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The Environmental Biology of the Embryos, Egg Masses and Nesting Sites of the Lingcod, *Ophiodon elongatus*

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THE ENVIRONMENTAL BIOLOGY
OF THE EMBRYOS, EGG MASSES AND NESTING SITES
OF THE LINGCOD, OPHIODON ELONGATUS^{1/}

by

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TABLE OF CONTENTS

	Page
List of Tables.....	iv
List of Figures.....	vii
INTRODUCTION.....	1
Natural History of the Lingcod.....	1
The Egg Stage; Ventilation and Hypoxia.....	4
MATERIALS AND METHODS.....	9
Egg Collection and Incubation.....	9
Instrumentation.....	12
Staging of Embryological Development.....	14
Field Observations and Sampling Procedures.....	15
Interstitial Environment of the Egg Mass.....	19
Embryo Tolerance to Hypoxia and Ammonia.....	23
Embryo Respiration.....	25
Effects of Hypoxia on Embryo Mortality and Development.....	26
RESULTS.....	29
Interstitial Oxygen and Embryo Mortality in the Field.....	29
Interstitial Environment; Laboratory.....	32
Embryo Tolerance to Hypoxia and Ammonia.....	41
Embryo Respiration.....	45
Bradycardia.....	49

TABLE OF CONTENTS (cont.)

	Page
Effects of Hypoxia on Embryo Mortality	
and Development.....	53
Nest Depth Distribution.....	58
Predation on Nests.....	60
DISCUSSION.....	62
Agents Causing Mortality.....	62
Nest Site Selection.....	68
Adaptations and Responses to	
Inadequate Ventilation.....	73
Larval Size: Ecological Considerations.....	79
Auxilliary Ventilation.....	81
Predation on Nests.....	82
Egg Mortality.....	85
SYNOPSIS.....	88
LITERATURE CITED.....	91
APPENDIX A: Supplemental Tables.....	100

List of Tables

Number	Page
1. Developmental stages of lingcod embryos.....	16
2. Interstitial oxygen levels and condition of embryos contained in wild egg masses.....	30
3. Changes in oxygen, pH and ammonia at the nest interior during a two hour exposure to slack water.....	40
4. Changes in oxygen, pH and ammonia at the nest interior during a 70-hour exposure to slack water.....	42
5. Results of 96-hour oxygen bioassays.....	44
6. Effects of developmental stage and oxygen level on embryo respiration: two-way ANOVA.....	50
7. Newman-Keuls multiple range test: embryo respiration.....	51

List of Tables (cont.)

Number	Page
8.	Effects of different oxygen levels at the egg mass interior on hatching success and larval size at hatch.....54
9.	Two-way ANOVA: Effects of time of hatching and interstitial oxygen on the size of larvae.....56
10.	Newman-Keuls multiple range test: larval size at hatch.....57
A-1.	Mortalities of embryos incurred during oxygen bioassays.....101
A-2.	Respiration data used for two-way analysis of variance: developmental stage x oxygen level.....102
A-3.	Bradycardia regression data used for analysis of covariance.....103

List of Tables (cont.)

Number	Page
A-4. Standard lengths of larvae hatching from nest pieces incubated at different current velocities.....	104
A-5. Depth of lingcod nests below mean lower low water.....	105
A-6. Temperature and salinity at two depths in Dabob Bay.....	106
A-7. Incidence of guardian males and predator sightings at lingcod nests.....	107

List of Figures

Number	Page
1. Airlift powered egg incubator.....	11
2. Field study locations in Dabob Bay and near San Juan Island.....	17
3. Diagram of current velocity tank.....	21
4. Interstitial oxygen levels at the center of three egg masses at various water velocities.....	33
5. Diagram of egg mass 5 designating positions of sampling ports.....	35
6. Interstitial oxygen levels at several locations in an egg mass at various current velocities.....	36
7. Oxygen depletion at the interior of two egg masses during slack water conditions.....	37

List of Figures (cont.)

Number	Page
8. Changes in oxygen, pH and ammonia at the interior of the egg mass during a two hour exposure to slack water.....	39
9. Survival of advanced embryos at four time periods and six oxygen levels.....	43
10. Mean oxygen uptake of lingcod embryos at ambient oxygen levels.....	46
11. Oxygen uptake of embryos at several different oxygen levels.....	48
12. Depth distribution of lingcod nests in Dabob Bay and the San Juan study sites.....	59

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INTRODUCTION

Natural History of the Lingcod

The range of the lingcod, Ophiodon elongatus, extends along the Pacific coast of North America from Kodiak Island, Alaska to Pt. San Carlos, Baja California (Miller and Geibel 1973). This species supports extensive sport and commercial fisheries in British Columbia, Canada, and in the United States. The major features of this species' life history have been identified, but specific details, especially regarding the early life history stages are limited.

