Acute Effects of Gas Supersaturation on Juvenile Cultured White Seabass

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Abstract

White seabass *Atractoscion nobilis* reared at a production and research hatchery have been observed to occasionally suffer from ocular emphysemas. To identify a possible cause of these lesions, cultured juvenile white seabass were exposed to five gas saturation levels between 98% and 122% total gas pressure (TGP). Experiments were run for 96 h using fish weighing 3 and 22 g at water temperatures of 17.9 ± 0.3°C or 23.0 ± 0.3°C. Fish were observed every 8 h for symptoms of gas bubble disease. Throughout the trial, no fish died when exposed to 98, 102, or 109% TGP and only one fish died at 116% TGP. When fish were exposed to 122% TGP, however, mortality was 5 percentage points greater for both small and large fish in 23°C water than for those at 18°C and 20 percentage points greater for the large than for the small fish at both temperatures. The most prevalent and severe lesions were in large fish exposed at 23°C, followed by small fish exposed at 23°C, large fish exposed at 18°C, and small fish exposed at 18°C. Corneal emphysemas were the most common lesion, affecting even the control group of large fish in 23°C seawater. For fin emphysemas, only the large fish at 18°C were affected at both 116% and 122% TGP. These fish also had the highest incidence of fin emphysemas (50%), followed by the large fish at 23°C (38.3%), small fish at 18°C (6.7%), and small fish at 23°C (1.7%). These results indicate that temperature, size, and gas supersaturation all play roles in the onset of gas bubble disease for white seabass. These factors should be investigated in any hatchery setting when ocular emphysemas arise.

The white seabass *Atractoscion nobilis* is the largest sciaenid found in the waters off California and Baja California (Miller and Lea 1974). White seabass have long been prized by both commercial fishers and sportfishing enthusiasts because of their size and quality as a food fish (Moser et al. 1983). However, during the 1960s the number of white seabass declined so rapidly that in 1984 an experimental restocking program was established to help ameliorate this problem (Vojkovich and Reed 1983). Because of this, the Ocean Resource Enhancement and Hatchery Program was created to evaluate the feasibility of using

stock enhancement to augment populations of declining marine fishes in Southern California. In 1995 the Leon R. Hubbard, Jr., hatchery was constructed and began rearing white seabass. The primary goal of the hatchery was to develop and implement techniques and protocols that would provide a steady supply of healthy and vigorous white seabass to test stock enhancement concepts through a tag-and-release program. To accomplish this goal, a great deal of effort has gone into controlling both water temperature and gas saturation levels. This control is necessary because rapid changes in temperature or biological processes can increase gas saturation levels sufficiently to cause gas bubble disease (GBD) in fish (Woodbury 1941; Renfro 1963).

Although the hatchery has made considerable improvements in maintaining overall water quality since its initial construction in 1995, incidences of GBD, specifically gas-related ocular lesions, continue to occur in hatchery-reared fish. White seabass have been observed to suffer from exophthalmia (abnormally protruding eyeball, or pop-eye) and intraocular emphysema (abnormal accumulation of air in the eyeball) in both hatchery and net-pen environments. Since white seabass with severe ocular lesions are routinely culled from hatchery pools, it is imperative to understand and identify the cause of such abnormalities.

Although exophthalmia is not solely symptomatic of GBD (Dukes and Lawler 1975; Stroud et al. 1975), we did find that gas supersaturation (GSS) levels in the hatchery seawater were relatively high, ranging from 99.8% to 116% total gas pressure (TGP). Because GSS has been known to cause ocular emphysemas in fish (Weitkamp and Katz 1980), we decided to test the hypothesis that this was responsible for the observed eye lesions. Timmons et al. (2002) also indicated that water quality in regards to GSS should be maintained below 110% TGP with no more than 103% of that being nitrogen gas.

We developed a series of 4-d exposure trials to study the causative elements of GBD and to determine the susceptibility of white seabass to GSS. This type of study is commonly used with teleost fish to test their susceptibility to supersaturation, metals, and organic xenobiotic contaminants (Backman et al. 1991; Krise and Smith 1991). Some researchers have even compared different size-classes of fish in multivariate dose–response tests (Gray et al. 1985; Jensen et al. 1986). In this study, we sought to gain a broader understanding of the causative factors associated with GSS and GBD in white seabass, so that practical solutions can be developed to prevent or treat this disease through all rearing stages.

