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SOME EFFECTS OF GAS-SUPERSATURATED SEAWATER ON *SPISULA SOLIDISSIMA* AND *ARGOPECTEN IRRADIANS*

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ABSTRACT

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Two size classes of the surf clam, *Spisula solidissima*, and the bay scallop, *Argopecten irradians*, were exposed to different concentrations of gas-supersaturated seawater in a flowing seawater system. Both species tested experienced no mortality when held in the control treatment maintained at 96% oxygen and 109% nitrogen. Mortality, gill tissue damage, gas emboli, membranous tissue blisters, and abnormal secretion of shell material were induced experimentally at elevated levels of gas supersaturation. Results indicate significant mortalities of surf clams and scallops held at 114% O_2 and 195% N_2, and at higher levels of gas concentration. These values suggest a point of reference for the bivalve culturist in identifying potential problems which can be caused by gas-supersaturated seawater.

INTRODUCTION

The pathological effect of exposure of aquatic organisms to gas-supersaturated seawater has been termed gas-bubble disease and has been well documented since the early part of this century as a hazard to fish (Marsh and Gorham, 1905). The effect of gas-supersaturated seawater on invertebrates has been reported only recently (Hughes, 1968; Malouf et al., 1972; Lightner et al., 1974; Johnson, 1976).

The occurrence of pathologies in mollusks due to exposure to gas-supersaturated seawater has been noted (Malouf et al., 1972); however, the lethal levels of these gases have not been defined. By experimentally exposing mollusks to known concentrations of supersaturated seawater, this study

attempts to define the lethal levels of gases and also investigates the occurrence of sub-lethal effects.

METHODS

Highly supersaturated seawater was available throughout these experiments from the Milford Laboratory seawater system. In the winter, seawater is heated in a closed-system, impervious carbon heat exchanger through a ΔT of 25 to 30°C. This system prevents atmospheric gases from leaving the seawater as they normally would when water is heated in an open vessel. The seawater retains excess gas even as it is cooled with unheated seawater to produce temperatures suitable for bivalve culture.

Vigorous aeration of supersaturated seawater can reduce the level of supersaturation. Using this principle, a degassing apparatus (Fig. 1) was devised to deliver seawater continuously at saturation and at four different concentrations of gas supersaturation. The degassing apparatus consists of five, 4-l battery jars, arranged on a stairstep platform. Supersaturated seawater at 20°C is introduced into the top jar and flows sequentially through each jar in the system. The four lower jars are aerated by airstones and, by controlling the amount of aeration, the quantity of excess gas driven out of solution can be regulated. In this way, seawater at decreasing levels of gas-supersaturation is available. Seawater is then siphoned from each of the five jars at 500 ml min^{-1} into five, 10-l fiberglass trays used as holding containers for the experimental animals.

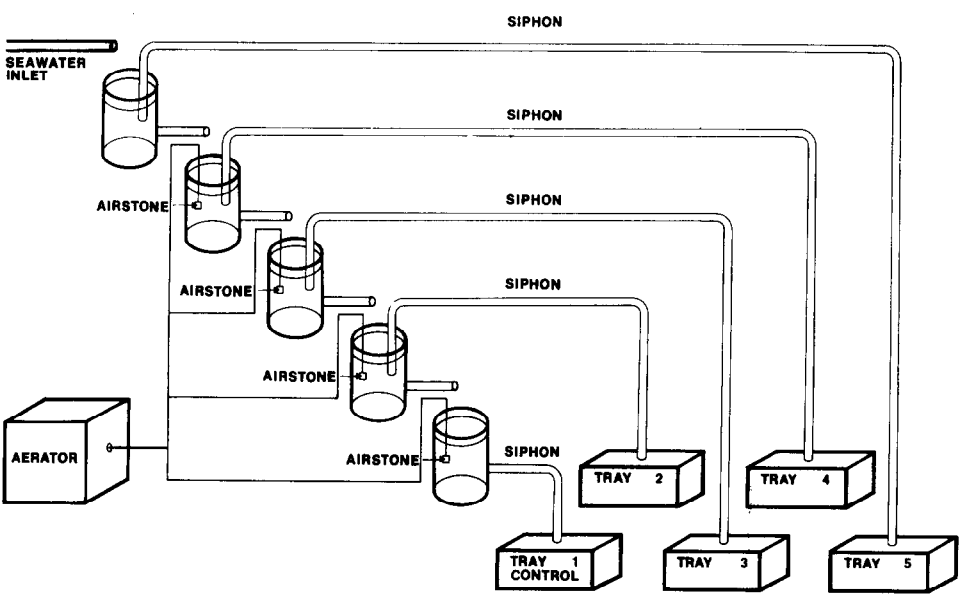

Fig.1. Diagram of differential degassing apparatus.

