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AN ANALYSIS OF LINGCOD (*Ophiodon elongatus*) EGG MORTALITY

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INTRODUCTION

The recent decline of lingcod stocks in Puget Sound (Ilg et al. 1979) is so severe that a fishing moratorium was imposed by the Washington State Department of Fisheries in 1978. The urgency of the situation prompted the initiation of numerous research efforts including this study. This paper addresses a portion of a more extensive early life history investigation that was initially funded in 1978 by the recreational fisheries program at the Northwest and Alaska Fisheries Center in Seattle, Washington.

In the course of procuring wild eggs for use in laboratory experiments, it was apparent that egg masses from a particular nesting area contained embryos spanning an array of developmental stages, the most advanced of which were located near the peripheral surface of the mass. In one instance, dead embryos were observed at the interior of the mass. Egg masses displaying these characteristics were located in Dobob Bay, a water system characterized by low tidal current velocities (Kollmeyer 1962). Examination of a few egg masses from a high current velocity area (San Juan Island) revealed no differential embryo development or interior mortalities. Given the massive nature of the lingcod nest (two to three litres is a typical nest volume) and the prevailing water movement characteristics at the two locations, it seemed plausible that the mortalities could be a result of inadequate nest ventilation. Both Wickett (1954) and McNeill (1966) contend that the high embryo mortalities they witnessed in chum and pink salmon redds are a direct consequence of poor water circulation and the resultant low dissolved oxygen within the redd. When herring spawn (*Clupea* sp.) is deposited in a thick layer, egg mortalities can be substantial (Taylor 1971; Hempel 1971; Outram and Humphrey 1974). The authors suggest that insufficient oxygen or metabolite accumulation resulting from poor water circulation is the cause, but experimental evidence is lacking.

The purpose of this investigation was to assess the role of nest ventilation in embryo development and survival. To accomplish this, it was necessary to conduct a program utilizing both field and laboratory observations and experiments.

INTERSTITIAL OXYGEN AND EMBRYO CONDITION; WILD NESTS

Field studies were conducted at five nesting areas in Puget Sound using SCUBA. Two, Pulali Pt. and Wawa Pt., were located in Dabob Bay, a fjord on Hood Canal (Fig. 1). The other three areas, Turn Island, North Cove, and Henry Island, were located near San Juan Island. Maximum tidal current velocities in San Juan Channel are approximately 215 cm/sec at a three meter tide fluctuation (NOAA Tidal Current Tables, 1979), while mid-channel velocities in Dabob Bay attain an approximate maximum of only six cm/sec during a similar tide; the latter value was calculated from data presented by Kollmeyer (1962) and Sverdrup et al. (1942).

Interstitial oxygen was mesured by extracting a five mL water sample from near the centre of the mass using a glass syringe fitted with a 100 m long anesthetic needle; oxygen was subsequently measured with a polarographic microelectrode. Nests were then corred with a 25 mm pipe possessing a sharpened edge. Embryos in the core were examined for viability and stage of development.

Samples were taken at Dabob Bay during tidal current excursions (at vertical fluctuations >2.0 t), when interstitial oxygen levels are probably near peak levels. Interstitial oxygen ranged from 2 to 35% saturation; mean = 16% (Table 1). Oxygen in amabient water at nest sites ranged from 85 to 87% saturation. All thirteen nests examined at Dabob Bay exhibited retarded embryo development at the interior with the more advanced stages present at the periphery. Of the three masses transported to the laboratory for experimentation, two contained concentrations of dead embryos at their interiors. Of the ten remaining in the field, eight incurred mortalities at their interiors. Two nests could not be relocated. Thus, ten of thirteen, or 77% of the nests examined at Dabob Bay incurred mortalities at their interiors. I suspect that the remaining 23% (three nests) would have also incurred mortalities if they had been relocated or remained in situ and could be resampled near term.

The nature of the mortality observed in these masses is quite distinct; it is first evident at the innermost portion of the mass or near the substrate contact surface, no viable eggs occur within the necrotic volume of the mass; the necrotic volume increases in size from the interior towards the peripheral surface as the egg mass develops.

Mortality was estimated in near-term egg masses (as determined by the presence of developmentally advanced embryos at the periphery) by sampling them just prior to hatching. Of course, mortality could continue to occur until hatching; therefore, these are necessarily conservative estimates. Embryo mortality in eight near-term nests at Dabob Bay ranged from 5 to 95% (mean = 59%).