METHODS

Operational parameters.—A total of 12 experiments were completed during this study. Experiments were subdivided into four sets of three trials each. For a given experimental set, three trials were run using similarly sized fish under matching water quality parameters. Trials were initiated 1 week apart, which allowed time for system maintenance between experiments. Two

weeks were allotted between experimental sets to stabilize TGP treatments under the new temperature regime.

Before fish were added, treatment conditions were established by mixing an air-equilibrated water source maintained at 623.2 mm Hg (above atmospheric pressure) with a saturated seawater source passed through a degassing tower. The control saturation level (98% TGP) was achieved by means of a vacuum degasser. Temperature was computer controlled, and seawater flow through each 75-L aquarium was regulated, ranging from 1.2 to 1.7 L/min. For a full description of the system, along with specific partial pressures, see Smiley and Drawbridge (2008).

Experimental design.—Two size-classes of fish were used in this study. The "small" class was 53–67 d posthatch and weighed 3 ± 1.3 g (mean $\pm$ SD), while the "large" class was 98–114 d posthatch and weighed 22 ± 6.1 g. To begin each experiment, fish were screened for eye lesions and transferred with dip nets from their original culture pool into 1 of the 10 treatment aquaria until all aquaria had 10 fish (two replicates for each of the five saturation levels). There were four experimental groups. Group A consisted of large fish in 18°C water, group B of small fish in 18°C water, group C of large fish in 23°C water, and group D of small fish in 23°C water (Table 1). For the first six trials (groups A and B), fish were acclimated to 18°C over a 5-d period before each experiment in their original culture pools. This was accomplished by decreasing seawater temperature 1°C per day from the initial culture temperature of 23°C and then maintaining them at 18°C for 2 d before starting any experiments. The second series of six trials (groups C and D) were conducted at 23°C and did not require temperature acclimation. The gas saturation in the culture pools ranged from less than 100% to 101.6% TGP.

Data collection.—Water quality measurements and fish observations were made every 8 h. Water quality measurements were taken by submerging a Hydrolab MiniSonde (Hach Hydromet, Los Altos, California) into a mesh holder in one corner of the aquarium. The variables measured included salinity (±0.1‰), total dissolved gas (±1.0 mm Hg), dissolved oxygen (±0.1%, ±0.1 mg/L), temperature (±0.1°C), and barometric pressure (±1.0 mm Hg). Observational data were recorded by counting the number of fish suffering from cutaneous emphysemas, ranking and counting the number of fish suffering from ocular emphysemas, and counting and removing any fish that died. Cutaneous emphysemas were characterized by discrete, oval to linear, refractile gas bubbles trapped beneath the skin and recorded as either present or absent in the fish. The locations of emphysemas were also recorded but severity was not (Figure 1). The ocular emphysemas, which included thin-walled, translucent, refractile, raised, and fixed lesions within the cornea and exophthalmia, were ranked quantitatively (Figure 2).

It was not possible to track individual fish throughout the experiments, so a mean corneal lesions score was calculated for each treatment group. To calculate the mean score, all 10 fish in each tank were ranked based on eye lesion severity and the most

TABLE 1. Summary data for small and large white seabass showing the measured temperature, fish age at the start of the trial, actual weight, and length for each trial.

Experimental group	Trial	Measured temperature (°C)	Fish age (d)	Weight (g)[a]	Length (cm)[a]
Large fish; 18°C	1	17.8 (0.2)	114	26.4 (4.3)	14.6 (0.92)
	2		120		
	3		114		
Small fish; 18°C	4	18.0 (0.3)	57	2.6 (1.0)	6.7 (0.97)
	5		63		
	6		57		
Large fish; 23°C	7	22.9 (0.3)	98	18.3 (5.1)	13.0 (1.38)
	8		105		
	9		112		
Small fish; 23°C	10	23.1 (0.2)	53	3.7 (1.4)	7.7 (0.94)
	11		60		
	12		67		

[a] Means (SDs in parentheses) for all trials with the specified groups.

severe lesion was reported. The ranks were then averaged for that time series and graphed over time to indicate progression. At the end of each trial, fish were euthanized with a lethal dose of tricaine methanesulfonate (0.3 g/L MS-222; Western Chemical, Ferndale, Washington) before total length (± 0.1 cm) and wet weights (± 0.1 g) were taken. A final semiquantitative corneal emphysema score for each fish eye was determined at this time.