Spawning behavior is initiated during late autumn and early winter when males establish residence at potential nesting sites, primarily in rocky habitat. Nest sites appear to be concentrated near shore (Jewell 1968, Moulton 1977, Low and Beamish 1978, LaRiviere et al. in press). Females are rarely seen at the spawning grounds. It is assumed that they move into the areas for only a brief period to deposit eggs, then vacate the locale. Typically, a male guards each nest until hatching (Jewell 1968, Low and Beamish 1978, LaRiviere et al. in press). Actual deposition of eggs has only been observed once (Wilby 1937). Eggs and a clear, viscous, gelatinous matrix are

typically extruded into crevices and rocky interstices, forming a compact mass. Within 24 to 48 hours, the eggs are firmly cemented to each other but not to the substrate. Presumably, the gelatinous matrix forms the adhesive since it is no longer recognizable in its previous form once the mass has set. Unoccluded interstices are present among the eggs where the matrix formerly occurred. Incubation time ranges from 42 to 59 days (Jewell 1968) during which time the nest is usually guarded by the male (Jewell 1968, Low and Beamish 1978, LaRiviere et al. in press). Eggs are about 2.8 mm in diameter when deposited and approach 3.5 mm after water hardening (Wilby 1937). Phillips (1958) reported fecundities of 60,000 and 518,000 eggs for 762 mm and 1,041 mm (TL) females, respectively.

Based on collections in Canada, the epipelagic larvae occur in the upper 3 meters of the water column from March through May (Phillips and Barraclough 1977). The standard length at hatch ranges from, approximately 8.5 to 11.5 mm. First food includes small copepods and their eggs. As larvae grow, copepods and fish larvae constitute the bulk of their diet. Near 3 months of age (total length approximately 80 mm), juveniles leave the pelagic community and take up residence on the bottom nearshore. In Canadian waters, juveniles appear to select shallow areas (less than 20 m) of sandy to sparsely rocky substrate (Phillips and Barraclough 1977), while in California small recently

settled juveniles were captured at depths to 55 m (Miller and Geibel 1973). Food items for these young juveniles include an assortment of crustaceans and small fishes, especially sandlance and flatfish (Giorgi, unpublished data). Juveniles are about 350 mm total length when they first move into more rugged rocky areas that are typical adult habitat (Miller and Geibel 1973).

Tagging studies indicate that adults are rather sedentary (Hart 1943, Chatwin 1956, Reeves 1966, Miller and Geibel 1973, Mathews et al. 1979) only relatively few venturing farther than a few kilometers from the original tagging sites. Hart (1943) observed greater adult movement than other authors, with 36% of tagged and recaptured fish making long excursions of several kilometers from tagging sites. Several authors contend that there is convincing evidence of a spawning migration of adults from deep to shallow water, as indicated by increased nearshore catches prior to and during the spawning season (Wilby 1937, Phillips 1958, Miller and Geibel 1973). Adults are apparently quite long lived; using annular rings on vertebrae, Chatwin (1956) estimated the age of the oldest female in a sample at 15 years.

The recent decline of lingcod stocks in Puget Sound (Ilg et al. 1979) was 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 several research programs, including a Sea Grant population study and an enhancement effort by Washington Department of Fisheries. This paper describes some of the research funded by the recreational fisheries program at the Northwest and Alaska Fisheries Center of the National Marine Fisheries Service in Seattle, Washington, to study the early life history of the lingcod.

The Egg Stage; Ventilation and Hypoxia

While gathering wild eggs for use in laboratory experiments, I noticed that lingcod egg masses from a particular nesting area often 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 Dabob 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 massiveness of the lingcod egg mass (2 to 3 liters is a typical nest volume), its compact nature with eggs tightly cemented together and the prevailing water movement characteristics at the two locations, it seemed plausible that the mortalities

observed in Dabob Bay lingcod egg masses could have resulted from inadequate nest ventilation.

It is suspected that poor water circulation within dense concentrations of demersal fish eggs can account for a substantial amount of egg mortality in a variety of species. Herring (Clupea sp.) deposit mats of eggs which can be quite thick; up to 16 egg layers, when aggregations of spawning adults are sizable (Outram and Humphreys 1974). The extent of embryo mortality observed in such situations increases with the thickness of the egg mat (Hempel 1971, Taylor 1971). One hypothesis offered is that interior-most eggs, those proximal to the substrate, die from insufficient oxygen or metabolite accumulation, however, experimental confirmation is lacking.

Oxygen levels in water near eggs has been found to be closely related to water current velocity. Phillips and Campbell (1961), using artificially constructed redds, demonstrated that steelhead (Salmo gairdneri) embryo survival increased with the oxygen level in the gravel, which was correlated with the apparent velocity in the redd. In a similar study, Coble (1961) established a positive correlation between steelhead embryo survival and dissolved oxygen, which again was closely related to the water flow in the gravel. Both Wickett (1954) and McNeil (1966) contended that the high embryo mortalities they witnessed in chum (Onchorhynchus keta) and pink salmon (O.

gorbuscha) redds were a direct consequence of poor water circulation and resultant low dissolved oxygen within the redd.

