1987

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Journal of Shellfish Research, Vol. 6, No. 2, 79–83, 1987.

EFFECT OF AIR-SUPERSATURATED SEAWATER ON *ARGOPECTEN IRRADIANS CONCENTRICUS* (SAY) AND *CRASSOSTREA VIRGINICA* (GMELIN)

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ABSTRACT *Argopecten irradians concentricus* and *Crassostrea virginica* were exposed to several different levels of supersaturated seawater at temperatures ranging from 10 to 21°C. Gas bubble trauma occurred at a total gas saturation level of 116%, causing mortality in juvenile *A. i. concentricus* and reduced growth in juvenile *C. virginica*.

KEY WORDS: Gas bubble trauma, air-supersaturated seawater, *Argopecten, Crassostrea,* cultured bivalves.

INTRODUCTION

Gas bubble trauma, commonly referred to as gas bubble disease, is a noninfectious disorder of aquatic animals caused by the physical formation of gas bubbles in the tissues and vascular system due to the uncompensated hyperbaric pressure of total dissolved gases (Bouck 1980; Colt 1986). Weitkamp and Katz (1980) reviewed the literature on the effects of air-supersaturated water. Harvey (1975) summarized its cause and effect in fish, and Colt et al. (1984) reported gas bubble trauma in bullfrog tadpoles. A number of commercially important invertebrates are adversely affected by supersaturated seawater, including abalone, clams, oysters, scallops, lobsters, shrimp and crabs (Hughes 1968; Malouf et al. 1972; Lightner et al. 1974; Johnson 1976; Supplee and Lightner 1976; Goldberg 1978; Elston 1983; Brisson 1985).

The effect of gas bubble trauma can be acute and terminal or chronic, often leading to secondary disease, reduced growth and gradual mortality. In bivalves it is often characterized by formation of gas blisters in soft body tissues and buoyancy of the whole animal (Malouf et al. 1972; Goldberg 1978; Bisker and Castagna 1985).

Supersaturation, created by heating ambient seawater during winter to temperatures about 20 C, caused gas bubble trauma in adult surf clams *Spisula solidissima* (Dillwyn), and adult and juvenile bay scallops *Argopecten irradians* (Lamarck) at gas saturation levels of 114% oxygen and 195% nitrogen and higher (Goldberg 1978). Malouf et al. (1972) reported gas bubble trauma in adult eastern oysters *Crassostrea virginica* (Gmelin) and adult hard clams *Mercenaria mercenaria* (Linne), but did not give saturation levels. Current research indicates that supersaturation studies should report the total excess gas pressure (mm Hg) or percent total gas saturation. It is this difference between the total gas pressure and the barometric pressure or hyperbaric pressure which causes gas bubble formation (Colt 1983). Gas bubble trauma has been reported at ambient seawater temperatures in juveniles of the coot clam *Mulinia lateralis* (Say), the soft shell clam *Mya arenaria* Linne and the hard clam *M. mercenaria* (Linne) at total gas saturation levels of 108%, 114% and 115%, respectively (Bisker and Castagna in 1985).

Determination of dissolved gas concentrations which may affect various cultured bivalves either acutely or chronically would be useful, since procedures can be initiated to degas seawater to more tolerable levels. This study examined the effects of air-supersaturation on juvenile *A. i. concentricus* and *C. virginica*.

MATERIALS AND METHODS

This experiment was conducted from April to May 1986 using flowing seawater pumped from Finney's Creek, Wachapreague, VA. Compressed air was introduced through a needle valve installed on the intake (vacuum) side of the pump to supersaturate the seawater during delivery under normal pumping pressure. This supersaturated seawater was degassed by cascading down a stairstep arrangement to produce four different supersaturation levels as detailed in Bisker and Castagna (1985). The lowest saturation level, which was similar to that of the ambient seawater, was designated the control. Each saturation level was replicated twice.

Experimental animals were held in ambient flowing seawater prior to the experiment. Each container received 100 *A. i. concentricus* of approximately 13 mm shell height (SH) held in mesh bags (15 × 12 cm, with 6 mm mesh), and 100 *C. virginica* of approximately 17 mm SH held on a sieve. *Argopecten irradians concentricus* were photocopied for convenient determination of initial SH measurements (Haines 1973), while SH of *C. virginica* was measured directly due to their irregular shape. Final SH measurements were determined on the live animals at day 28.

Dissolved gas levels of seawater in each experimental container and of ambient seawater were measured five times each week. Hyperbaric gas pressure was measured

Contribution No. 1372 from Virginia Institute of Marine Science.

TABLE 1.

Gas saturation levels (mean ± standard deviation and range).