In San Juan nests, oxygen ranged from 43 to 85% saturation (mean = 69%). Only one of thirteen nests examined contained developmentally retarded embryos. No interior mortalities that might be attributable to poor ventilation were observed in any near-term egg mass (n = 6).

INTERSTITIAL ENVIRONMENT OF THE EGG MASS; LABORATORY EXPERIMENTS

A number of laboratory experiments were conducted to examine the nature of the interstitial environment under a variety of water current conditions.

Egg masses were deployed in a 60 cm deep, 122-cm diameter, circular fibreglass tank with a central floor drain. A 52-cm diameter perforated lucite cylinder was placed eccentrically over the drain, which was fitted with

EMBRYO TOLERANCE TO HYPOXIA AND AMMONIA

Static bioassays were conducted to assess the effect of reduced oxygen on developmentally advanced embryos. Thirteen to fourteen eggs were rinsed well with ambient laboratory seawater and placed in each 300 mL BOD bottle. Test water of the appropriate oxygen content was prepared by gassing with nitrogen, then siphoned into the bottle allowing to overflow one volume. Bottles wer then sealed with ground glass stoppers and immersed in an open system ambient seawater bath. Ambient laboratory seawater ws used; pH = 7.80, salinity = 29.0‰ temperature = 8.5-9.5°C. Oxygen levels of the test solutions were: 2, 6, 12, 22, 50, and 85% saturation, the latter being the ambient control. Two replicate bottles were run for each oxygen level. Data was collected at 4-, 24-, 48-, and 96-h intervals. Criteria for death was cessation of heart beat. Since the chorion of the lingcod egg is opaque, it was necessary to dissect it from the embryo under a dissecting scope in order to view the heart. Care was taken to avoid thermal shock. Eggs wer dissected in 1-mL depression slides containing water from the particular oxygen treatment. Depression slides were kept cool in a vessel of ambient seawater between dissections. All eggs were dissected, but those resulting in damage to the embryo were discarded and not recorded as mortalities.

Similar assays were conductd to test the acute lethal response to ammonia. Test solution concentrations of NH_4CL were:

Total ammonia-N (ppm)	NH_3-N (ppb)
0.02 (ambient water)	negligible
0.25	2
1.00	9
10.00	89
50.00	444

Control (85% oxygen saturation) mortality never exceeded 10% in 96 hours. As earl as four hours, mortalities were observed in both the 2 and 6% saturation treatments. By 48 hours, mortalities reached 95% at 2% saturation, 75% at 6% saturation, and 30% at 12% saturation. By 96 hours, moralities at oxygen levels $\leq$ 22% saturation were $\geq$ 60%, with evidence that mortalities at 50% saturation were starting to occur.

LC_{50} and LT_{50} values with 95% confidence intervals were calculated using a logit analysis program (Table 2). Since the response data at 22% saturation did not meet the program requirements, this LT_{50} value was estimated by log-probit plot.

The mortality data indicates that oxygen levels in nests at Dabob Bay (Table 1) are certainly low enough to be lethal to developmentally advanced embryos. Oxygen in some San Juan nests also drops to potentially lethal levels, but typically this condition lasts for only a brief period during the tide change, by no means long enough to induce mortalities.

a 25-cm high standpipe. A high pressure raw seawater line was used to produce the water current. Current velocity was adjusted with a polyvinylchloride (pvc) ball value and measured with an Ott velocity meter. All egg masses were oriented lengthwise into the current. Masses did not posses peninsular-like projections or large indentations. Interstitial oxygen, ammonia, and pH were monitored at different current velocities.

Water samples were extracted from the interior of the egg mass with a five mL glass syringe and a 100 mm, stainless steel anesthetic needle. Needles remained imbedded at the same position and depth in the egg mass throughout the experiments. Only nests comprised primarily of developmentally advanced embryos were used.