Wickett (1954) stressed that the delivery rate of oxygen to a developing embryo in a redd is a function of water velocity as well as oxygen concentration. Other investigators (Coble 1961, Silver et al. 1963, Shumway et al. 1964) demonstrated experimentally that sufficiently reduced water velocity resulted in decreased larval size at hatch. But water velocity did not affect larval size to the degree that oxygen level did. DeMartini (1976) subjected egg masses of the painted greenling, Oxylebius pictus, to several current velocities and demonstrated that decreased velocities could significantly delay hatching.

Hypoxia is a stressful condition that can be expected to occur when ventilation is restricted. Embryo mortality is the extreme consequence of severe or chronic hypoxia. Sublethal effects of reduced oxygen on fish embryos are well known and can ultimately affect the survival of the organism to later life history stages (Rosenthal and Alderdice 1976). Numerous investigators studying a wide assortment of freshwater and marine fish species have established that incubation of embryos at reduced oxygen levels results in retarded development and reduced larval size at hatch (Silver et al. 1963, Shumway 1964, Brannon 1965, Alderdice and Forrester 1971, Oseid and Smith 1971,

Carlson et al. 1974, Carlson and Siefert 1974, Siefert and Spoor 1974). Morphological abnormalities during embryo development have been reported in Oncorhynchus keta (Alderdice et al. 1958) and Clupea harengus (Braum 1973) exposed to hypoxic water.

Mobile, free swimming stages of fishes often leave or avoid hypoxic locales (Davis 1975). When avoidance of the hypoxic condition is not possible, metabolic adjustments are necessary. These adjustments may include decreasing the metabolic rate, increasing ventilation of respiratory surfaces, shunting of blood to auxillary respiratory surfaces, bradycardia and perhaps, in rare cases, switching to anaerobic metabolism (Smith in press, Garey 1962, Randall and Shelton 1963, Randall 1968, 1970, Holeyton 1971, Congleton 1974). Investigations examining respiratory, circulatory and ventilatory responses of fish embryos to hypoxia are limited. Hayes et al. (1951) experimentally demonstrated that sufficiently low oxygen levels can depress metabolism in Salmo salar embryos. Both Wickett (1954) and Alderdice et al. (1958) established critical oxygen levels (oxygen concentrations below which metabolism is oxygen dependent) for salmonid embryos.

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

experiments. As previously mentioned, preliminary observations indicated that embryo mortalities in the interior of lingcod egg masses appeared to be related to current velocities in the spawning areas. Therefore, to assess the nature and extent of embryo mortality, egg masses from spawning grounds characterized by either low or high current velocities were compared. Oxygen levels of interstitial water of the egg masses were examined in the field and laboratory. Additionally, ammonia and pH changes were monitored in the laboratory. Lethal limits of oxygen and ammonia were examined as were selected responses of embryos to hypoxia. Since predation of lingcod nests has been documented as constituting an important cause of embryo mortality (Jewell 1968, Low and Beamish 1978, LaRiviere et al. in press), predation was also investigated. Finally, possible environmental criteria for nest site selection by spawning lingcod are discussed in light of data presented in this paper.

MATERIALS AND METHODS

Laboratory experiments were conducted at the National Marine Fisheries Service Aquaculture Field Station at Manchester, Washington.

Unless otherwise specified, seawater used for egg incubation and experimentation was coarse sand-filtered, then cannister-filtered to 5 microns, and finally sterilized in a Refco model RL-10-1P ultraviolet water purifier. Supply lines were constructed of polyvinylchloride (PVC) pipe and Tygon tubing. Seawater used in the laboratory was at ambient temperature and salinity. Temperature was monitored daily, while salinity, oxygen and pH were monitored intermittently.

Egg Collection and Incubation

Lingcod eggs used in the laboratory were collected subtidally at Hood Canal using SCUBA. In most cases, pieces were removed from the masses, but for certain experiments entire egg masses were collected. Eggs were transported in 200 liter polyethylene containers which had been leached in seawater for at least one month. Containers were filled with seawater at the collection site and supplied with aeration. Temperature during transport never changed more than 1.5°C.

At the laboratory, whole egg masses were held in a round, fiberglass tank, 122 cm in diameter, fitted with a central standpipe and supplied with a jet of fresh seawater creating a current velocity in excess of 50 cm/sec. Water depth was adjusted to assure total immersion of the egg masses.

Small lots of eggs were incubated in an airlift device designed to provide ample ventilation with minimal agitation (Figure 1). The incubators were constructed of clear acrylic tubing. Each had three major components: a central air-lift chimney, two water intake chambers, and two incubator baskets which held the eggs. The central chimney was fitted with a fine glass bead airstone at its base. The two intake chambers, located 180° apart, were confluent with the chimney just below the airstone. Both the bottom and removable top of the incubator baskets are fitted with Nitex cloth of desired mesh size (0.5 mm). A retainer ring glued to the outer wall of the incubator basket supported it in the chamber. When air was introduced into the base of the chimney, water was drawn through the incubator basket and chamber into the base of the chimney and out the top opening. Incubators were leached in running seawater at least two weeks prior to use. Incubators were deployed in a 100 liter acrylic

