiently complete understanding of GBT in juvenile salmonids.

Our data conform well to the suggestion that GBT can be divided into two types--chronic and acute (Alderdice and Jensen 1985; Jensen et al. 1986). We noted distinct differences in etiologies, rate processes, and mortality between our chronic exposure at 112% DGS and exposures at 120% and 130% DGS. Such a distinction between chronic and acute GBT is useful in assessing which signs of GBT would be most useful to a monitoring program. Several key characteristics of our data indicate that not all signs of GBT are relevant to all TDG levels, thus some consideration must be given to

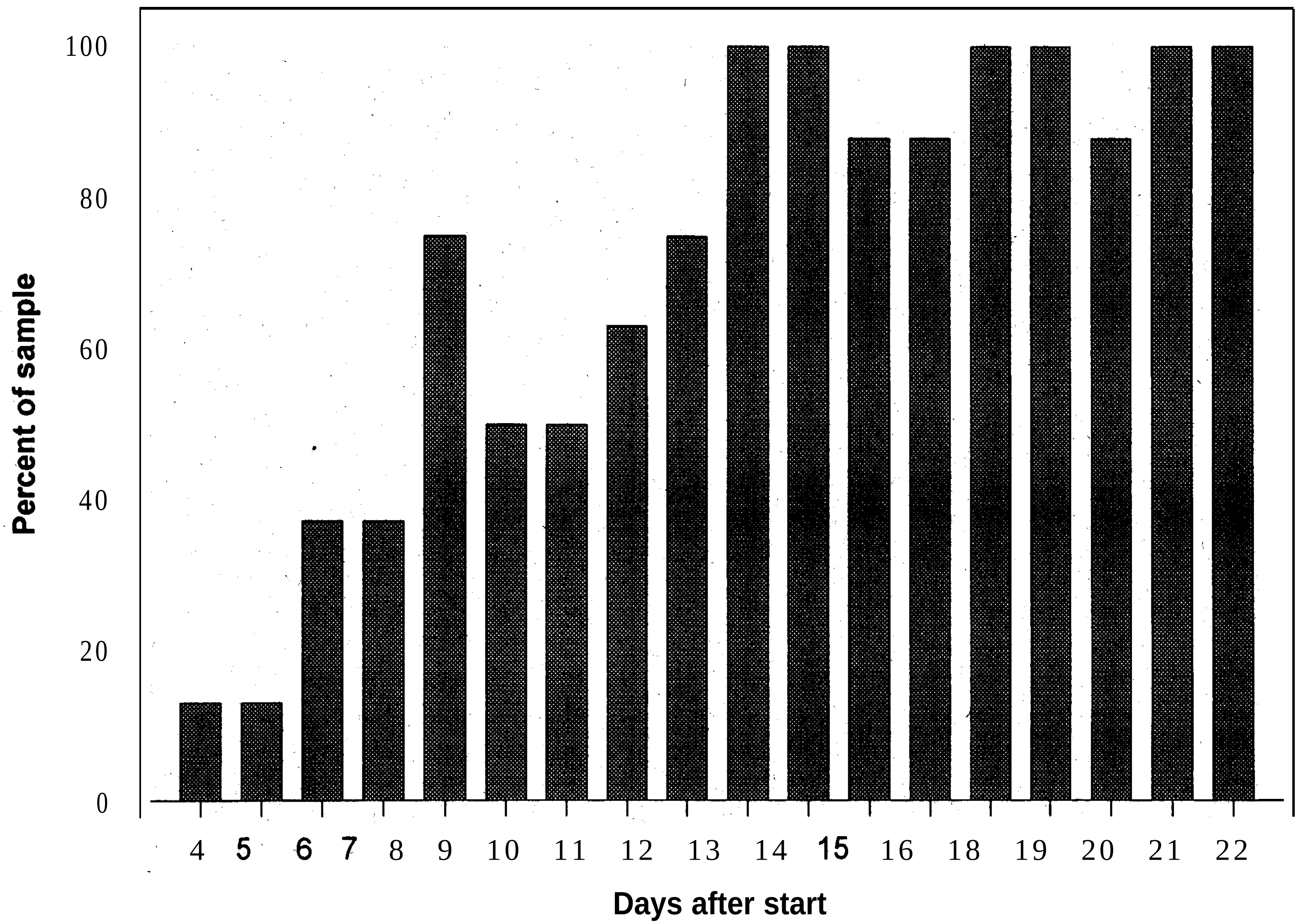


Figure 19.–Prevalence (N = 8/bar) of fin bubbles in juvenile chinook salmon during exposure to 112% TDG at 12°C.

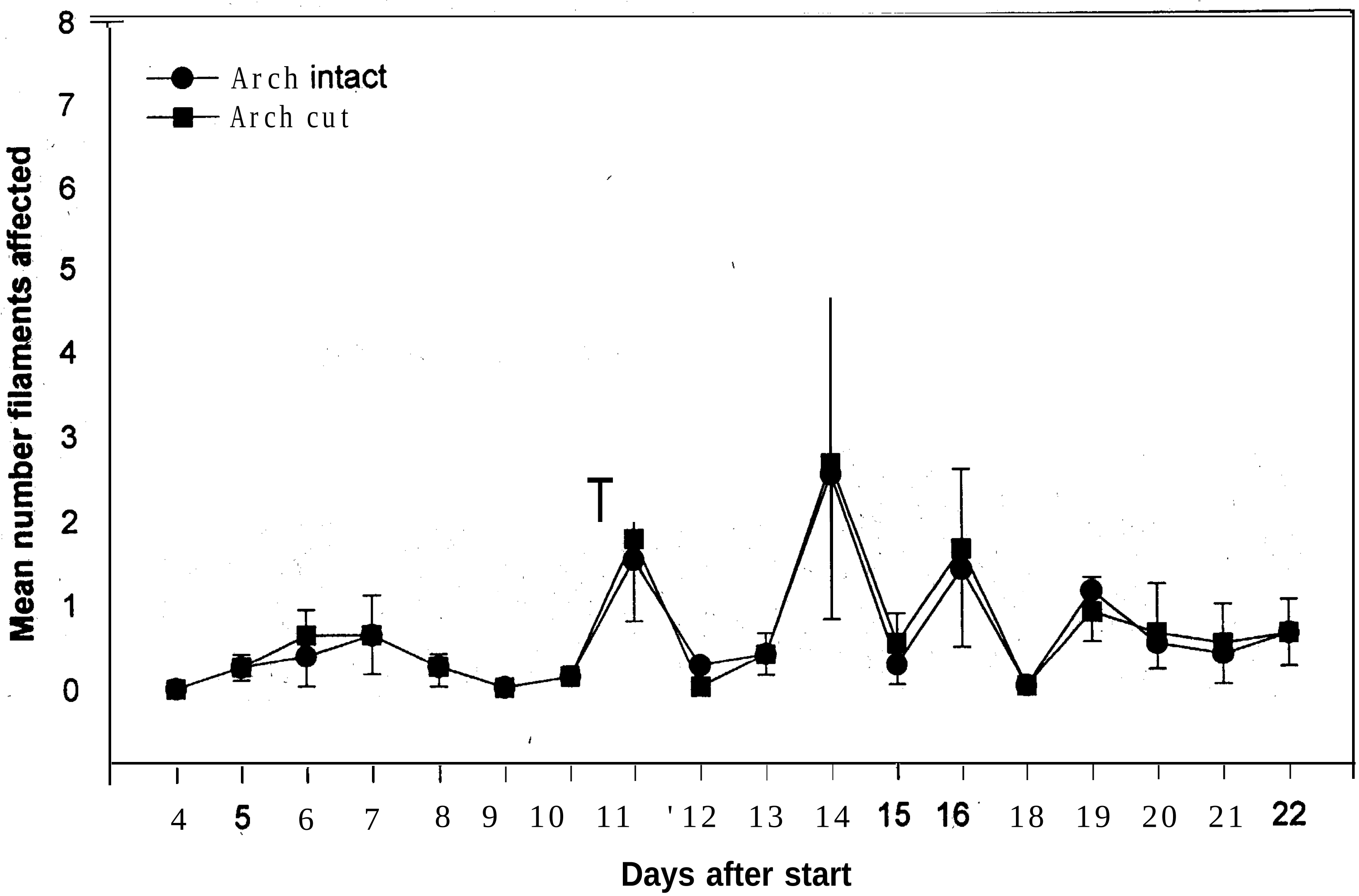


Figure 20.--Mean (and SE) number of gill filaments with bubbles in juvenile chinook salmon during exposure to 112% TDG at 12°C. Circles are data from a single, intact gill arch; squares are, data from gill filaments cut off the arch.

deciding which signs of GBT would best monitor fish condition under a wide range of environmental conditions.