TABLE I

Average daily measurements of physical parameters in exposure trays

Experiment	Temperature (°C)	Salinity (‰)	Dissolved gases				
			Tray 1	Tray 2	Tray 3	Tray 4	Tray 5
1976	19.8	25.5					
1977	20.1	26.8					
1976 O_2 mg l^{-1}			7.7	8.5	9.3	9.9	10.8
1976 O_2 % Saturation*			97	108	118	126	137
1977 O_2 mg l^{-1}			7.5	8.3	9.0	10.3	11.0
1977 O_2 % Saturation*			96	105	114	131	140
1977 N_2 ml l^{-1}			11.0	14.5	19.7	23.4	27.5
1977 N_2 % Saturation**			109	143	195	232	272

*100% oxygen saturation in seawater at sea level at 26 p.p.t. and 20°C is 7.82 mg l^{-1} (from Green and Carritt, 1967).
**100% nitrogen saturation in seawater at sea level at 27 p.p.t. and 20°C is estimated at 10.08 ml l^{-1} (from Murray et al., 1968).

Two sets of experiments were performed in the winters of 1976 and 1977. During the experimentation, dissolved gas levels, temperature, and salinity were recorded daily from each of the exposure trays. The Winkler titration method was used in 1976 to monitor dissolved oxygen. The availability of a Scholander microgasometric titration apparatus (Scholander et al., 1955) allowed measurement of both dissolved oxygen and nitrogen in 1977. This added capability allowed measurement of the two major constituents of air, believed to be the prime causative agents of gas-bubble disease in fish (Rucker, 1972). Since nitrogen levels were not measured in 1976, it was assumed that they were similar to those measured in 1977. This assumption is based on the facts that the experimental conditions were unchanged and the oxygen values remained similar from 1976 to 1977.

Table I summarizes the average daily measurements of the physical conditions in the exposure trays. Exposure time of all experiments was 40 days.

During the first trial in 1976, using this apparatus, three test groups were exposed to five levels of gas saturation. Five *Spisula solidissima* between 55 mm and 71 mm in length, ten juvenile *Argopecten irradians* between 8.6 mm and 15.3 mm in length, and five adult scallops ranging in length from 51 mm to 67 mm were maintained in each of the five treatments.

In 1977 a second trial was conducted in which both oxygen and nitrogen

levels of the test trays were monitored. Ten surf clams between 42 mm and 46 mm were maintained in each of the five treatments.

RESULTS AND DISCUSSION

Lethal effects of supersaturation

Table II represents the average number of days each group of animals survived at different gas concentrations. Average survival time is calculated by adding the number of days each animal survived and dividing by the total number of animals in the group.

One-way analyses of variance and Student Newman Keuls tests were performed (Table II) to determine if the average survival times were significantly different among the treatments (Sokal and Rohlf, 1965). In the 1976 tests, juvenile scallops at 108% oxygen concentration showed no significant difference in survival time from the control treatment (Table II). At oxygen supersaturation levels of 118% and above, survival times were significantly reduced when compared to the control treatment. No significant difference was found between the survival times at the highest levels of 126 and 137% oxygen. Similarly, adult scallops in the 1976 tests showed increasing mortality as the oxygen level increased.