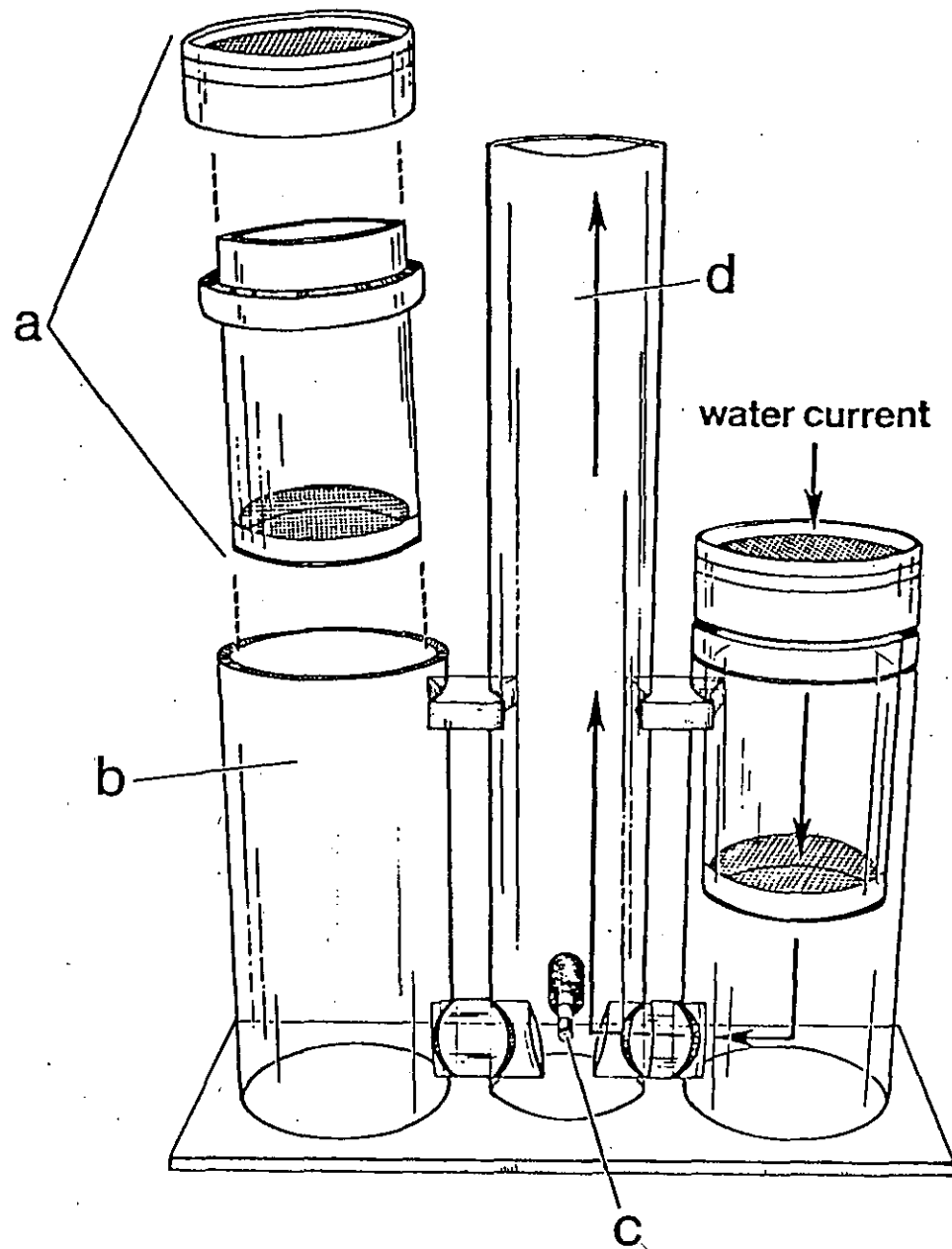


Figure 1. Airlift egg incubator: a) Incubator basket; b) Water intake chamber (7.0 cm o.d., 3 mm thick sidewall, height = 15.0 cm); c) Fine glass-bead airstone; d) Airlift chimney (5.1 cm o.d., 3 mm sidewall, height = 30 cm). Short pieces of tubing (2.5 cm o.d., 3 mm sidewall) connect the chimney and intake chambers. The chimney and both intake chambers are fixed to a 24.0 x 9.5 cm, 6 mm thick base.

aquaria with an open seawater supply system. Water replacement in the aquarium exceeded 4 liters per minute.