Using lateral line occlusion as a sign of GBT has several advantages. At 120% and 130% DGS, lateral line occlusion was the earliest sign of GBT observed, showed a consistent, progressive increase over time, had low inter-individual variation, and had a high prevalence. In addition, with the proper equipment, bubbles within the lateral line canal are easy to see and the examination is relatively straightforward, fast, and easily learned. Despite these advantages, there are several problems with this GBT sign. First, it may not be a relevant sign of GBT in fish receiving chronic exposures to low levels of TDG. At 112% DGS, we saw very few bubbles in the lateral line over a 22 d period; we do not know if lateral line occlusion would have been more severe with a longer exposure or if a threshold level of TDG exists where bubbles in the lateral line become a more consistent sign of GBT. Second, recent evidence from studies in our laboratory and at Battelle Laboratories, Richland, WA, suggests that lateral line bubbles can collapse and disappear within a short time after fish enter normally saturated water or experience high pressures when descending to deeper water. Although more research is necessary to confirm these findings, the possibility that bubbles in the lateral line are not an overly persistent sign of GBT may confound their use for monitoring the severity of GBT. Finally, the relation between average lateral line occlusion and cumulative mortality is not clear, which essentially precludes the use of this sign alone as a predictor of future mortality. However, recent information from our laboratory indicates that extensive lateral line occlusion may increase the vulnerability of juvenile salmon to predation (M. Mesa, unpublished data).

Monitoring bubbles in the fins also has several advantages. First, bubbles in the fins showed high prevalence at all the TDG levels we examined. Second, like the lateral line, average and maximum severity rankings of fin bubbles showed a progressive increase over time. Third, there are several “sample sites”, or fins, on fish that can be scanned for bubbles. Although our results indicate that the caudal fin accounts for most of the bubbles in fins, this was not always the case and the ease of scanning fins for bubbles (another advantage) makes it desirable to examine as many fins as possible. Finally, recent work at our laboratory suggests that fin bubbles may be a more persistent sign of GBT and therefore less likely to disappear rapidly with changes in pressure or decreases in TDG, but this notion requires further experimentation. One problem with monitoring the fins for GBT involves the subjectivity and lack of detail in assigning ranks to severity of bubbles. The range of fin surface area covered with bubbles associated with the ranking system, particularly rank 1, may be too broad to specifically account for the trauma observed. For example, we believe there may be substantial differences in the severity of trauma experienced by fish with 5% versus 20% of their fin area covered with bubbles, yet both would receive a rank of 1. This type of problem may be solved by simply assigning a percentage value, not a rank, to the amount of fin surface area covered by bubbles along with a description of the trauma. This would likely require more training and take more time, but in the end may lead to more relevant data. Another problem, based on some recent experiments in our laboratory, is that fin bubbles may not appear when fish are exposed to very high, acutely lethal levels of TDG. This is due to bubbles growing rapidly in the circulatory system and killing fish before bubbles can form in the fins. Finally, there is, like the lateral line, lack of a clear relation between fin bubble severity and mortality. As our data for fish exposed to 112% DGS indicate, fin bubbles can become quite severe with no associated mortality, which contrasts with our results at higher TDG levels. Therefore, without information on exposure history, bubbles in the fins, by themselves, may not be a good indicator of potential mortality.

Examination of gill tissue for intravascular bubbles due to GBT offers an intriguing catch-22. On the one hand, such bubbles appear to be the proximate cause of death in fish receiving lethal exposures to DGS.   For example, examinations of fish that had died or were moribund during our trials revealed that almost all gill filaments were occluded with long, rod-shaped bubbles. These bubbles often extended the length of the filament and clearly caused a massive hemostasis that eventually led to death. We believe these bubbles form in the afferent filamental artery and their growth may be rapid once they get started, thus locating just one or two of these relatively large bubbles in the gills may be important in assessing fish condition. In fact, because large, intravascular gill bubbles are directly related to mortality, one might presume that examination of this sign alone is sufficient--and the most relevant--for assessing the severity of GBT. However, examining the gills is also fraught with difficulties. Of all the signs we examined to assess the progression of GBT, bubbles in the gills provided the least satisfying information. First, bubbles in the gills may only be relevant at high TDG levels, since we saw few of them in fish exposed to 112% TDG. Thus, like all other signs of GBT, it would be necessary to consider exposure history when using the gills to assess the severity of GBT. Second, the average number of gill filaments with bubbles showed little if any progressive change over time, thus, although intravascular gill bubbles may be a proximate cause of death, they may not be a good predictor of mortality. Third, bubbles in the gills showed an extreme amount of inter-individual variation, which could lead to sampling (and statistical) difficulties in field situations. Fifth, recent evidence suggests that intravascular gill bubbles, like the lateral line, may easily collapse with increases in hydrostatic pressure (Montgomery Watson 1995). Sixth, the significance of microscopic bubbles in the tips of some filaments, which we commonly observed (in fact, such bubbles comprise a large part of our data and were used to derive averages), is unknown and can therefore confound prevalence data. Finally, examining and finding bubbles in the gills is difficult and, consequently, could have the propensity for a high degree of error. This would be particularly true for field applications, where the emphasis should be on examining live fish as they migrate down the river. As we discuss in the next chapter, gill examinations on live, anaesthetized fish are more difficult than those on dead fish or excised gill arches.

Despite the many disadvantages of the signs used to monitor the progression of GBT in juvenile salmonids, we believe many of the problems can be overcome and that our data can be used to establish a biologically sound GBT monitoring program for salmonids in the Columbia and Snake rivers. Based on our results, there are several aspects to consider if the signs we examined are to be used in a monitoring program. First, it is clear, not only from our work but also from past research (Meekin and Turner 1974; Dawley and Ebel 1975; Schiewe and Weber 1975), that GBT in juvenile salmonids is a *progressive* trauma. That is, many of the signs of GBT become progressively worse over time. This notion contrasts with the idea that signs of GBT may respond only at certain TDG thresholds and is extremely useful to applying our methods in field situations. The severity of GBT in fishes is based essentially on two factors--TDG level and exposure time. There are, of course, other modifying influences (e.g., species, fish size and activity, water temperature) that might affect rate processes but not the eventual outcome--that fish exposed to high TDG levels for a sufficient amount of time will develop GBT. Therefore, if fish in the wild encounter high TDG levels and are exposed for a sufficient time, the progressive nature of GBT indicates that sublethal signs of GBT would be present in a representative sample of fish. In other words, given the progressive nature of GBT, extreme individual variation in susceptibility to GBT, and a rigorous fish sampling program in the field, it should be entirely possible to detect sublethal signs of GBT in fish if, in fact, *fish are actually experiencing sufficient exposures to DGS*. Another aspect to consider when applying our data to field situations is that prevalence and severity of GBT signs should be used when trying to assess potential population effects. Both prevalence and severity are necessary to completely understand and account for exposure history, individual variation, and possible mortality. Finally, we believe that no single sign of GBT can alone meet the objectives of a GBT monitoring program. Several indicators of the prevalence and severity of GBT in migrating fishes are necessary to make informed decisions regarding fish management on the Columbia River system.

How would such a multivariate approach be implemented in a Columbia River system-wide GBT monitoring program? In fact, the approach of examining samples of fish for GBT as they out-migrate, using data from the lateral line, fins, and, to a lesser extent, gills, is already in place. We believe our data affirms that this type of approach is necessary. What is missing from the program is some criteria that allow fishery managers to determine when fish populations may be at increased risk due to GBT. We offer the following as an example of how our data could be used to define specific criteria for assessing the potential effects of GBT on out-migrating juvenile salmonids.

Our approach basically involves first determining the LT_{10} for a given TDG level--or the time it takes to kill 10% of the population--and then listing the average prevalence and severity of GBT signs in the population at that time. All of this information can be derived from our data and an example of this approach is presented in Table 1. Since we can consider laboratory bioassay TDG exposures worst-case scenarios because fish have little or no ability to depth compensate, data such as that

Table 1.--General characteristics of gas bubble trauma (GBT) in a sample of juvenile chinook salmon exposed to 120% total dissolved gas (TDG) at 12°C. The time of exposure was 36 h, which closely approximates the LT_{10}--or the time required to kill 10% of the population.