Gas Treatment	Hyperbaric Gas Pressure mm HG	% Total Gas	% Oxygen	% Nitrogen	N
1	122.8 ± 9.73 (98–137)	116.2 ± 1.30 (112.8–118.1)	110.2 ± 2.94 (105.6–115.8)	118.1 ± 1.87 (113.9–121.4)	21
2	62.5 ± 5.12 (51.5–69.5)	108.2 ± 0.68 (106.8–109.2)	105.1 ± 2.49 (100.8–110.1)	109.3 ± 1.14 (106.6–110.6)	21
3	29.9 ± 5.30 (20.0–42.0)	103.9 ± 0.69 (102.6–105.5)	102.5 ± 2.37 (98.7–107.6)	104.4 ± 1.14 (102.6–106.8)	21
Control	6.3 ± 2.57 (1.5–12.0)	100.8 ± 0.34 (100.2–101.6)	100.1 ± 2.44 (95.2–104.4)	101.0 ± 0.83 (99.8–103.3)	21
Ambient	6.4 ± 9.96 (−11.0–35.0)	100.8 ± 1.31 (98.6–104.6)	96.1 ± 5.25 (85.2–104.4)	102.1 ± 1.75 (99.6–105.9)	20

with a gasometer (Bouck 1982). Concurrent dissolved oxygen (D.O.) measurements were taken using a YSI Model 58 oxygen meter with an oxygen probe Model 5775 (Yellow Springs Instrument Co., Yellow Springs, Ohio). The D.O. meter was air calibrated. Water temperature, salinity, and barometric pressure were measured for determination of total dissolved gas, percent oxygen saturation (% O_2), and percent nitrogen saturation (% N_2) as described by Bouck (1982). Experimental animals were monitored daily for flotation, noticeably visible air bubble formation in tissues and removeal of dead animals.

Hyperbaric gas pressure (GP) and percent total gas saturation (% TG) were compared between saturation levels using one-way analyses of variance (ANOVA) with replication. The mean survival in days of animals at each treat-

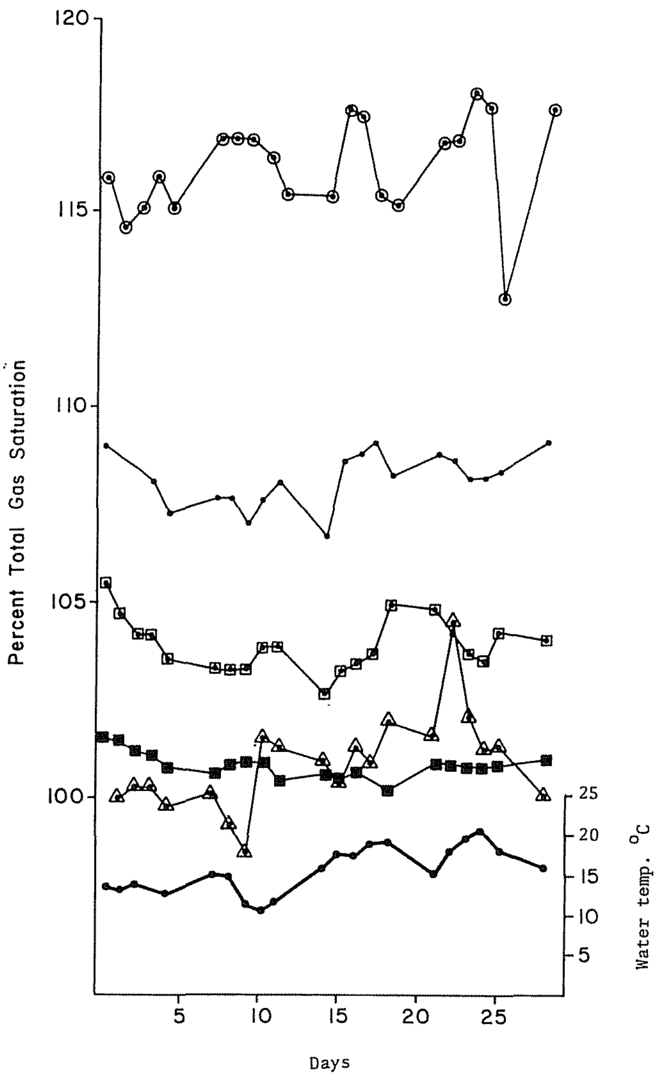

Figure 1. Mean percent total gas saturation for treatments 1 (○), 2 (●), 3 (□), and control (■) and for ambient seawater (△), with mean water temperatures.