Statistical analyses.—The prevalence of corneal emphysemas was compared among trials and treatments by means of Cochran's Q test for equal proportions. A contingency table was used to compare corneal emphysema severity among treatments. Fish lengths and weights were compared by means of

a Kruskal–Wallis one-way analysis of variance. This test was chosen because the population was not normally distributed (Shapiro–Wilk test: $P < 0.05$). All statistical results were deemed significant at the 95% level.
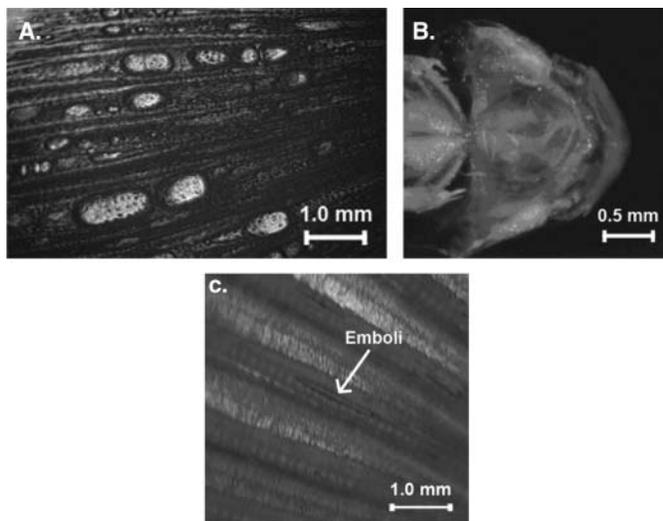
FIGURE 1. Examples of emphysemas in the **(A)** pectoral fin, **(B)** roof of mouth, and **(C)** gills of white seabass exposed to saturation levels of 116% and 122% total gas pressure.
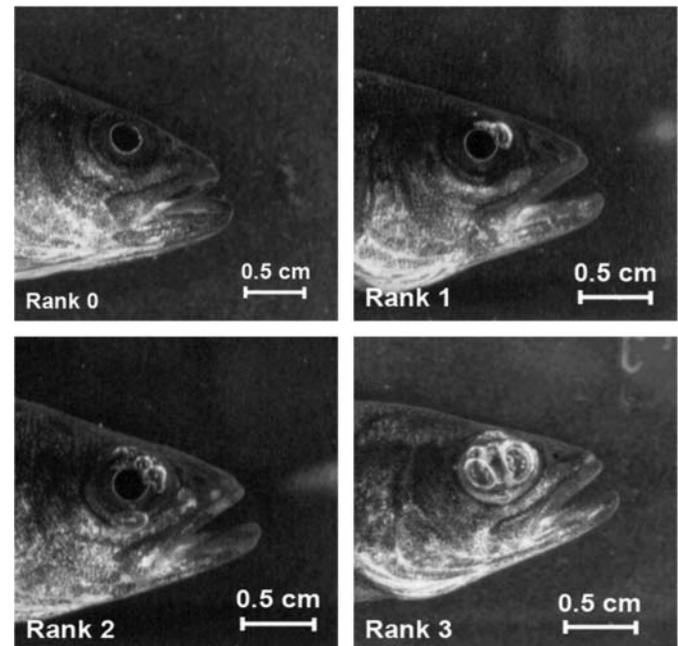
FIGURE 2. Examples of rank categories for corneal emphysemas observed in white seabass exposed to saturation levels ranging from 98% to 122% total gas pressure over 96 h at 18°C and 23°C. Rank 0 indicates that no corneal emphysema was present. If the emphysema covered less than one-fourth of the eye it was scored as mild (rank 1); if it covered more than one-fourth but less than one-half of the eye, it was scored as moderate (rank 2); and if it covered more than one-half of the eye or the fish was suffering from a corneal lesion and exophthalmia, it was scored as severe (rank 3). When corneal emphysemas were observed in both eyes, the score for the more severe eye was recorded.