GBT sign	Sample characteristics
Mean lateral line occlusion	21 $\pm$ 9.9%
Lateral line prevalence	100%
Mean severity rating in fins	0.267 $\pm$ 0.069
Maximum severity rating in fins	75% with $\geq$ rank 1
Fin bubble prevalence	75%
Mean gill filament bubbles	4.4 + 4.4 (arch intact); 10.3 + 10 (arch cut)
Gill bubble prevalence	37%

in Table 1 can be compared to similar information taken from fish in the wild to assess the extent and severity of GBT in migrating fish. If representative samples of fish in the wild have GBT signs similar to those of fish from the laboratory bioassay data, then it may be assumed that some mortality in the population has occurred and corrective actions could be taken. The criteria could be made more conservative by using signs of GBT that may be present in fish at the LT_5 or LT_1 (i.e., 5% and 1%) level of mortality. The advantages of this approach are that it uses prevalence and severity of multivariate data, it is based on worst-case TDG exposure scenarios, and it can provide managers with unequivocal criteria for rapid decision making. The disadvantages of this approach are that it is based on limited data and would require representative samples of fish from the wild. This approach could also be affected by the disappearance of GBT signs in wild fish due to the increased hydrostatic pressure fish encounter as they swim to deeper water. This could happen, for example, if fish with signs of GBT approach a dam and sound to depths required to enter bypass facilities--which may collapse some or all of their GBT signs. If this did occur, GBT monitoring personnel at the fish bypass facilities would probably be unable to account for the disappearance of GBT signs and data gathered from such monitoring may be considered suspect. However, theories involving the disappearance of GBT signs require further study.

It is surprising to us that, given the history of dissolved gas supersaturation in the Columbia River system and the large amount of research on the subject, we still have substantial gaps in our understanding of DGS and GBT. Today, these subjects are scientifically and politically contentious, which may ultimately prove to be detrimental to the resource. Is there a problem with DGS and GBT in the Columbia River system today? At present, we cannot answer that question but believe an answer is possible in the not too distant future with a focused research effort dealing with specific, relevant questions that will allow us to achieve a more complete understanding of DGS effects on juvenile salmonids in the Columbia River.

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CHAPTER 1, APPENDIX A

GILL EXAMINATIONS

During the 1995 mid field season we received an immediate request from cooperative agency officials to implement gill exams to the field protocol of the smolt GBT monitoring program. Even though the gills can be susceptible to GBT it was not included in the GBT exams because of the high risk of inspecting a live fish's gill tissue mass. At this point we had only performed gill exams on live fish that were dosed in lethal anaesthesia from our gas supersaturation experiments.

We conducted some laboratory experiments to evaluate and possibly improve our gill examinations and also to develop a non-lethal method of examining gills in the field. Our objective was to compare counts of bubbles in gill filaments between scan observations of the whole gill tissue mass and examining each individual gill arch. Initially, we performed examinations of dead fish to establish and refine our methods. We then conducted examinations on live, anaesthetized fish to evaluate the methods for use in the field.

Methods

We subjected groups (N = 44) of age-1 spring chinook salmon (FL range = 120-160 mm) to 120 and 130% TDG for a time sufficient to produce signs of GBT usually from 4 to 48 h depending on TDG level. We then sampled 2-3 fish every 0.5 to 1 h thereafter, placing them into a lethal dose of buffered MS-222 prepared with supersaturated water.

We removed fish from the anaesthetic, placed them left side up on a moist paper towel, and removed the opercle with curved surgical scissors. We examined the entire gill tissue mass without any physical manipulation of the gill arches under a dissecting scope (10-40x) and counted the number of gill filaments with intravascular bubbles. We then made a second count, but this time used a blunt probe to lift each arch out of the way to facilitate counting of bubbles in each individual arch. We summed the counts obtained from each arch to yield a total count. The entire procedure was repeated on the right side of the fish and counts were compared for the entire sample using the non-parametric sign test. Fish that had a majority of their gill filaments with bubbles could not be counted quickly and were recorded as too numerous to count (TNC).

After completion of the examinations using dead fish, we repeated the procedure using live fish (N = 16) that had been anaesthetized in 50 mg/L MS-222. Fish were removed from the anaesthetic and placed in a plastic weighing dish slightly filled with supersaturated water. We exposed the gill tissue using blunt, serrated-tip forceps to grasp the opercle and lift it out of the way and conducted the examinations as described above. After the examination, fish were placed in a tank with normally saturated water and monitored for delayed mortality.

Results

Examinations using dead fish

We examined a total of 44 fish that were alive at the end of the TDG exposure. In addition, we examined 8 fish that had died; all of these fish had gill bubbles that were TNC and all had bubbles in at least one fin. Of the live fish we examined, only 4 of the 44 fish had no signs of GBT. Fourteen of the 40 fish with GBT had no gill bubbles but did have other signs of GBT. There were 26 of 44 fish with gill bubbles; of these, only 2 had exclusively gill bubbles. Of these 26 fish, 11 of them had gill bubbles that were TNC and 2 of them were discarded because of procedural anomalies. Thus, we used data from 9 fish for the gill examination comparisons.

The number of filaments with bubbles was highly variable among fish. In general, counts obtained by examining each individual arch and summing for a total count were higher than those obtained by whole tissue scans (Table). However, examining each individual arch was more time consuming, was difficult to do on live fish (discussed below), and had the potential to be more injurious to fish. Differences in gill bubble counts between the right and left side of the fish were negligible. Although we could discern no distinct trend in the location of bubbles amongst the arches, we noted that just 3 of the 13 fish had bubbles in only the 4th arch.

Examinations using live fish

We examined 16 live fish after exposure to 120% TDG for about 54 h. Although all fish had signs of GBT, only 6 of 16 had gill bubbles and none of these fish had only gill bubbles. There were always other signs (primarily fin bubbles) associated with the presence of gill bubbles. Fish generally had low numbers of bubbles in the gills (range from 0-12 filaments affected), and counts obtained by scanning the tissue mass vs. summing the counts from individual arches were similar but not statistically comparable due to the small sample size. Although fish were anaesthetized during these examinations, they often jumped and moved about excessively when the opercle was lifted or the gill arches were being moved. After the examinations, all fish were placed in normally saturated water and eventually showed full recovery from GBT with no delayed mortality.

Discussion

Despite of our small sample size for gill examinations we found non-lethal gill exams to be difficult in handling fish as they were sensitive to the manipulation of the opercle and/or gill arches and that the fish's reflex from touching these areas could result in injury. There is also uncertainty with the amount of time to employ a non-lethal gill exam, particularly an individual arch exam, adding to the difficulty of recovery. Even though individual arch counts revealed higher counts of gill filaments with bubbles than scanning, the majority of these filaments were still evident from a scanning exam except for the few that occurred in the fourth arch. Without further experimentation we remain optimistic as to which non-lethal technique is most suitable to examine gill bubbles; one that is passive and fairly indicative of GBT or an aggressive method for an absolute count. We have observed through our laboratory studies and literature review that GBT effecting the gills is widely variable depending on TDG levels. However, TDG levels above 120% can cause intravascular bubbles in the gills with only minor prevalence of gas emboli on the external anatomy (e.g. fins and lateral line).

CHAPTER 2

Gas Bubble Trauma Signs in Juvenile Salmonids at Dams on the Snake and Columbia Rivers

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Introduction

In 1994 a management decision was made to spill water to reduce turbine-related mortality of juvenile salmonids migrating past hydropower dams on the Snake and Columbia rivers. Spilling water over dams can cause gas-supersaturated water which in turn can cause gas bubble trauma (GBT) in aquatic organisms. Supersaturation occurs when the pressure of gases in the water is higher than barometric pressure and can be created when air is entrained in water spilling over dams. Gas supersaturation can also exist in natural aquatic environments.

Gas supersaturation in the Columbia and Snake rivers was a serious problem in the late 1960s and early 1970s when spill at dams caused supersaturated water for long stretches in the reservoirs (Ebel et al. 1975; Meekin and Turner 1974). Losses of adult and juvenile salmon were experienced during high flow years due to gas supersaturation and in low flow years due to juvenile fish going through the turbines (Meekin and Turner 1974; Ebel et al. 1975). River gas saturation levels reached 140% and were sustained through the reservoirs at 118% (Meekin and Turner 1974). These fish kills precipitated a number of studies to determine the cause and effects of gas supersaturation on juvenile salmon. In the 1970s the Army Corp of Engineers began studying structural changes to the dams which could reduce the creation of supersaturated water. Spillway deflectors, also known as flip lips, which reduce air entrainment were determined to be the best device to achieve this goal (Ebel et al. 1975). Conditions in the Columbia and Snake rivers improved significantly by 1975, and by 1979 it was believed that the problem of gas supersaturation was solved (Weitkamp and Katz 1980). The spill program has again focused attention on potential GBT problems in the Columbia and Snake rivers.