In both the 1976 and 1977 tests, surf clams showed no mortality at gas

TABLE II

Results of analyses of variance and Student Newman Keuls tests for each of four trials

Experiment	*F* value	df	Average survival time (days)								
			Control Tray 1 97% O_2		Tray 2 108% O_2		Tray 3 118% O_2		Tray 4 126% O_2		Tray 5 137% O_2
1976 *Spisula* 55—77 mm	23.4**	(4, 20)	40	=	40	>	32	>	20	=	18
1976 *Argopecten* 8—15 mm	5.15**	(4, 45)	40	=	38	>	15	>	9	=	8
1976 *Argopecten* 51—67 mm	95.5**	(4, 20)	40	=	38	>	24	>	15	>	9
			Tray 1 96% O_2 109% N_2		Tray 2 105% O_2 143% N_2		Tray 3 114% O_2 195% N_2		Tray 4 131% O_2 232% N_2		Tray 5 140% O_2 272% N_2
1977 *Spisula* 42—46 mm	17.84**	(4, 45)	40	=	40	>	28.4	>	10.8	=	8.4

** $P < 0.01$.

> denotes a significant difference between adjacent values ($P < 0.05$).

= denotes no significant difference between adjacent values.

Fig.2. Photograph showing tissue blisters on siphons of juvenile *Spisula solidissima*.

saturation levels up to 105 or 108% O_2, and 143% N_2. At levels exceeding 118% O_2 in 1976, and 114% O_2, 195% N_2 in 1977, the rate of mortality significantly increased with increasing concentrations of saturated gases (Table II).

For both these species and for the various sizes of animals tested, oxygen levels of 5 to 8% above saturation were apparently harmless over the 40-day period. At oxygen levels of 114 or 118% and nitrogen levels of about 195% saturation, significant stress, as manifested by an increased rate of mortality, was evident.

Sub-lethal effects of supersaturation

When an aquatic organism is placed in an environment where gas pressure exerted from the medium is greater than that exerted by the tissues of the organism, the tissues become laden with dissolved gases equal to the level of dissolved gases in the medium. The gas-laden tissues tend to equilibrate with the atmosphere, causing formation of air bubbles within the organism.

Tissue blisters were observed on the siphons of many of the surf clams that had been exposed to supersaturated seawater in all treatments except the control. These tissue blisters (Fig.2) are pockets of trapped air that have accumulated under the membrane that sheaths the siphon. Surf clams with

this condition were subsequently held in flowing seawater below saturation for several months and the tissue blisters remained.

Many surf clams exposed to highly supersaturated seawater secreted a layer of calcareous shell material, which surrounded air bubbles that had formed between the mantle and the inner shell. This is similar to the conchiolin blisters and subsequent deposition of new shell that has been reported in oysters exposed to supersaturation (Malouf et al., 1972). In surf clams these walled-off bubbles caused a buoyancy which floated many of the test animals. This occurred in animals held at and above the 118% oxygen level in 1976, and in animals held at and above 114% O_2, 141% N_2 in 1977. Surf clams with this calcareous chamber were maintained below saturation for several months and the condition persisted.

Scallops exhibited similar symptoms when exposed to treatments at and above 118% O_2 in 1976 and 114% O_2, 195% N_2 in 1977. In many of the scallops, gas bubbles appeared to be imbedded in the tissue along the mantle edge. Serious deterioration of gill tissue was also seen in the scallops. Accumulation of minute air bubbles on the gill surfaces ultimately caused a breaking of the interlamellar junctions and a feathering of the fragile gill tissue.

Several behavioral effects resulting from exposure to gas supersaturation were observed in the course of the experiment, but not quantified. Surf clams held at high supersaturation levels showed a marked decrease in pumping behavior and fecal production. Scallops produced far less fecal material than mucus-like pseudo-feces.

Significance to the bivalve culturist

The causes of air supersaturation of seawater are varied. Man-made causes, such as closed-system heat exchangers, pumps with faulty impellers, and thermal effluents, can all create supersaturation. In nature, mixing of hot and cold water masses can produce supersaturation.

In the present study it has been shown that both oxygen and nitrogen exist at abnormally high levels after closed-system heating of cold seawater. It is speculated that the etiologic agent of the observed pathologies is the combined partial pressures of these two gases.

The bivalve culturist should be aware of the causes of air supersaturation of seawater and should, ideally, degas supersaturated culture water to below saturation. Caution must be taken in temperature tolerance studies to assure that gas supersaturation is not an underlying variable.

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