TABLE 2.   Fin emphysema prevalence, mortality, and eye lesion occurrence (in fish that expired) for white seabass exposed to 116% and 122% total gas pressure (TGP). Mortality was summed for all trials in each experiment and divided by 60 (the number of fish at the start of each trial) to create percentages. No fish held below 116% TGP exhibited fin emphysemas or mortality.

Fish size	°C	TGP (%)	Fin emphysema prevalence (%)	Mortality (%)	Eye lesions (%)
Small	18	116	0	2	0
	18	122	7	20	0
	23	116	0	0	0
	23	122	2	25	67
Large	18	116	8	0	0
	18	122	50	40	22
	23	116	0	0	0
	23	122	38	45	63

RESULTS

There were no statistical differences in length or weight among small and large white seabass within experimental groups (Kruskal–Wallis: $n = 100$, $P > 0.05$). There were, however, statistical differences between groups (Kruskal–Wallis test: $P < 0.05$).

Mortality

No deaths occurred among small or large white seabass exposed to the three lowest TGP treatment levels (98, 102, and 109%), and only a single small white seabass died when exposed to 116% TGP at 18°C. At 122% TGP, mortality was 5 percentage points greater for both small and large fish in warm water than in cool water and 20 percentage points greater among large fish than among small fish at both temperatures (Table 2). The time to 20% mortality was between 56 and 64 h for large fish at 23°C, 64 h for large fish at 18°C, 72 h for small fish at 23°C, and 96 h for small fish at 18°C. The first deaths occurred at 16 h for large fish at both temperatures, at 24 h for small fish at 23°C, and at 40 h for small fish at 18°C (Figure 3). No correlation could be made between gas loading (eye lesion progression) and mortality. Some fish died with no external signs of GBD.

Behavioral Observations

The behavioral signs associated with exposure to increasing levels of GSS in the white seabass included loosing buoyancy control, lethargy, bumping the walls and bottoms of the aquaria, darting sporadically, shaking violently, opening their mouths rapidly and flaring their gills (cough), orienting to other fish (schooling; nonswimming), and swimming against the bottoms of the aquaria.

When fish lost buoyancy control it was obvious because they attempted to swim downward. This behavior did not occur at exposures of 116% TGP or less. However, at 122% TGP, the prevalence of downward swimming was 18 times greater in the large fish exposed at 23°C (10.8%) than in the large fish exposed at 18°C (0.6%) and 13 times greater in the small fish exposed at 23°C (10.5%) than in the same size fish exposed at 18°C (0.8%). The onset of this behavior in the large fish began 32 h into the

exposure period at 23°C and at 64 h at 18°C. The small fish followed the same trend.

Cutaneous Emphysemas

Cutaneous emphysemas were observed in all major fins (except pelvic fins) and affected only the fish exposed to 116% TGP or more. The number of fish affected was significantly greater at 18°C for both size-groups ($P < 0.008$), and there was a significant difference between size-classes, the large fish exhibiting more lesions ($P < 0.001$). The time of onset was also much earlier for the large fish (16 h) than for the small fish (88 h; Figure 3). Not all fish suffering from cutaneous emphysemas were observed to have corneal emphysemas. Occasionally, in the fish exposed to the highest gas saturation level, emphysemas and gas emboli were observed in the roof of the mouth (hard palate), subcutaneous tissues over the head, and blood vessels in the gills (Figure 1).

Corneal Emphysemas: Prevalence and Severity

The prevalence and severity of corneal emphysemas (as indicated by cumulative and average scores) increased sharply with increasing gas saturation levels. The most prevalent and severe lesions for a group were in the large fish exposed at 23°C, followed by the small fish exposed at 23°C, the large fish exposed at 18°C, and the small fish exposed at 18°C (Table 3).

TABLE 3.   Percent prevalence of corneal emphysemas in small and large white seabass exposed to gas saturation levels ranging from 98% to 122% total gas pressure (TGP) in seawater at 18°C and 23°C.