Instrumentation

Oxygen tensions were measured with an Instrumentation Laboratory model 113pH/gas analyzer equipped with a polarographic oxygen micro-electrode. Water samples in syringes were injected, via a two-way stainless steel stopcock, into the micro sample chamber (capacity = 0.3 ml) which houses the sensory face of the electrode. Both the electrode and sample chamber were encased in a temperature-controlled circulating water jacket. An external cooling line was installed on the analyzer, enabling the jacket temperature to be lowered below ambient room temperature. The cooling line was immersed in a running water bath with ambient temperature seawater. Temperature range over the experimental period was 7.9 to 9.5°C. Since the water sample, sample chamber, and electrode were at the same temperature during determinations, the oxygen partial pressure, in mm Hg, could be read directly from the meter scale. The electrode and sample chamber were allowed to stabilize at the ambient seawater temperature for 30 minutes prior to calibration. Calibration required setting zero on the meter scale while slowly bubbling nitrogen gas through the sample chamber containing seawater. After at least two minutes, gas

injection was ceased and the entrapped nitrogen was allowed to temperature stabilize. In a similar fashion, room air was bubbled through the sample chamber and the meter needle was adjusted to read the partial pressure of oxygen in room air where:

$$P_{\text{oxygen}} = 0.209 (P_{\text{atm.}} - P_{\text{w.v.}})$$

Atmospheric pressure ($P_{\text{atm.}}$) was read from the laboratory barometer which was periodically calibrated with the local weather service. Water vapor pressure ($P_{\text{w.v.}}$), which varies with temperature, was taken from tables of Sienko and Plane (1966). Because of the low electrode temperatures, it usually required three to four minutes for the meter to stabilize from the time of initial sample injection. All procedures regarding usage and calibration of this instrument were in accordance with those presented in the operator's manual. Readings were checked with Winkler titration (Strickland and Parsons 1972).

In this text, oxygen is usually expressed as percent saturation but where necessary is converted to oxygen concentrations by using the appropriate solubility factor from Carpenter's (1966) tables. Salinities were measured with a hydrometer to the nearest 0.5‰.

Certain experiments required seawater with dissolved oxygen concentrations below that of ambient water. To

provide the necessary water, a 10-liter aspirator bottle was filled with seawater and gassed with nitrogen until the desired oxygen level was attained. The top of the vessel was then loosely sealed with parafilm and water was extracted from the bottom port of the vessel while nitrogen gas was concurrently released into the dead space to displace oxygen.

Ammonia was measured with an Orion ammonia electrode model 95-10 coupled with an Orion microprocessor/analyzer model 901. Gilbert and Clay (1973) found this electrode useful down to 10 ppb as NH_3 . The electrode was calibrated according to the operator's manual prior to each series of measurements. If determinations were required over the entire day, the electrode was recalibrated every two hours as necessary. All standard solutions were made with freshly distilled and deionized water. Total ammonia levels in ambient laboratory seawater ranged from 16 to 36 ppb. These values are similar to those noted at the New England Aquarium by Gilbert and Clay (1973), using the same electrode.

Staging of Embryological Development

Since it was not possible to estimate the age of wild lingcod embryos used in laboratory experiments, a series of developmental stages from fertilization to hatching was defined, in order to compare the relative performance of

embryos during various experiments. Morphological features used as embryological landmarks included but were not limited to those used by other authors (Knight 1963, Ballard 1973). Developmental stages and corresponding morphological characteristics are presented in Table 1. Hatching occurs during stage 14. The opaque chorion was removed prior to embryo classification.

Field Observations and Sampling Procedures

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 characterized by low tidal current velocities (Figure 2). 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 midchannel velocities in Dabob Bay attain an approximate maximum of only 6 cm/sec during a similar tide; the latter value was calculated from data presented by Kollmeyer (1962) and Sverdrup et al. (1942).

Field observations included: frequency of occurrence and activity of guardian males, macrofauna associated with nests, evidence of predation, and nest depth. Depths were measured with both an oil filled and capillary depth gauge

Developmental stage	Embryological events
1	Fertilization to early blastodisc formation.
2	Blastodisc extends half the distance to the equator; no neural plate evident.
3	Blastodisc envelops one-third of the yolk; neural plate is visible.
4	Embryo encircles half of the yolk; optic vesicles are apparent.
5	Embryo encircles three-quarters of the yolk; melanophores first appear; red blood cells are first evident.
6	Eye pigment is first apparent; pectoral fin buds are easily recognizable.
7	Eye pigment darker; dorsal melanophores arranged in rows, lateral melanophores now evident; dorsal and anal fin folds present.
8	Melanophores present on dorsal gut surface.
9	Embryo completely encircles yolk; a few melanophores appearing on head.
10	Many stellate melanophores on head; yolk sac vascularization evident.
11	Two rows of melanophores on each side of head, three rows on dorsal body surface.
12	Mouth visible; caudal fin fold becoming lobate.
13	Melanophores present on opercle area.
14	Lower jaw even with or slightly protruding beyond upper jaw; hatching occurs during this stage.

Table 1. Developmental stages of lingcod embryos with corresponding morphological characteristics.