Interstitial oxygen as a function of increasing current velocity is depicted in Fig. 2. At least 15 min elapsed between current velocity increases and oxygen measurement. This was ample time for oxygen to stabilize. Prior to extraction of the "zero" velocity sample, each nest was situated in an open system for two hours without current directed at it. Even though the masses differ considerably in size and configuration, interstitial oxygen reponds similarly to increasing current velocity (Fig. 2). At velocities approaching 20 cm/sec, oxygen in the three egg masses was near 80% saturation. The largest mass required slightly higher velocities to attain the same ventilation efficiency as the smaller ones. With some allowance for variation due to mass size, current velocities less than approximately 7.5-10 cm/sec produce only limited ventilation of the masses. It is well to note that in the laboratory experiments virtually all facets of the mass are exposed to ventilating currents. This is clearly not the case at natural nest sites where nests are extruded into rocky crevices and interstices. In the latter situation, somewhat faster currents would probably be required to provide comparable ventilation.

During slack tide periods, the high density of embryos in conjunction with the associated interstitial fauna (primarily harpacticoid copepods and the gastropods (Amphissa sp. and Calliostoma sp.) may deplete interstitial oxygen and produce excretory products to change pH and increase ammonia concentrations in interstitial water. To determine the extent of these changes, a short-term slack water simulation was conducted. Oxygen, ammonia, and pH were concurrently monitored at a single port inserted near the center of egg mass no. 1 (volume = 3.2 lt, weight = 3.7 kg). The egg mass was exposed to a current velocity in excess of 40 cm/sec for 2 hours prior to cessation of the current to assure ample flushing of the nest.

Oxygen decreased for 45 min then leveled off at 8 to 13% saturation. PH remained relatively stable for the first hour then decreased to 7.05 at 2 hours (Fig. 3). Total ammonia increased for 2 hours with no indication of an upper limit. Ammonia (NH_3) concentrations increased steadily to 6 ppb the first hour, then fell to 1.9 ppb at 2 hours due to the concomitant decrease in pH from 7.70 to 7.05. During a chronic exposure (70 hours) to slack water, all three factors reached their extreme levels at the end of the exposure period: oxygen = 4% saturation, pH = 6.60, total ammonia = 7.14 ppm (the NH_3 fraction = 4 ppb). The continued decrease in pH servers to prevent the NH_3 fraction from attaining appreciable concentrations.

Only two mortalities were observed during the 96-hour ammonia bioassay. Both occurred in the same vessel at an intermediate ammonia concentration (1.0 ppm at 96 hours). Since no other mortalities were observed at concentrations up to 50.0 ppm (444 ppb as NH_3-N), it was concluded that the mortalities were chance events and not attributable to ammonia. This indicates that ammonia concentrations do not reach lethal concentrations in poorly ventilated nests, at least not until well after embryos had already expired from chronic hypoxia.

To further emphasize the role of adequate ventilation to the ultimate survivorship of the embryos, an egg mass of uniform configuration (loaf-like in appearance) was dissected into three near equal pieces, each of which was exposed to a different water current velocity until hatching. Current velocities were adjusted until the desired interstitial oxygen level (20, 50, and 85% saturation) was attained. Results are summarized:

Oxygen level (% saturation)		
nominal	range	% mortality
85	77-91	6
50	29-76	48
20	14-22	93

Mortality was estimated from the ratio of the weight of dead eggs remaining to the original weight of the egg mass pieces. Clearly, the extent of mortality increased with increasing hypoxia.

DISCUSSION

The mean egg mortality estimated for dabob Bay (59%) is in agreement with nest mortalities reported by Low and Beamish (1978) and LaRiviere et al. (in press), 77% and 60%, respectively. Although in the latter investigations, nest dislodgement and predation (not hypoxia) were the causes of mortality. Whether the mortality estimates discussed here are representative over the range of the species is uncertain. Demersal spawn which is guarded by a parent is often expected to realize high survivorship to hatching. In the case of lingcod, it may be that mortality which appears great to the biologist is actually quite moderate when viewed in the context of the species entire life history.

The likelihood that other areas exist which incur greater or lesser mortality is undesirable. It is not difficult to identify numerous oceanographic locales, within the range of the species, where current velocity or wave force is sufficient to provide adequate nest ventilation, but not so extreme as to cause extensive nest dislodgement. Additionally, concentrations of potential nest predators often vary substantially between spawning areas. Therefore, it is plausible to suspect that there is a substantial expanse of benthos which possesses both physical and biological features which are conducive to high embryo survivorship. Whether or not lingcod utilize such areas is uncertain; observations are very limited, usually restricted to the shallow (<40 m) near-shore habitat. More expansive surveys, including the deeper offshore basins and reefs, which quantify the incidence of spawning and attempt to estimate embryo mortality are necessary to better understand the role of the egg stage in the recruitment mechanism.