Severity of GBT can range from mild to fatal depending on level of supersaturation, species, life cycle stage, condition of the fish, and temperature of the water (Ebel et al. 1975; Meekin and Turner 1974; Weitkamp and Katz 1980). Symptoms can appear when saturation reaches 110% (Meekin and Turner 1974). However, mortalities increase dramatically as saturation levels increase above 120% - 125% (Dawley and Ebel 1975, Weitkamp 1974, 1976). All species of aquatic organisms can be susceptible to GBT; however, within a species the ability to tolerate supersaturated water can vary significantly (Nebeker and Brett 1976). It appears that death from GBT is caused by the formation of emboli in the cardiovascular system leading to a blockage of the circulatory system (Marsh and Gorham 1905). The emboli can fill the capillaries from the gill lamella back to the heart (Bouck 1980; Dawley et al. 1976). Bubbles can form in all internal organs and cavities, and disrupt neurological, cardiovascular, osmoregulatory, and respiratory function (Weitkamp and Katz 1980; Stroud et al. 1975). High arterial blood gases can cause an increase pressure in the swim bladder (Shrimpton et al. 1989). One of the most common external symptoms associated with GBT is subdermal emphysema of the skin, fins, eyes and mouth (Dawley and Ebel 1975; Marshal and Gorham 1905; Meekin and Turner 1974; Nebeker and Brett 1976). Bubbles can also appear in the lateral line, reducing the response to stimuli (Weber and Schiewe 1976). Some studies suggest that juvenile salmonids can avoid gas-supersaturated water. Because effective supersaturation decreases 10% for each meter of depth, fish can elude supersaturated water if compensation depth is available (Lutz 1995). In laboratory experiments by Stevens et al. (1980) coho, *Oncorhynchus kisutch* and chinook salmon *O. tshawsytcha* demonstrated lateral avoidance behavior. Meekin and Turner (1974) reported that chinook salmon given the choice between supersaturated or saturated water chose saturated water. Dawley et al. (1976) found that chinook salmon and steelhead may detect and avoid supersaturated water by sounding. Volition cage experiments by Meekin and Turner (1974) and Weitkamp (1976) indicated that fish spent enough time at adequate depths in the water column to avoid the effects of supersaturation.

Given the opportunity, fish can recover from GBT (Schiewe 1974; Meekin and Turner 1974). Recuperation from GBT, even in severe cases, is rapid and complete given adequate exposure to gas equilibrated water (Meekin and Turner 1974; Schiewe 1974). Mortalities from recovering fish are due primarily to secondary infections of lesions caused by subdermal emphysema (Weitkamp 1976). Repeated exposures may increase tolerance to supersaturated water (Weitkamp and Katz 1980). Cramer and McIntyre (1975) reported a genetic basis for differences in tolerance to GBT in some fall chinook salmon stocks in the Columbia River - implying that gas supersaturation was a mortality factor long before the construction of dams. The expectation that salmon can withstand some level of gas supersaturation is evident in the management decision to allow spill only to the extant that water below each dam not exceed 120% gas supersaturation.

The objectives of this study were to determine the proportion of juvenile salmonids migrating past dams on the Snake and Columbia Rivers that have signs of GBT based on non-lethal examination of the lateral line and fins; and to establish a database of percent of lateral line occluded with bubbles under various river conditions of total dissolved gas and temperature.

Methods

Fish were collected at Lower Granite, Little Goose and Lower Monumental dams on the Snake River and McNary, John Day and Bonneville dams on the Columbia River. Sampling was conducted 3 days a week by Columbia River Research Laboratory (CRRL) staff when the total dissolved gas (TDG) was below 120%; when TDG was above 120% sampling was conducted 7 days a week and Smolt Monitoring Program staff sampled on days when CRRL staff were not on site. Collections were made between 12:00 pm and 12:00 am and varied from site to site. At sites where there were bypass/collection systems, fish were collected from the separator. At John Day and Bonneville dams fish were collected by dip-basket or air-lift and fish were taken as quickly as possible from those structures.

Prior to collecting any fish, all equipment was set up and checked to be sure it was functioning properly. Each site had five 5-gal plastic buckets - three buckets for holding fish and two for irrigating fish gills during examination. Two holding buckets contained MS-222, buffered with bicarbonate, at concentrations of 80 and 30 mg/L made with water from the site of fish collection. As fish were collected they were put in the 30 mg/L bucket and taken to the examination station and then transferred one at a time, just prior to examination, to the 80 mg/L bucket. The third holding bucket was the recovery bucket and contained clean water (without anesthetic) with an air stone vigorously aerating the water and a lid to insure that fish did not jump out after recovering from the anesthetic. Two buckets were used to irrigate the fish gills during GBT examination. A valve regulated the flow of water (containing buffered 30 mg/L MS-222) down a length of surgical tubing. The end of the tubing was inserted into the mouth of the fish allowing the water to flow over the gills during the examination. A catch basin under the examination tray directed the water into the fifth bucket on the floor.

Forty fish per species were examined each sampling day. Species sampled were spring/summer or fall chinook salmon and steelhead *O. mykiss*. Sampling was done without regard to fin clips (i.e., no distinction was made between hatchery and wild fish); however, adipose clips were noted. Only as many fish as could be examined within 15 minutes of capturing the first fish were collected at one time. An exception was at John Day Dam and Bonneville Dam where samples were collected once each hour. After a fish was fully anesthetized, we recorded the fish's forklength, and placed it on the examination tray with the left side of the fish up and the gill irrigation tube in its mouth. Using a dissecting microscope (4 - 40x), the biologist examined the dorsal, caudal, and anal fins and noted the presence of any gas bubbles. Based on the absence or presence of bubbles, each fin was rated on the following scale:

0 = no bubbles 1 = 1 - 25% of fin was covered with bubbles

2 = 26 - 50% of fin was covered with bubbles

3 = greater than 50% of fin was covered with bubbles.

We then placed a bubbleometer on the side of the fish, parallel to the lateral line. Bubbleometers are narrow, flexible, clear plastic strips with unit-less hatch-marks, spaced about every 0.5 mm along its length. Several bubbleometers of various lengths were available and we used one that was at least as long as the fish's lateral line. Again using a dissecting microscope, we examined the lateral line for bubbles and counted the number of bubbleometer units that were occluded with bubbles. Using the same bubbleometer, we measured the length of the lateral line from the end of the caudal peduncle in a straight line to the operculum. If there were no bubbles in the lateral line, its length was not measured. We worked as quickly as possible; fish were put in the recovery bucket as soon as possible. After all fish in the batch had been examined, they were returned to the collection system.

All measurements were recorded in the appropriate place in the data sheet. After all fish were examined, or at intervals through the day, the data were transferred to the computer data file. After all data was entered into the computer file, we proofed the computer file against the written data sheet and corrected any erroneous entries. The computer file was transferred electronically to CRRL. Staff at CRRL reviewed the data and transferred it electronically to the Fish Passage Center.

In addition to the regular sampling of chinook and steelhead, gill bubble examinations were done on hatchery steelhead at Lower Monumental Dam, McNary Dam, John Day Dam, and Bonneville Dam from May 12 to June 9. Hatchery steelhead were collected in the usual way and placed in a lethal dose of buffered MS-222 (200 mg\ml). The fish was then placed on a moist paper towel and examined for bubbles in the lateral line and fins. The operculum from the left side was removed with a dissecting scissors and the gill mass was examined at 10x-40x magnification. The fish was tilted and the focus adjusted so as to obtain a clear view of the gill area. A probe was used to move the gill mass when necessary. The examiner counted the number of long, rod shaped bubbles present in the gill filaments. Then with a blunt probe and tweezers the gill arches were lifted up one at a time and any previously unseen bubbles were added to the count. When a large number of bubbles were present in the gill filaments, a code of TNC for “too numerous to count” was recorded. The gill examination was then repeated on the right side of the fish.