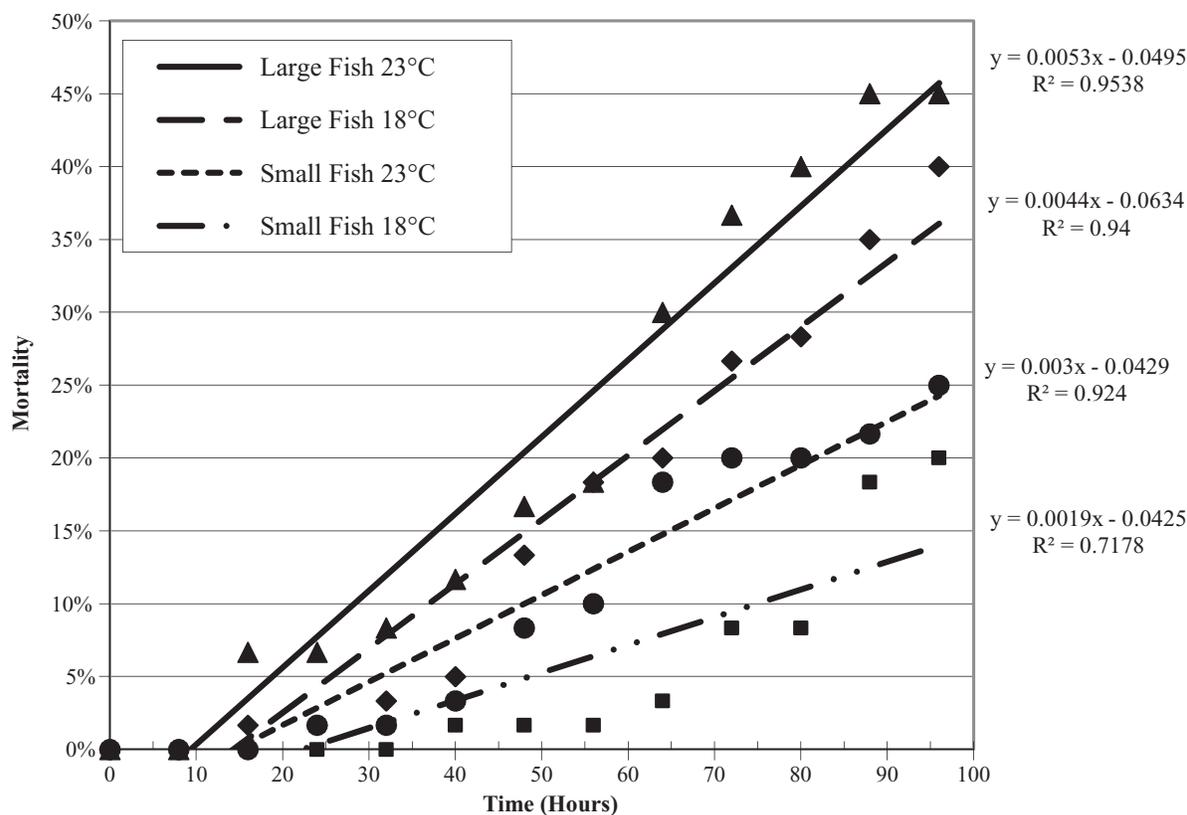| TGP (%)	Small fish		Large fish	
	18°C	23°C	18°C	23°C
98	0	0	0	2
102	0	5	5	10
109	0	23	18	53
116	5	43	25	58
122	12	65	33	63
Mean	3	27	16	37

FIGURE 3. Relationships between time of exposure and mortality for two sizes of white seabass exposed to two temperatures at 122% total gas pressure. Mortality was summed for all trials in each experiment and divided by 60 (the number of fish at the start of each trial) to create percentages. Linear trend lines, equations, and R^2 values have been added to the graph to allow extrapolation.

Among severely affected fish, multiple emphysemas were observed to coalesce, forming an irregular, multichamber layer covering the entire surface of the eye. Some fish with severe corneal emphysemas also exhibited moderate periocular hemorrhage.