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Table 1. Mean interstitial oxygen and condition of embryos within wild egg masses. The sample size is designated in parentheses. Mean oxygen in ambient water at the time of sampling is presented. Oxygen measurements in Dabob Bay were made during tidal excursions (>2 m vertical changes), while San Juan were taken only during slack tide.

	Mean oxygen % saturated; mL $O_2.l^{-1}$		Condition of egg masses	
			Frequency of egg masses containing developmentally retarded embryos (%)	Mean mortality in near-term egg masses (%)
Area	Ambient	Intersitial		
Dabob Bay	86; 6.18	16; 1.14	100	59
		(16)	(14)	(8)
San Juan	89; 6.11	69; 4.73	8	0
		(10)	(13)	(6)

Table 2. Oxygen LC_{50} and LT_{50} values and 95% confidence intervals for stage 10 embryos. Values were calculated with a logit analysis program written by Russ Kappenman, Biometrics, Northwest and Alaska Fisheries Center, Seattle, WA.

Exposure time (hr)	LC_{50} oxygen (% saturation)	95% C.I. oxygen (% saturation)
48	9.4	7.6 - 11.2
96	32.6	25.5 - 39.6

Oxygen level (% saturation)	LT_{50} (hr)	95% C.I. (hr)
2	26.2	17.5 - 34.9
6	40.1	32.1 - 48.1
12	62.6	51.5 - 73.8
22[a]	85.0	-----

[a]The LT_{50} value for 22% saturation was estimated by log-probit plot.