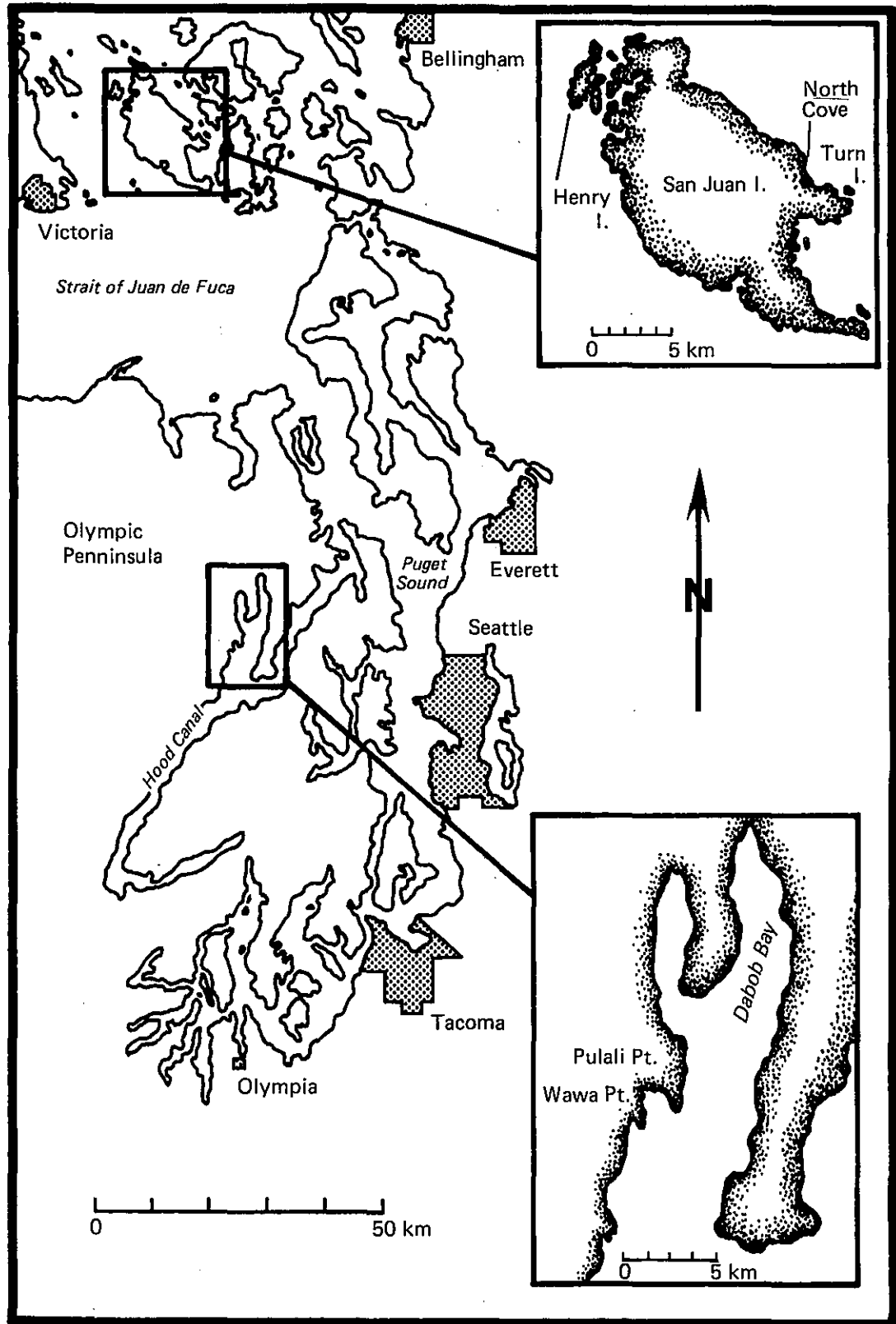


Figure 2. Field study sites near San Juan Island (North Cove, Henry and Turn Island) and in Dabob Bay (Pulali and Wawa Point).

and adjusted to mean low low water. Information was recorded on a plastic slate.

For identification and relocation purposes, numbered markers were deployed at specific nest sites. Each consisted of a 1 m strip of surveyor's tape fastened at one end to a styrofoam float (approx. 65 cm³) with a metal anchor (approx. 400g) at the other.

In a number of nests at each area, interstitial oxygen was measured and embryo condition was evaluated. For oxygen determinations, a 5-ml water sample was extracted near the center of each mass with a glass syringe via a 100-mm, 18-gauge anesthetic needle. The needle was then imbedded in a rubber stopper. Ambient oxygen in water surrounding nests was also measured. The water sample was placed in an ice-brine bath (-1.0 to 0.0°C) and immediately transported to either the Friday Harbor Laboratory on San Juan Island or Manchester facility for processing. Elapsed time from extraction to processing never exceeded three hours. In order to assure that this transport technique sufficiently fixed the oxygen content in the syringes until processing, the oxygen level in several syringes held in ice-brine was monitored for five hours. Specifically, three interstitial water samples were extracted from an egg mass held in the laboratory; oxygen was measured at the time of extraction and again at the end of the five hour period. There was no significant change in the oxygen

content of the syringes ($p < .01$; paired t-test). Syringes containing harpacticoid copepods (a common interstitial organism in the egg masses) were not processed.