Results

Snake River - About 1,000 to 1,320 spring chinook salmon and an equal number of steelhead were sampled at each of the three Snake River dams from mid April through August (Table 1). Of all fish examined at each site, between 0.3% and 0.7% had signs of GBT (Table 1). On a daily basis, the prevalence of fish with any signs was usually less than 5% and never exceeded 10% (Figs. 1, 2, & 3). Although the number of fish with signs and the number of days when any signs were seen were low, it appeared that both increased when comparing Lower Granite to Little Goose to Lower Monumental (Figs. 1, 2, & 3). Because the severity of GBT signs was low, we did not present the data as means and variances, but rather the maximum severity seen (Table 1). The maximum percent of lateral line occluded with bubbles in any fish was 2.3% and all fish with fin bubbles had one fin with a rank of 1, so the highest average rank of any fish at any Snake River dam was 0.33 (Table 1).

Columbia River - Between 860 to 960 spring chinook salmon and somewhat fewer steelhead (356 to 820) were examined at each of the three lower Columbia River dams (Table 1). In addition, between 1350 to 1475 fall chinook salmon were examined at each dam; however, fewer than 0.1% (4 of 4,273) of the fall chinook salmon had any signs and those signs were very minor (Table 1; daily prevalence data not shown). The proportion of fish with any sign of GBT was low but it appeared that there was a greater prevalence of signs in fish at Columbia River dams than at Snake River dams (Table 1). Considering only the spring chinook salmon and steelhead, 3.7% (164 of 4573) of the fish examined at lower Columbia River dams had signs of GBT compared to 0.5% (35 of 7175) of fish examined at Snake River dams. On a daily basis, the prevalence of signs in spring chinook salmon exceeded 10% on only one occasion, at John Day Dam on May 9 (Fig. 5). Prevalence of GBT signs in steelhead exceeded 10% on several of occasions; however, sample sizes on those dates were less than 20 fish, except for two dates in May at McNary Dam (Fig. 4) and one date at Bonneville Dam (Fig. 6). Severity of GBT signs was low -- less than 2.5% occlusion of lateral line; however, one spring chinook salmon examined at McNary Dam had 4.8% of its lateral line occluded with bubbles.

In general there was no correlation between GBT signs in the fins and signs in the lateral line. That is, signs in one place did not mean that there would be signs in the other (Figs. 1 through 6). However, GBT signs were more prevalent in the fins than in the lateral line. Of the 208 fish with signs (all fish combined), four had bubbles in fins and lateral line, 52 had bubbles only in the lateral line, and 152 had bubbles only in the fins.

Table 1. Prevalence and severity of gas bubble trama in juvenile spring chinook salmon and steelhead sampled at collection facilities located at dams on the Snake and Columbia Rivers in 1995 during downstream migration.

Site[1]	Species[2]	Total Fish Sampled	Total Fish with Any Signs	Prevalence[3]	Max. % Occlusion of Lateral Line [4]	Max. Fin Bubbles[5]
LGR	SPCH	1015	4	0.4 %	0.5	0.33
	STHD	1181	3	0.3 %	0.3	0.33
LGS	SPCH	1317	6	0.5 %	0.0	0.33
	STHD	1221	4	03%	0.4	0.33
LMN	SPCH	1223	9	0.7 %	1.7	0.33
	STHD	1218	9	0.7 %	2.3	0.33
MCN	SPCH	962	24	2.5 %	4.8	0.67
	STHD	820	36	4.4 %	1.0	0.67
	FACH	1448	1	<0.1 %	0.0	0.33
JDD[1]	SPCH	860	29	3.4 %	2.3	0.33
	STHD	710	22	3.1 %	1.4	1.00
	FACH	1473	2	<0.1 %	0.7	0.00
BON	SPCH	865	41	2.0 %	0.7	0.33
	STHD	365	17	4.6 %	0.6	0.33
	FACH	1352	1	<0.1 %	0.0	0.33

l-Bon = Bonneville Dam JDD = John Day Dam MCN = McNary Dam LMN = Lower Monumental Dam LGS = Little Goose Dam LGR = Lower Granite Dam

2-SPCH = spring chinook salmon FACH = fall chinook salmon STHD = steelhead

3-Prevalence represents the percent of all fish sampled with any bubbles in their fins or lateral line

4-% Occlusion of lateral line = (Bubble Units \ Lateral Line Units) * 106

5-Fin Bubbles represents the sum of fin code values divided by three. Maximum value = 3.0
Fin rating: 0 = no bubbles; 1 = 1-25%; 2 = 26-50%; 3 = >50% of fin occluded.

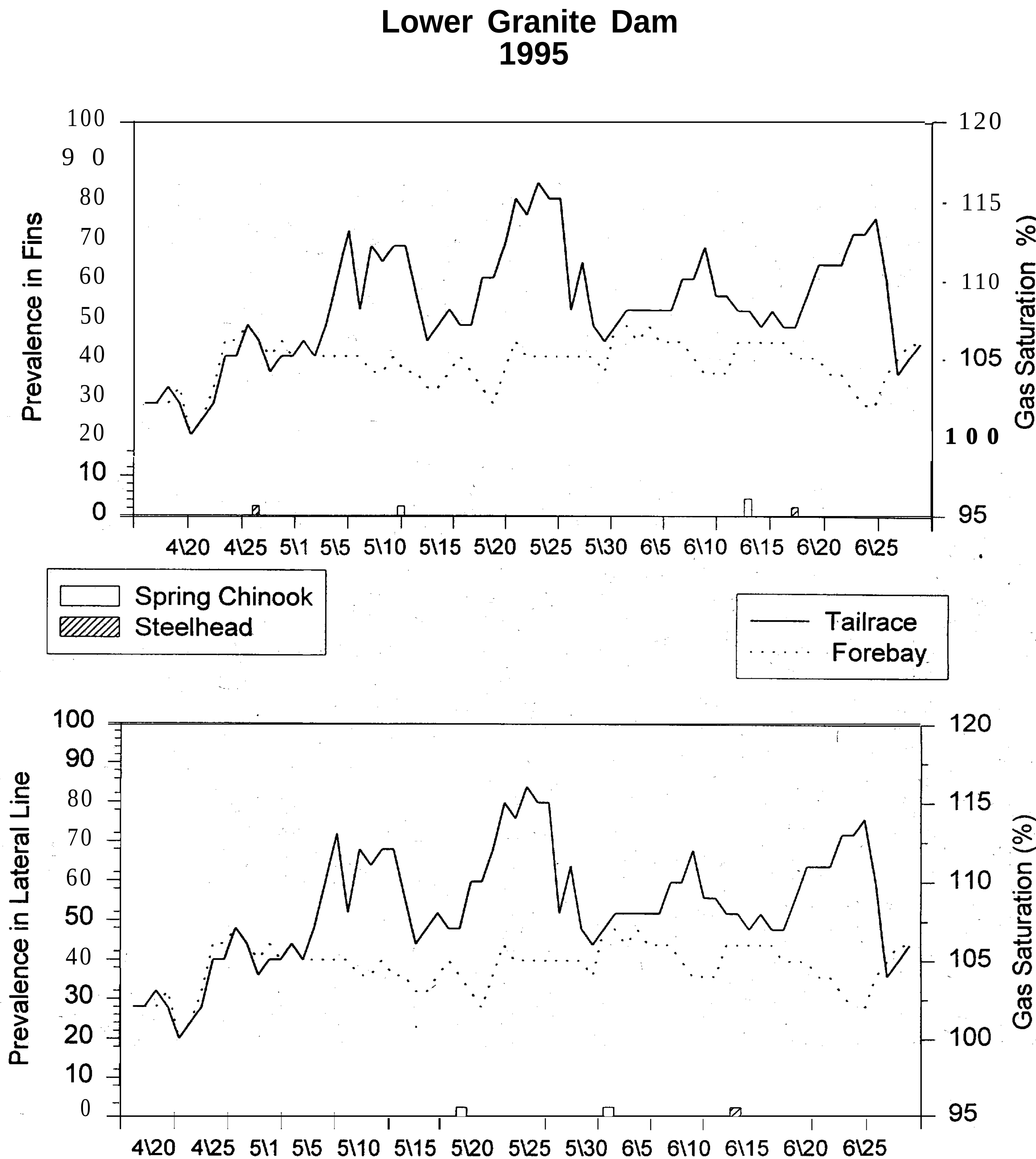

Figure 1. Prevalence (% positive) of bubbles in fins (top) and lateral line (bottom) of spring chinook salmon and steelhead and gas-saturation in the forebay and tailrace of Lower Granite Dam. The width of the graph bars in no way represents sample size, prevalence, or degree of seventy. Number above bar represents sample size when less than 40.