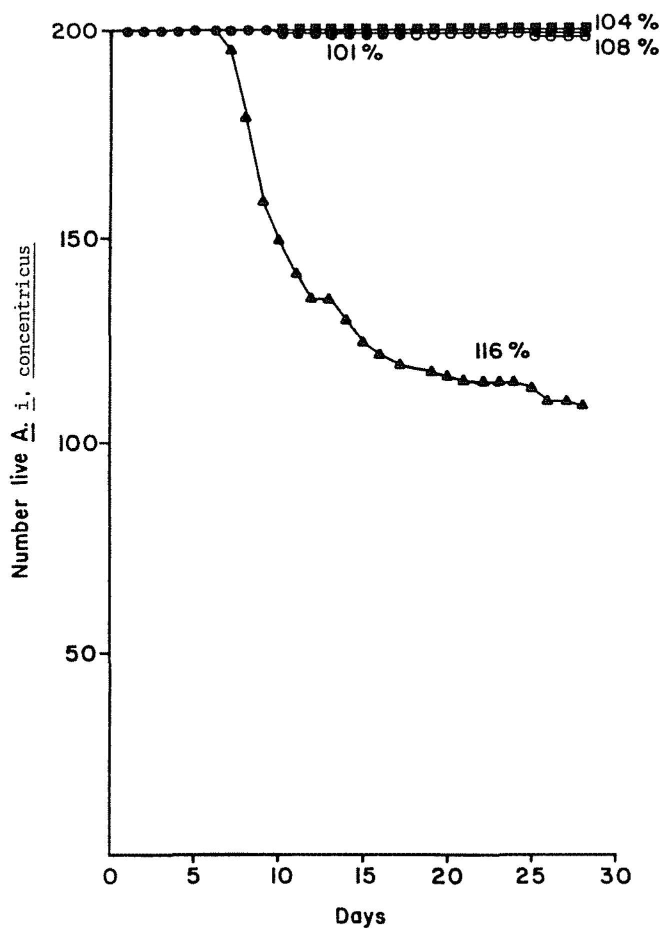

Figure 2. Survival of *Argopecten irradians concentricus* for treatments having mean total gas saturation of 116%, 108%, 104% and 101%.

TABLE 2.

Mean survival in days (arithmetic mean ± standard deviation) for *Argopecten irradians concentricus* and *Crassostrea virginica* at each gas saturation level.

% TG	*A. i. concentricus*	*C. virginica*	N
116	20.2 ± 9.19*	27.7 ± 2.35	200
108	27.9 ± 0.83	27.9 ± 1.27	200
104	28.0 ± 0.00	27.8 ± 2.03	200
101 (control)	27.9 ± 1.34	28.0 ± 0.00	200

* significantly different from control ($p = 0.01$)

ment level was calculated by adding the number of days each animal survived and dividing by the number of animals used (Goldberg 1978). Variances in survival between saturation levels were compared using one-way ANOVA after log x transformation of data for each species. Duncan's multiple-range was used to compare mean survival days of the scallops (Steel and Torrie 1960). Since no significant differences ($p = 0.01$) were found between replicate GP or % TG measurements, shell height data for each species were combined for analysis. Initial and final SH measurements of *A. i. concentricus* were compared using one-way ANOVA at each saturation level. Initial and final SH measurements of *C. virginica* were compared similarly after log x transformation of data.

RESULTS

The means and ranges of GP, % TG, % O_2, % N_2 and water temperature are shown on Table 1 and Figure 1. Hyperbaric gas pressure and % TG were not significantly different between replicates; however, they were significantly different ($p < 0.001$) between saturation levels.

Argopecten irradians concentricus exposed to a mean % TG of 116% demonstrated significant mortality after 7 days and only 55% survived to day 28 (Figure 2). Mean survival for scallops was significantly lower ($p < 0.01$) at 116% TG (Table 2). There was no significant difference ($p = 0.01$) between initial and final shell heights of scallops exposed to 116% TG (Table 3). Scallops exposed to 108%, 104% and 101% TG had significantly greater ($p < 0.001$) final SH than initial SH measurements. None of the scallops floated and no bubbles were observed in the tissues.

There was no significant mortality nor difference in mean survival ($p = 0.01$) of *C. virginica* observed at any gas saturation level (Table 2). There was no significant differences ($p = 0.01$) between initial and final shell heights of oysters exposed to 116% TG (Table 3). Oysters exposed to 108%, 104% and 101% TG were significantly larger ($p < 0.001$) after 28 days than at the beginning of the experiment. No oysters floated nor were bubbles observed in the tissues.

DISCUSSION

No air blisters or flotation of animals were observed in this study, but mortality and reduced growth did occur in scallops at 116% TG. No mortality was observed in oysters at 116% TG, although shell growth was reduced when compared to that of oysters exposed to lower levels of supersaturation. A previous study reported that total gas saturations of 108% and 114% caused gas bubble trauma in *M. lateralis* and *M. arenaria,* respectively (Bisker and Castagna 1985). Air blisters in the tissues and flotation were observed at these levels. Significant mortality occurred in *M. lateralis* at 114% TG, but *M. arenaria* had no significant mortality even at levels as high as 120% TG. No mortality was observed in *M. mercenaria* exposed to 115% TG in that study, although shell growth was reduced when compared to that of clams exposed to lower levels of saturation.