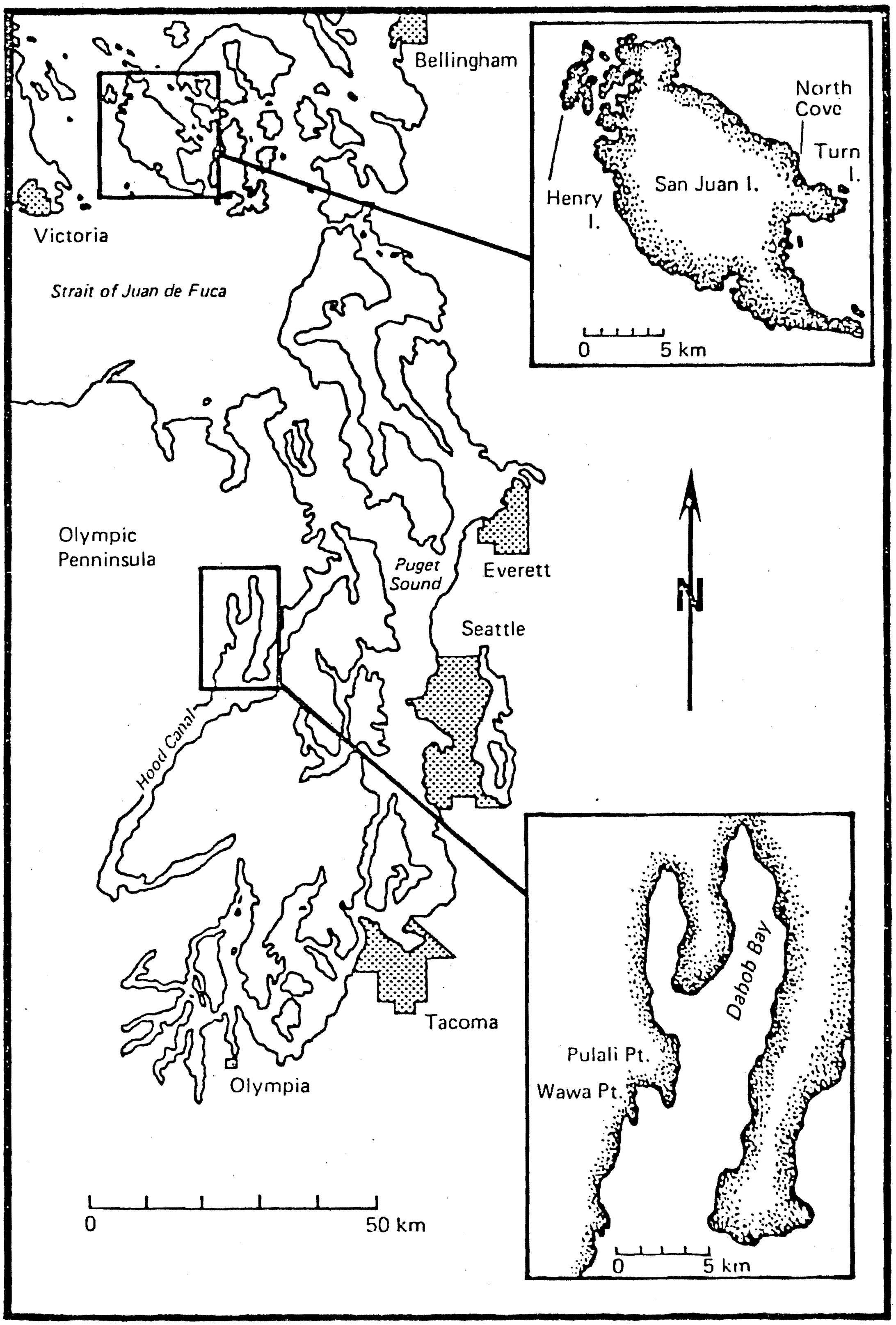

Fig. 1. Field study sites near San Juan Island (north Cove, Henry and Turn Island) and in Dabob Bay (Pulali and Wawa Point).

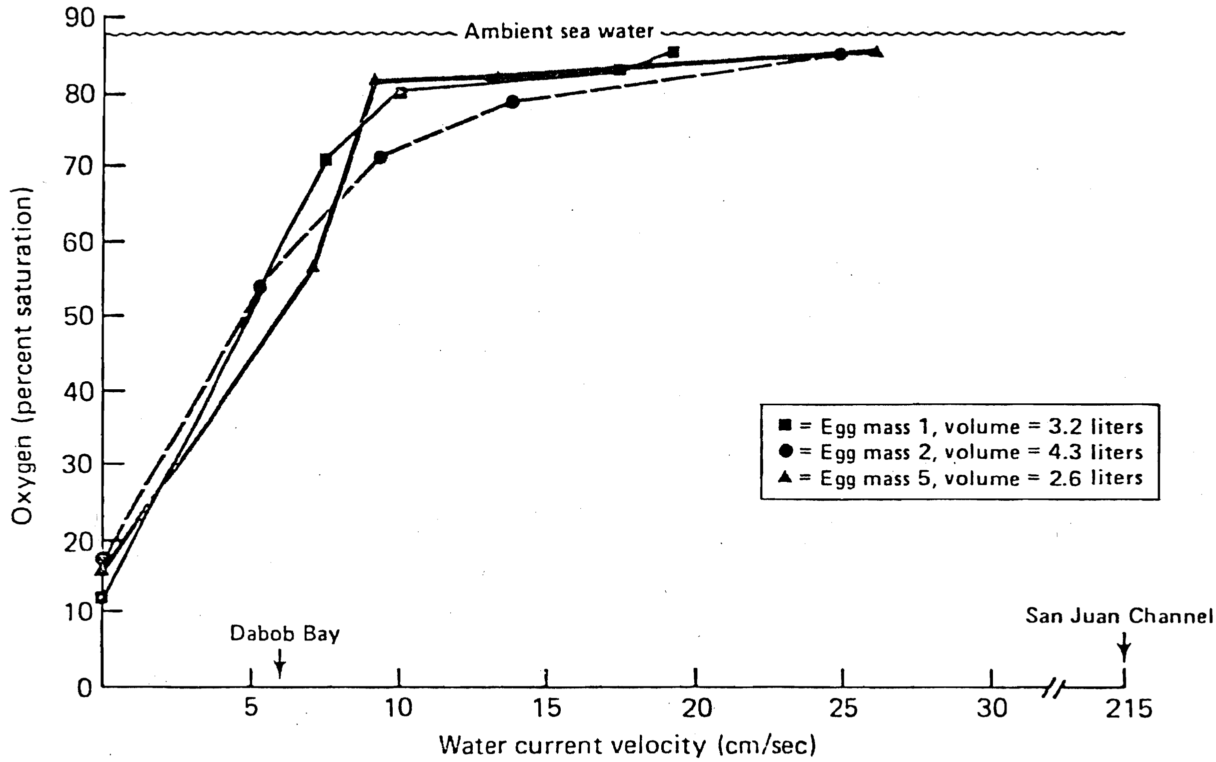

Fig. 2. Interstitial oxygen at the center of three egg masses. Samples were taken at increasing current velocities at least fifteen minutes after current adjustment. Ambient water properties; oxygen = 86-87% saturation, temperature = 8.5-9.0°C, salinity = 28.5‰. Arrows indicate maximum tidal current velocity during a three meter tide fluctuation at Dabob Bay and San Juan Channel.