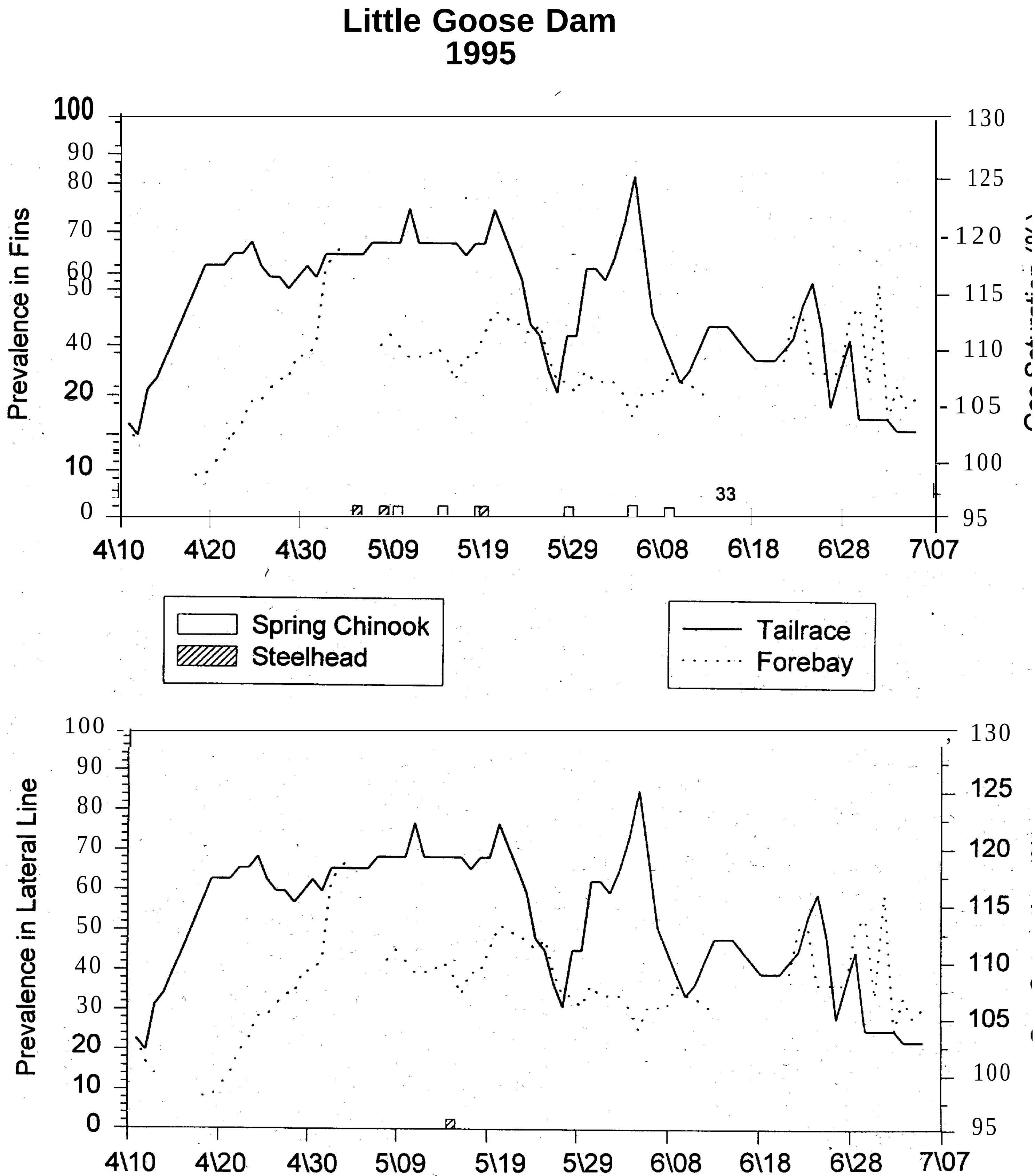

Figure 2. Prevalence (% positive) of bubbles in fins (top) and lateral line (bottom) of spring chinook salmon and steelhead and gas saturation in the **forebay** arid **tailrace** of **Little** Goose Dam. The width of the graph **bars** in no way represents sample size, prevalence, or degree of severity. Number above bar represents sample size when less than 40.

Lower Monumental Dam
1995

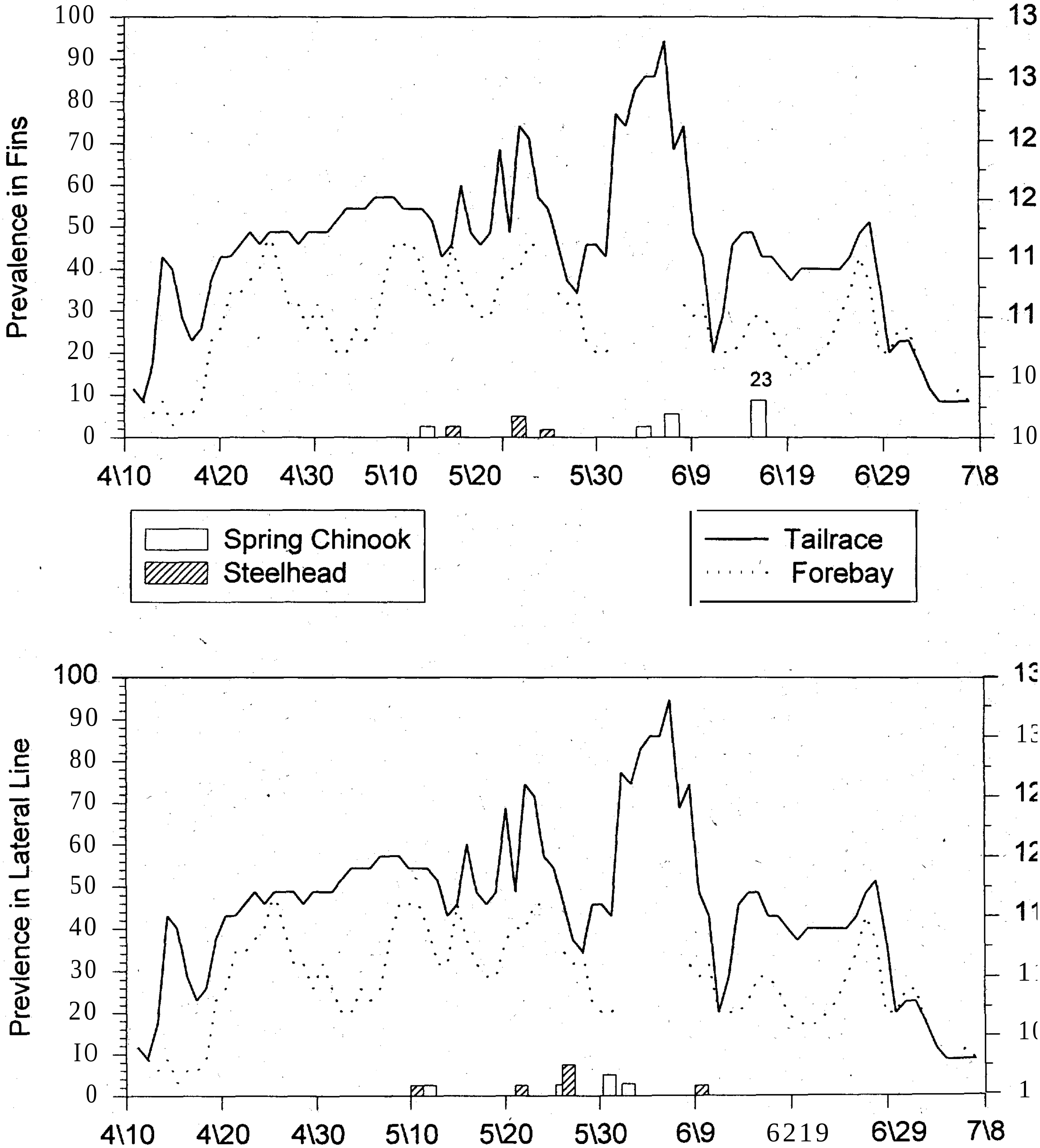

Figure 3. Prevalence (% positive) of bubbles in fins (top) and lateral line (bottom) of spring chinook salmon and steelhead and gas saturation in the **forebay** and **tailrace** of tower Monumental Dam. The width of the graph bars in no way represents sample size, prevalence, **or** degree of severity. Number above bar represents sample size when **less** than 40.