The size of the fish was an important determining factor in the development of ocular lesions. In comparisons made with the same GSS levels and temperatures, small fish consistently had fewer and less severe corneal lesions than large fish. The small white seabass exposed to gas saturation levels at 18°C had the lowest mean corneal emphysema scores and were only affected at 116% and 122% TGP. The large fish exposed to 18°C water had higher mean corneal emphysema scores for both 116% and 122% TGP and were affected at 102% and 109% TGP as well. There were similar findings at 23°C; large fish consistently had higher mean corneal emphysema scores and prevalence rates.

Temperature also influenced the prevalence and severity of corneal lesions. Although the most severe lesions were generally observed in large fish, the small fish exposed to saturation levels ranging from 109% to 122% TGP at 23°C had higher corneal emphysema scores and prevalence rates than the large fish subjected to the same treatment levels at 18°C (Table 3). Comparisons of temperature influences within specific size-classes also revealed that the large fish exposed at 23°C had mean scores and prevalence rates more than twice those for similarly sized fish at 18°C for almost all GSS levels. Among the small fish at 18°C, corneal lesions only developed in fish exposed to the two highest TGP levels. In contrast, corneal emphysemas were observed at all saturation levels above the control level when the small fish were exposed at 23°C (Table 3).

For the two lowest treatment groups of 98% and 102% TGP, the only significant increase in the prevalence of corneal emphysemas occurred in the large fish at 23°C ($P = 0.025$). Above 102% TGP, the prevalence of corneal emphysemas increased significantly with increasing gas saturation level for the small fish at 23°C ($P = 0.001$) and the large fish at 18°C ($P = 0.008$). There was no significant increase in the prevalence of corneal emphysemas for the small fish at 18°C until they were exposed to 122% TGP. For the large fish at 23°C, the prevalence of corneal emphysemas increased significantly at 109% TGP but not at 116% or 122% TGP. The severity of corneal emphysemas was not independent of the saturation level for all trials at 23°C ($P = 0.083$).
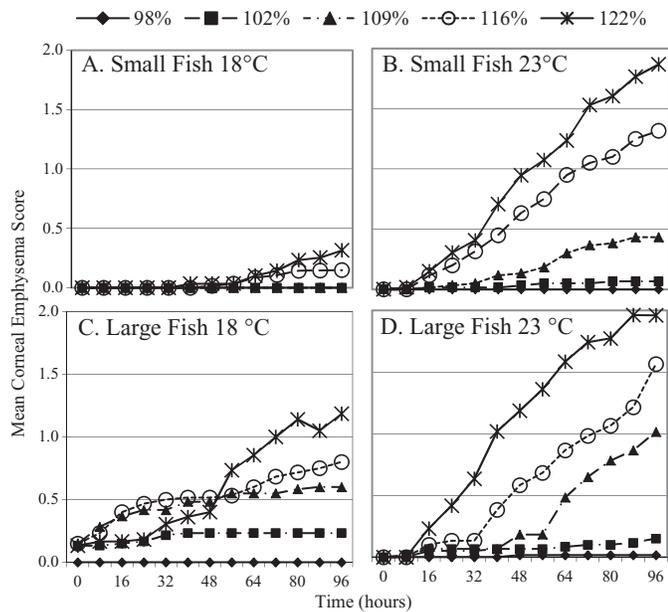
FIGURE 4.   Mean ranks for corneal emphysemas scored every 8 h for 96 h at saturation levels ranging from 98% to 122% total gas pressure in small and large white seabass held in seawater at 18°C and 23°C. Corneal emphysemas were ranked from 1 to 3 based on severity (see Figure 3), and scores were averaged for surviving fish.

Corneal Emphysemas: Onset and Progression

The onset and progression of corneal emphysemas was generally faster among larger fish, at higher TGP levels, and in warmer water. Among the small fish exposed to 116% and 122% TGP in 18°C water, the onset of corneal emphysemas was observed 40 h post-initiation of GSS treatment (Figure 4A). In contrast, similarly sized fish exposed to the same GSS levels at 23°C began to develop corneal lesions at 16 h (Figure 4B). Among larger fish, the onset of corneal emphysema differed only slightly between temperatures. At 18°C, corneal lesions first were observed 8 h after the GSS treatment was initiated (Figure 4C). At 23°C, corneal emphysemas were noted 16 h into the experiment (Figure 4D).