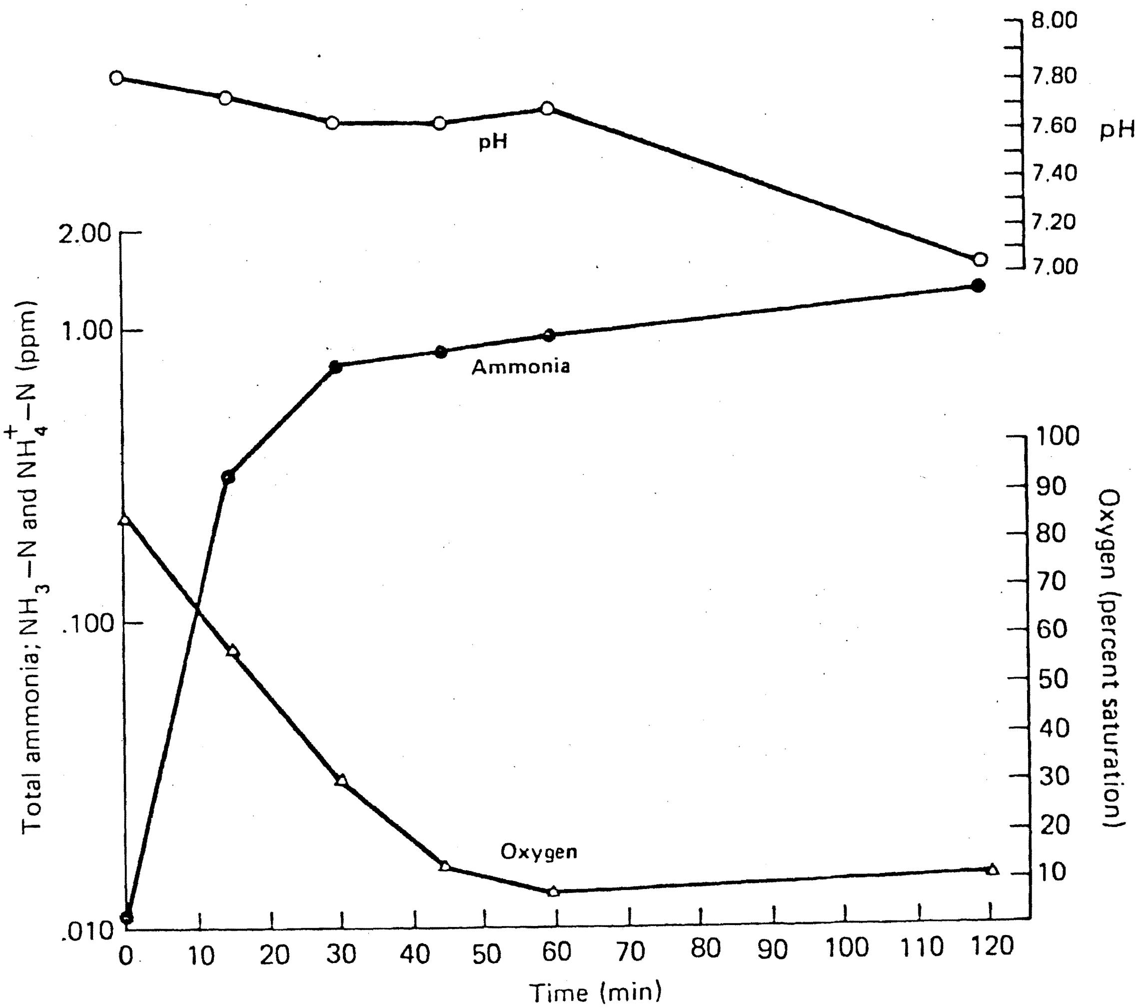

Fig. 3. Interstitial ammonia, pH and oxygen of egg mass #1 (volume = 3.2 lt, weight = 3.7 kg) monitored for 2 hr following cessation of current (>40 cm/sec) at time zero. Water samples were extracted from a single port near the center of the mass.