Gill bubble exams - As with the fish examined for bubbles in the fins and lateral line, the hatchery steelhead examined for gill bubbles showed few signs of GBT. Of the 940 fish sampled, 13 had gill bubbles; 10 of the 13 were examined at McNary Dam (Table 2). None of the 30 fish examined at John Day Dam had gill bubbles. One fish (out of 172) at Bonneville Dam and two fish (out of 390) at Lower Monumental were observed with gill bubbles. Of the 10 fish (out of 348) at McNary which had bubbles in the gills, three also had fin bubbles. However, none of these fish had a fin code rank above 1. None of the fish with bubbles in the gills also had lateral line bubbles. Of the 13 fish with bubbles present in the gills none had more than 8 filaments occluded with bubbles.

Discussion

Based on the GBT sampling conducted at Snake and Columbia river dams, it appears that gas supersaturation did not pose a threat to migrating juvenile salmonids during 1995. This is most evident in the Snake River where only 0.5% of all fish sampled had signs of GBT. In laboratory studies, Mesa et al. (Chapter 1, this report) indicated that spring chinook salmon held in shallow tanks at 12°C, and 120% or 130% supersaturation had a minimum of 25% prevalence of bubbles in fins and 100% prevalence in lateral line when the first mortalities occurred. There was no mortality in fish held at 112% supersaturation for 22 days even though prevalence of bubbles in fins and lateral line reached 100% and 75%, respectively. Mean severity of lateral line occlusion was at least 5% and mean ranking of bubbles in unpaired fins was 0.1 to 0.9 when first mortality occurred in the laboratory. In the monitoring program there were too

McNary Dam
1995

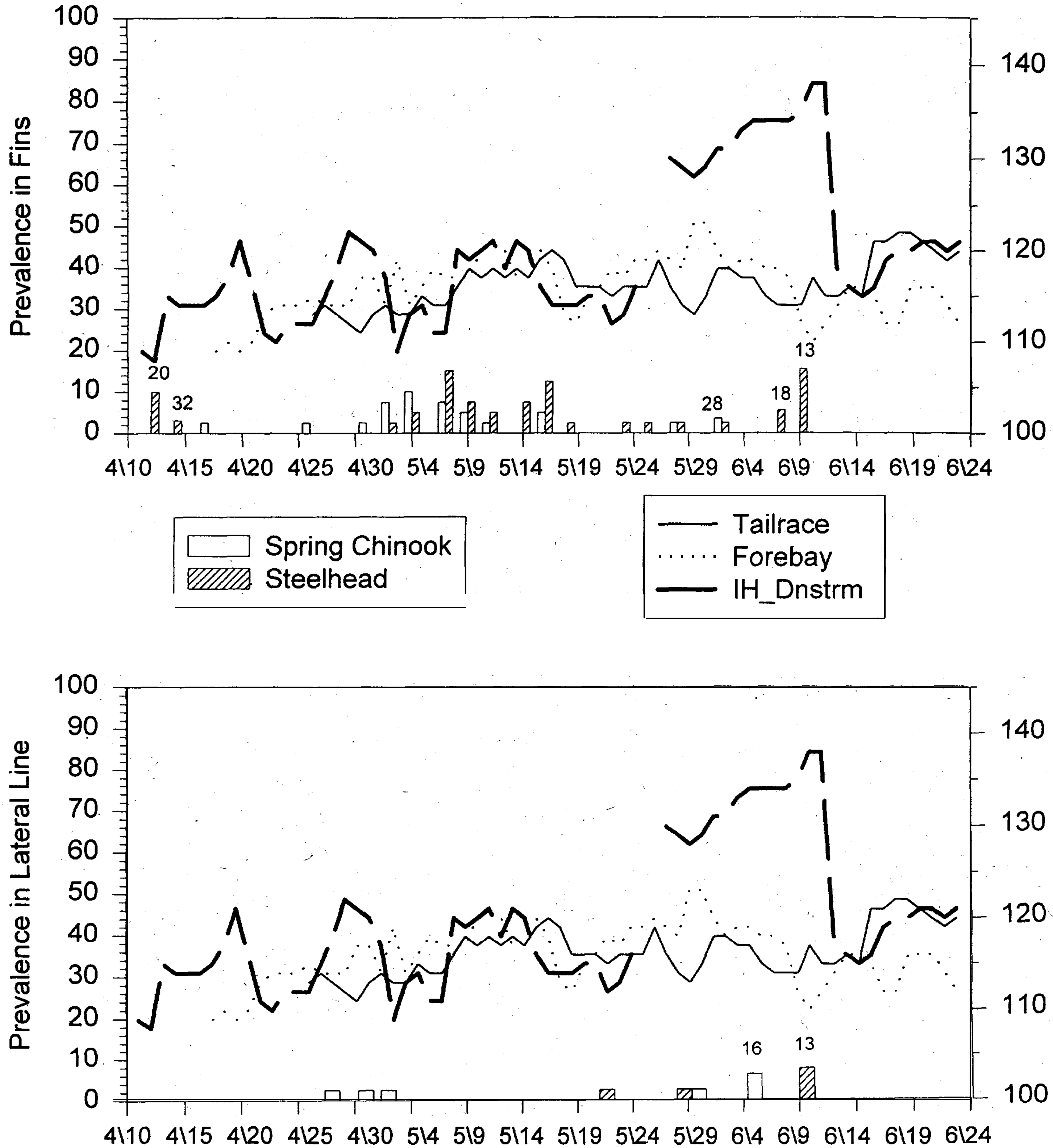

Figure 4: Prevalence (**%** positive) of bubbles in fins (top) and lateral line (bottom) of spring chinook salmon and steelhead and gas saturation downstream of Ice Harbor Dam (**IH_Dnstrm**) and in the **forebay** and **tailrace** of **McNary** Dam. The width of the graph bars in no way represents sample size, prevalence, or degree of severity. Number above bar represents sample size when less than 40.