Cores were extracted from egg masses with a 25-mm diameter, thin-walled steel pipe with a sharpened edge. The cores passed through the center of the masses to the substrate. They were transported to the lab in either a 16 or 200 liter plastic vessel filled with seawater (temperature $< 10^{\circ}\text{C}$). Embryos along the lengths of the cores were examined for viability and developmental stage.

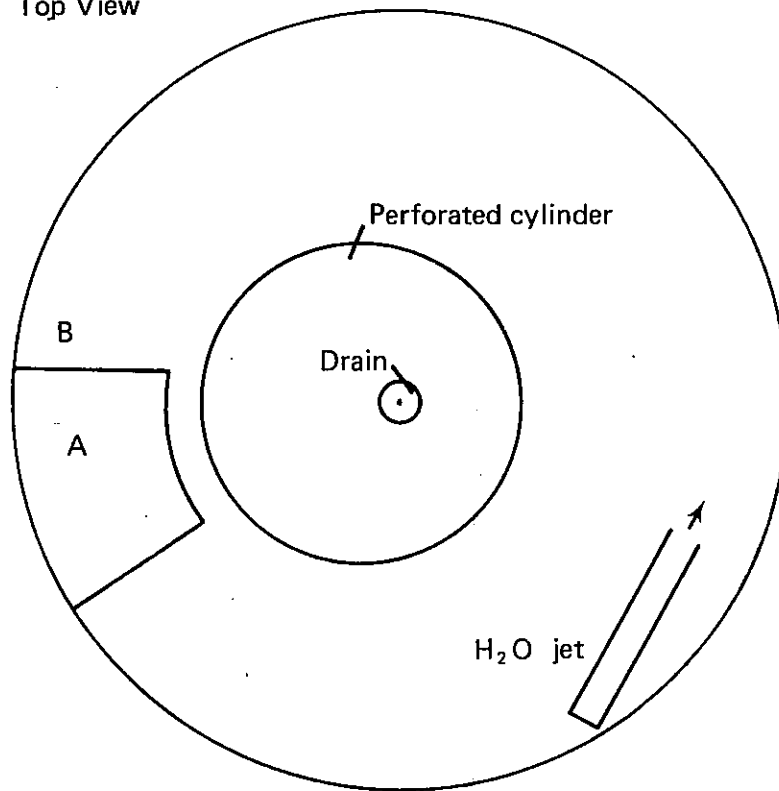
Interstitial Environment of the Egg Mass

Oxygen uptake of fish embryos increases with development (Alderdice et al. 1951, Braum 1973, Hempel 1979) while resistance to hypoxia decreases (Alderdice et al. 1958). Therefore, developmentally advanced lingcod embryos are expected to be both the most effective stages in depleting oxygen within an egg mass and the most sensitive to any resulting hypoxia. Consequently, advanced embryos (stages 10-11) and egg masses containing primarily stage 10-13 embryos were used in the laboratory experiments. The only exception occurred in the respiration experiment (to be described later), where performance over a range of developmental stages was of interest.

Laboratory experiments were conducted to examine the nature of the internal environment of the egg mass under a variety of water current conditions including slack water. Egg masses were deployed in a 60 cm deep, 122-cm diameter, circular fiberglass tank with a central floor drain. A 58-cm diameter perforated lucite cylinder was placed eccentrically over the drain, which was fitted with a 25-cm high standpipe (Figure 3). The eccentric positioning of the cylinder resulted in the most uniform flow across the deployment area as determined with an Ott current velocity meter. A high pressure seawater line was used to produce the water current. During experiments the current meter was secured at position "B", Figure 3. Inlet water flow was adjusted until the desired velocity registered on the current meter. Positions of the deployment area, water inlet, lucite partition, and current meter were the same for all experiments. All egg masses were oriented lengthwise into the current. Masses did not possess peninsular-like projections or large indentations.

Water samples were extracted from a number of locations within egg masses with a 5-ml glass syringe and an 18-gauge, 100 mm, stainless steel anesthetic needle. Needles remained imbedded at the same position and depth in each egg mass throughout each experiment. Oxygen, ammonia, and pH were monitored while egg masses were exposed to