The progression of lesions followed similar trends. The mean corneal emphysema score was less than 0.2 at 116% TGP and less than 0.4 at 122% TGP at 96 h for the small fish exposed at 18°C (Figure 4A). In contrast, the mean emphysema score for the small fish exposed to 116% TGP at 23°C was greater than 0.6 at 48 h and greater than 1.2 at 96 h. For the small fish exposed to 122% TGP at 23°C, the mean score was greater than 0.9 at 48 h and greater than 1.8 at 96 h (Figure 4B). For the large fish exposed to 122% TGP at 18°C, the mean score was greater than 0.5 at 48 h but 1.2 or less for the highest treatment group at 96 h (Figure 4C). For the large fish at 23°C, the mean score was 1.2 at 48 h and 2.0 at 88 h (Figure 4D). The progression of corneal emphysemas in the three highest TGP treatment groups was almost twice as fast in the large fish exposed at 23°C.

DISCUSSION

The results of this study indicate that there are clear differences in the sensitivity and response of cultured white seabass to supersaturated seawater with respect to fish size, water temperature, and TGP level. Responses to acute GSS exposure ranged from mortality and changes in behavior to variations in the type, prevalence, and severity of ocular and cutaneous lesions.

Mortality and Tolerance

In general, the white seabass were resistant to the lethal effects of GSS during 96-h exposures at levels up to 116% TGP. However, exposure to 122% TGP was lethal, and deaths were presumed to have resulted from gas emboli blocking vascular flow to critical organs. This was evidenced by emboli found in the gills and hemorrhage seen in the eyes. The white seabass in 23°C seawater died faster and at a higher rate than the fish in 18°C water. The time to 20% mortality for 122% TGP exposures conducted at 18°C was achieved at 64 h for the large fish and 96 h for the small fish. When 122% TGP exposures were conducted in 23°C water, the time to 20% mortality was between 56 and 64 h for the large fish and 72 h for the small fish. Factors that could have contributed to the earlier attainment of 20% mortality in the warm water are as follows: (1) a faster rate of swim bladder overinflation, (2) a larger volume of swim bladder gas, (3) a greater number of gas emboli with a faster rate of formation, (4) a larger mean size of circulating gas emboli, and (5) a change in gas permeable membranes.

At 122% TGP, the large white seabass were significantly ($P < 0.02$) more sensitive to the lethal effects of GSS exposure than the small fish. The discovery that small white seabass were more tolerant of higher GSS levels than large fish is consistent with research conducted on Atlantic salmon *Salmo salar* and lake trout *Salvelinus namaycush* (Krise and Herman 1991), European seabass *Dicentrarchus labrax* and striped mullet *Mugil cephalus* (Gray et al. 1985), and Chinook salmon *Oncorhynchus tshawytscha* (Meekin and Turner 1974). This difference may be due to differential metabolism, loss of emboli from the blood vessels, and even differences in the target organs because of the fish's being in a different development stage.

When comparing temperature effects, both size-classes of white seabass were less tolerant of GSS when exposed to the warmer temperature. Similar trends have been reported by Gray et al. (1985) in European seabass and are believed to be caused by increased ventilation in warm water. Other possible causes include an increase in gas diffusion at warmer temperatures, the higher rates of gas production by the gas gland (swim bladder) and choroid rete mirabile (eye) under GSS conditions, and the greater expansion of gas at higher temperatures. Owing to the increased buoyancy caused by excess gas in the circulatory system, many fish will migrate vertically to deeper water to escape any harmful effects (Knittel et al. 1980). However, if fish cannot access an adequately deep water column (which is common in hatchery settings), then increased mortality is expected (Ebel and Raymond 1976).