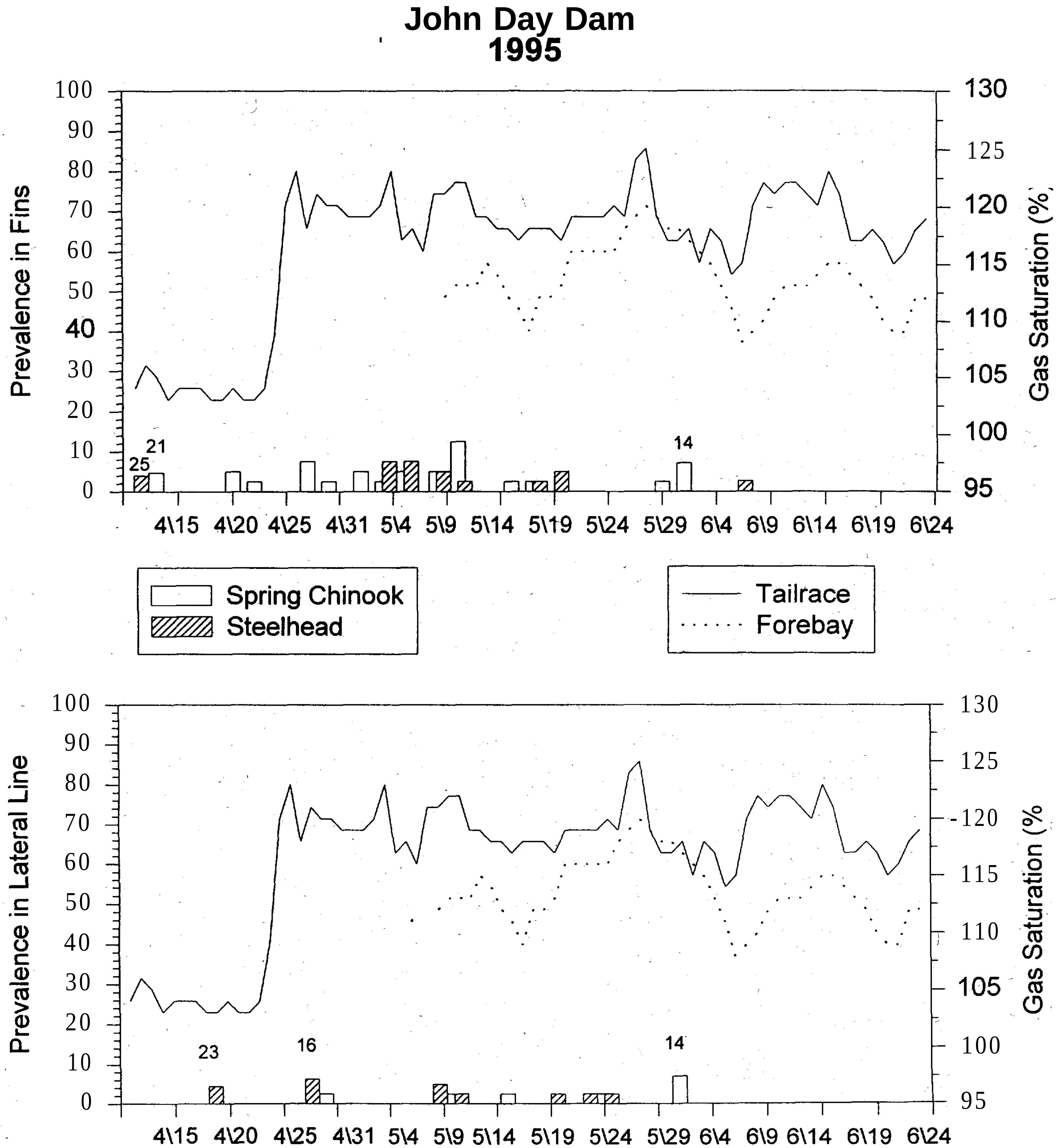

Figure 5. Prevalence (% positive) of bubbles in fins (top) and lateral line (bottom) of spring chinook salmon and steelhead and gas saturation in the **forebay** and **tailrace** of John' Day Dam. The width of the graph bars in no way **represents** sample size, p&valence, or degree of severity. **Number above** bar represents **sample** size when less than 40.

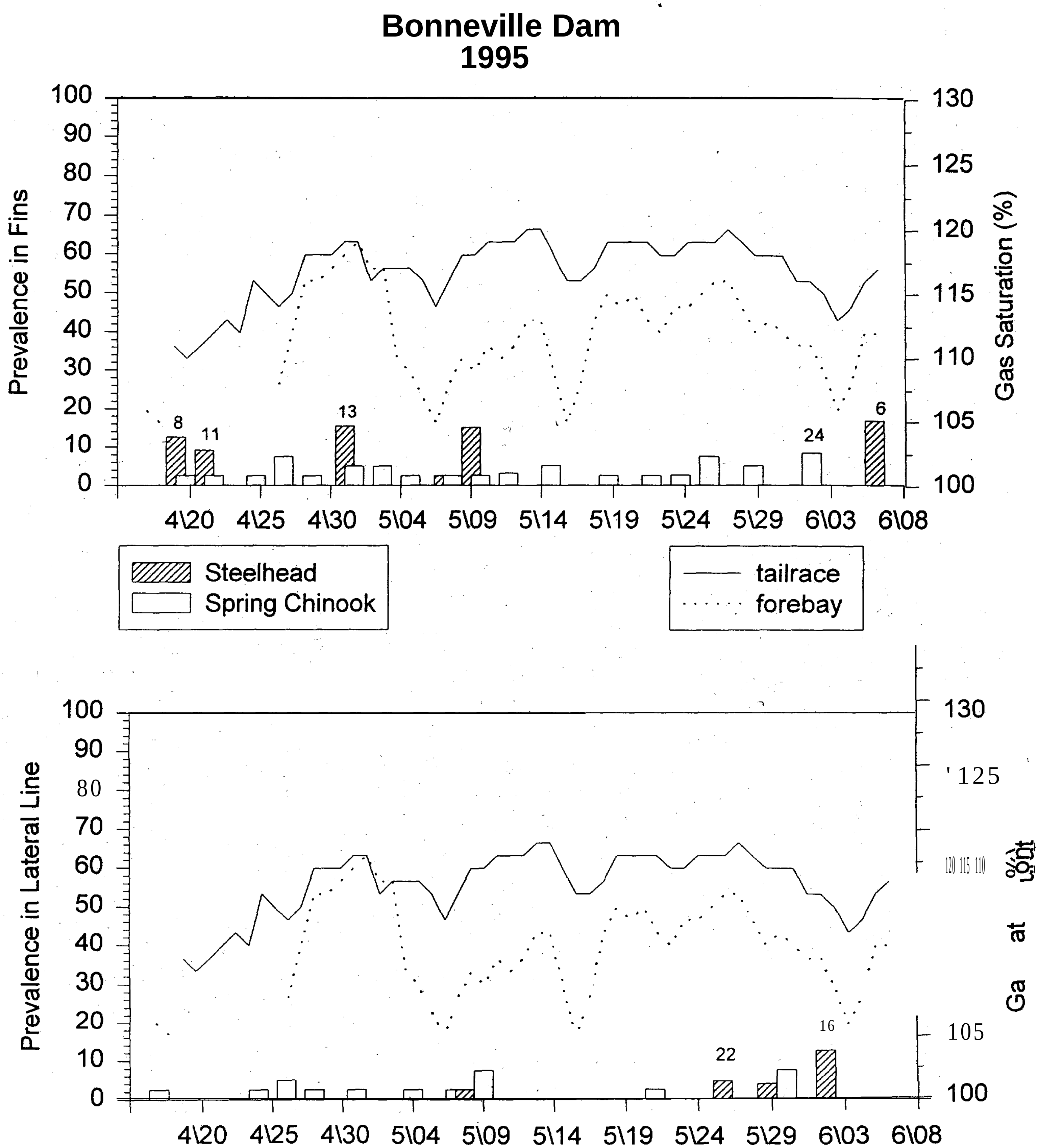

Figure 6. Prevalence (% positive) of bubbles in fins (top) and lateral line (bottom) of spring chinook salmon and steelhead and gas saturation in the **forebay** and **tailrace** of Bonneville Dam. The width of the graph bars in no way represents sample size, prevalence, or degree of severity. Number above bar represents sample size when less than 40. **Tailrace** TDG % taken at Warrendale, **OR,** four miles down river from Bonneville Dam.

Table 2. Number of hatchery **steelhead** with bubbles in the gills. Fish were sampled at collection facilities located at **dams** on the Columbia and Snake Rivers.

Site[1]	Total Fish Sampled	Total Fish with Gill Bubbles	Total Fish with Fin Bubbles[2]
BON	172	1	0
JDD	30	0	0
MCN	348	10	3
LMN	390	2	0
Total	940	13	3

I-Bon = **Bonneville** Dam JDD = John Day Dam MCN = **McNary** Dam LMN = Lower Monumental Dam

2-Equals the number of fish sampled with bubbles in the gill and also in the fins. None of the fish examined had bubbles in the gills and lateral-line.

few fish with GBT signs for mean values to be meaningful, but the low maximum values for individual fish with GBT signs would suggest that GBT was not a problem at the population level. Furthermore, the fact that there were consistently more fish with GBT signs in the fins than fish with GBT signs in the lateral line, and that the lateral lines were always less than 5% occluded, is suggestive of the lower level, potentially non-lethal TDG-exposure seen in the 112% laboratory experiment as opposed to the fatal exposures at 120% and 130% (Mesa et al. Chapter 1).

Even though there was low prevalence and severity of GBT signs in fish collected at the dams, trends in the data are consistent with what one would expect if one assumes a worst-case exposure history. That is, assuming that all fish sampled were from the same population and experienced similar TDG-exposure histories beginning at Lower Granite Dam and continuing downriver. Fish collected at Lower Granite had the lowest prevalence and among the lowest severity of GBT; fish collected at dams successively further downstream in the Snake River had increasingly higher prevalence and severity of GBT. There was an apparent increase in overall prevalence of GBT in spring chinook salmon and steelhead sampled at McNary Dam as compared to those in the Snake River suggesting that either (1) fish migrating from the upper and mid Columbia River had TDG-exposure histories that caused GBT signs greater than those of fish from the Snake River and/or (2) Snake and Columbia river fish were affected by the higher gas supersaturation caused by the uncontrolled at Ice Harbor Dam, the last dam on the Snake River.

There are several possible explanations for the low proportion and severity of GBT signs in the fish examined at the dams, including: (1) fish with serious GBT died in the river before reaching the dams, (2) fish lost signs of GBT moving from the river through the fish collection systems at the dams, as the result of changing hydrostatic pressure, and (3) the impact of GBT was minimal. Limited in-river sampling (Dr. Thomas Backman, Columbia River Inter-Tribal Fish Commission, unpublished data) suggests that the prevalence and severity of GBT in fish sampled at the dams was the same as that of fish in the river. The rule of parsimony requires taking the simplest explanation that explains the data and that explanation is that GBT was not a problem for juvenile salmonids migrating in the Snake and Columbia rivers in 1995.

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