Crassostrea virginica, M. arenaria and *M. mercenaria* tended to have better survival than *A. i. concentricus* and *M. lateralis* when exposed to % TG of 114–116%. This may be attributed to differences in the bivalves' ability to sustain valve closure, and thus isolate themselves from the external environment. Shell closure by oysters, coot clams and hard clams essentially seals off the soft body tissues from the outer environment. Oysters and hard clams can live under anaerobic conditions for extended periods when temperatures are below 20°C (Dunnington 1968; Crenshaw and Neff 1969; Hammen 1969). *Mulinia lateralis* lacks this ability and thus may expose their tissues to the supersaturated water more frequently. Shumway et al. (1983) im-

TABLE 3.

Shell heights (mean ± standard deviation) in mm of *Argopecten irradians concentricus* and *Crassostrea virginica* before and after 28 days exposure to gas saturation.

	A. i. concentricus				*C. virginica*			
% TG	Initial	N	After exposure	N	Initial	N	After exposure	N
116	12.8 ± 1.96	200	12.9 ± 1.79	109	17.6 ± 8.04	200	16.8 ± 8.34	200
108	13.0 ± 2.13	200	19.8 ± 2.42*	198	16.7 ± 7.07	200	21.1 ± 7.99*	200
104	13.4 ± 2.03	200	20.0 ± 2.35*	200	17.2 ± 8.35	200	21.4 ± 8.85*	200
101	13.4 ± 2.21	200	20.1 ± 2.53*	199	16.8 ± 7.96	200	20.8 ± 9.05*	200

* significantly different from initial measurement ($p = 0.01$)

TABLE 4.

Lowest mean hyperbaric gas pressure (GP in mm Hg), percent total gas saturation (% TG), percent oxygen (% O_2) and percent nitrogen (% N_2) which caused gas bubble trauma in bivalves.

Species	GP mm HG	% TG	% O_2	% N_2
A. i. concentricus	<u>123</u>	<u>116</u>	<u>110</u>	<u>118</u>
C. virginica	123	116	110	118
M. mercenaria	113*	115*	111*	116*
M. lateralis	58*	108*	106*	108*
M. arenaria	104*	114*	111*	114*
S. solidissima			<u>114**</u>	<u>195**</u>

<u>x</u> = mortality observed at this level.
* From Bisker and Castagna (1985).
** From Goldberg (1978).

plies that the short survival times of coot clams under anoxic conditions is due to their unusually high rate of anaerobic glycolysis. *Mya arenaria* can make its mantle cavity watertight, undergo anaerobic metabolism for extended periods and regulate the hydrostatic pressure of its internal tissues (Trueman 1966; Ricketts and Calvin 1968). Trueman (1966) recorded hydrostatic pressures of over 73 mm Hg during shell adduction in soft shell clams. A positive hydrostatic pressure in internal tissues should aid in the compensation of the hyperbaric pressure of total dissolved gases in exposed tissues thus reducing bubble formation (Weitkamp and Katz 1980). Scallops lack the ability to completely close off tissues from the environment, undergo extended periods of anaerobic metabolism and significantly increase the hydrostatic pressure of internal tissues, all of which should make them more susceptible than oysters to supersaturation (Van Dam 1954). The detrimental effects of gas bubble trauma would be lessoned by reducing exposure time of internal tissues to supersaturation. Bivalves can reduce this exposure by sealing off these tissues through shell closure, undergoing anaerobic metabolism, reducing metobolic rate, or increasing internal hydrostatic pressure. Although brief periodic exposure of internal tissues to the supersaturation may be necessary, this may not be detrimental at the saturation levels tested.

Significant mortality occurred after 7 and 12 days of exposure to 115–116% TG in *A. i. concentricus* and *M. lateralis*, respectively. After 28 days of exposure to this saturation level, survival of scallops and coot clams was about 50% and 15%, respectively. Although mortality occurred sooner in the bay scallop, this species seemed to become more tolerant of the supersaturated water with time.

The lowest mean saturation levels which have caused gas bubble trauma in six bivalve species are shown in Table 4. The water quality upper limit of 110% TG (GP = 76 mm Hg) proposed by the United States Environmental Protection Agency (1976) should be low enough to prevent gas bubble trauma in juvenile marine bivalves. The monitoring of gas saturation levels with a device such as a gasometer would detect problems in seawater culture systems and allow the implementation of methods which would lower the saturation levels.

ACKNOWLEDGEMENTS

The authors would like to thank N. Lewis, J. Moore and J. Watkinson for their valuable assistance. We also thank M. Gibbons, G. Grant and D. Stilwell for their editorial advice.

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