While direct comparisons are not available for white seabass, sensitivity across species indicates similar mortality patterns. Mesa et al. (2000) reported times to 20% mortality for juvenile Chinook salmon exposed to 120.2 ± 0.2% TGP at 12.2 ± 0.5°C that were similar to those for white seabass (40–120 h). Juvenile and adult cutthroat trout *Oncorhynchus clarkii* and steelhead *Oncorhynchus mykiss* exposed to 121 ± 1% TGP at 12.2 ± 0.2°C were less tolerant than all white seabass groups except the large fish exposed to 23°C (Nebeker et al. 1980; Mesa et al. 2000). In contrast, speckled dace *Rhinichthys osculus* exposed to 120 ± 1% TGP were more tolerant of acute GSS exposure going 420 h before experiencing 20% mortality (Nebeker et al. 1980).

The mortality curves reported by Bouck (1980) for nonlarval salmonids do not agree with those determined in this study. Bouck (1980) reported a period of rapid mortality followed by a period of long-term survival with persistence of GBD symptoms. In our study, white seabass exhibited GBD symptoms before death rather than after a mass-mortality incident. This seems to be specific to white seabass and will allow for observation of GBD symptoms before any mortality problems occur.

Behavior

Behavioral changes may be the best initial indicator of GSS problems in captive teleosts. The behaviors exhibited by white seabass in response to acute GSS exposure matched 8 of the 13 behaviors reported by Backman et al. (1991) in American shad *Alosa sapidissima* and 1 reported by McCutcheon (1966) in 12 species of marine physoclists. The "cough" observed in white seabass was similar to the "yawn" response reported by McCutcheon (1966) and may be a good initial sign of problems associated with GSS since no white seabass in the control aquaria exhibited this behavior.

Another key behavioral indicator of acute GSS exposure in white seabass was loss of buoyancy control, which was presumed to be caused by swim bladder overinflation. This behavior was observed only at the highest treatment level (122% TGP) and was seen more frequently (10% more) in both size-classes when they were exposed to the warmer 23°C water. The loss of buoyancy began 24 h sooner in the warm water than in the cold water for both size-classes of fish.

Clinical Indications

The primary external lesions associated with acute exposure of juvenile white seabass to supersaturated water were gas emboli in fin capillaries and corneal emphysemas. Cutaneous fin emphysemas were observed in all treatment combinations at 122% TGP. The large fish exposed to 18°C had the highest prevalence of fin lesions and were the only group affected at 116% TGP. Size was an important variable, as large fish were affected more than small fish when exposed at the same temperature. Water temperature was also an important variable, but a surprisingly higher prevalence of fin emphysemas was associated with cold, not warm, water. One possible explanation for this finding, which is the reverse of trends associated with lethal-

ity and corneal emphysemas, is that a reduction of perfusion in the vascular beds of the gills, skin, and fins (along with a slower rate of embolus formation) results in bubble formation in these fine capillary networks.

In contrast, corneal emphysemas were by far the most common lesions and were tightly correlated with temperature and TGP exposure. Both the prevalence and severity of corneal emphysemas increased with increasing TGP in all exposure groups. Similar to GSS-related behavioral changes and lethality, the prevalence and severity of corneal emphysemas were higher in the large white seabass and in fish exposed to the warmer 23°C water.

While corneal emphysema has not been reported for other fish species exposed to GSS conditions, exophthalmia secondary to elevated gas pressure behind the eye was a common finding (Weitkamp and Katz 1980; Krise et al. 1990; Krise and Herman 1991). Exophthalmia caused by subretinal hemorrhage in the choroid rete mirabile of the eye has also been commonly reported (Stroud et al. 1975; Smith 1988; Machado et al. 1987; Krise et al. 1990) and has been correlated to the level of GSS (Krise and Smith 1993). The reduction of GSS in surrounding seawater should then be an important part of the rearing process for white seabass.

To minimize the prevalence of corneal emphysemas in the hatchery, smaller seabass should be reared at less than 109% TGP at 18°C or less than 102% TGP at 23°C. Larger juvenile seabass should be reared in seawater that is less than102% TGP at 18°C and less than100% TGP at 23°C. These TGP levels are lower than those reported in Timmons et al. (2002) and suggest that white seabass are more sensitive to the deleterious effects of GSS than other fish species. This information should be used cautiously to optimize culture conditions because nonlethal air supersaturation over periods longer than the 96 h tested in these experiments almost certainly will result in a higher prevalence of GBD and mortality (Mesa et al. 2000).

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