Top View



Cross Section

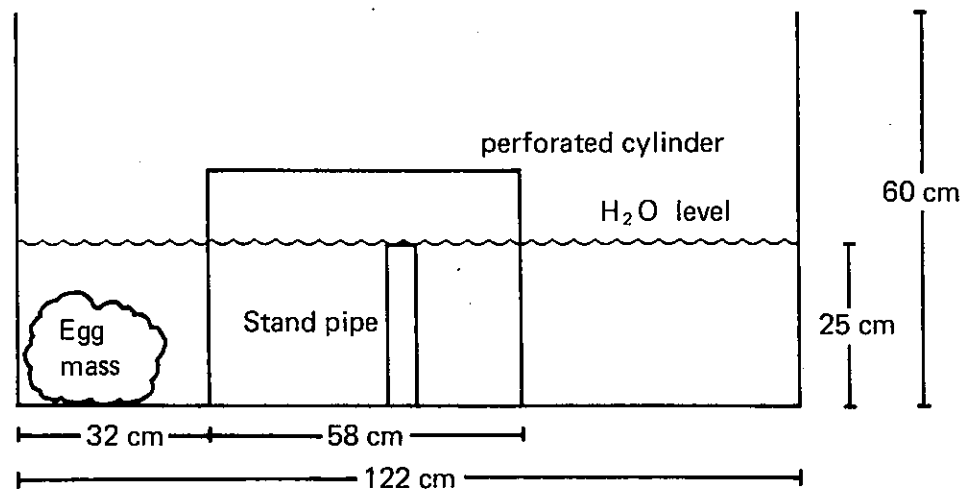


Figure 3. Circular (122 cm diameter), fiberglass, current velocity tank. The water jet was adjusted to provide the appropriate test velocity which registered on the current meter (B). The eccentrically positioned lucite cylinder provided the most uniform flow across the nest deployment area (A). Perforations in the cylinder wall permitted water to exit the system via the drain.

slack water. A 0.5-ml volume of water was extracted and discarded from the needle dead space before the actual water samples were extracted. The oxygen tension of a 1-ml sample was measured with the microelectrode. The pH of a 3-ml sample was measured with a Corning pH meter model 610 A. To measure ammonia a smaller 5-ml sample was used instead of the prescribed 200 ml water sample. The water sample and a midget stirring bar were placed into a glass vial, the inside diameter of which just fit over the electrode. The electrode was then inserted into the vial and the gap sealed with parafilm. All other procedures are identical to those presented in the operator's manual except that the volume of reagents was reduced proportionately with the water sample. There was no detectable difference between ammonia values determined with a 5 or 200-ml water sample.

In aqueous solution, ammonia occurs in two forms: the NH_4^+ ion and the un-ionized NH_3 molecule, the relative proportions of which vary with pH, temperature, and salinity. Values for NH_3 were taken from tables developed by Bower and Bidwell (1978). When the pH of the water sample was outside the range covered in the tables, NH_3 was calculated using the equation presented by Armstrong et al.

(1978) as taken from Albert (1973):

$$[\text{NH}_3] = \frac{[\text{Total ammonia}]}{1 + [10.0]^{(\text{pK}_a - \text{pH})}}$$

The pK_a values of ammonia in seawater were taken from Bower and Bidwell (1978).

Embryo Tolerance to Hypoxia and Ammonia

Static bioassays were conducted to assess the effect of reduced oxygen on advanced embryos (stage 10-11). Forty-eight samples of 13 to 14 eggs each were rinsed well with ambient laboratory seawater ($\text{pH} = 7.80$, salinity = $29.0^\circ/\text{oo}$, temperature = $8.5\text{--}9.5^\circ\text{C}$) and placed in 300 ml BOD bottles. Test water of the appropriate oxygen content was prepared by gassing with nitrogen as previously described, then siphoned into the bottle allowing it to overflow one volume. Bottles were then sealed with ground glass stoppers and immersed in an open system ambient seawater bath. Oxygen levels of the test solutions were: 2, 6, 12, 22, 50, and 85 percent saturation, the latter being the ambient control. Two replicate bottles were run for each oxygen level. Treatments were monitored for death at 4, 24, 48, and 96 hour intervals as defined by cessation of heart beat. Since the chorion of the lingcod egg is opaque, it was necessary to dissect it from the embryo at the end of the treatment period under a dissecting scope in

order to view the heart. Care was taken to avoid thermal shock. Eggs were 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 dissections resulting in damage to the embryo were discarded and not recorded as mortalities. Heart beat rates were recorded, but could not be estimated for all dissections, because many embryos died before accurate counts could be made and timed. Oxygen levels in BOD bottles were checked at the end of the exposure period; variation from initial measurements was less than 3 mm Hg.

Similar assays were conducted to determine the acute lethal concentration of ammonia. Test solutions of NH_4Cl in ambient laboratory seawater (pH=7.80, salinity=28.5‰, oxygen=85% saturation, temperature=8.7-9.3°C) were:

Total ammonia-N (ppm)	NH_3 -N (ppb)
.02 (ambient water)	negligible
.25	2
1.00	9
10.00	89
50.00	444

Embryo Respiration

Glass syringes (1-ml) were used as respirometers. Lührlock stainless steel needles imbedded in rubber stoppers sealed each respirometer. Between experiments, respirometers were washed with a 5% aqueous hypochlorite solution and rinsed thoroughly with hot tap water, followed by distilled water. Eggs of various developmental stages were carefully separated from the adhesive egg mass and rinsed with seawater. A single egg was placed in each respirometer. Care was taken to eliminate all air bubbles by repeatedly purging and loading the syringes while submerged in a glass vessel. Respirometers were visually examined and the procedure repeated as necessary. Once sealed the respirometers were placed in a running seawater bath at a temperature of 8.3-9.0°C. Respirometers were not agitated or stirred.

Salinity, temperature, and oxygen tension of respirometry water was measured at the beginning of each experiment. Experiments were conducted between the hours of 0700 and 1700 and lasted from 4.0 to 6.6 hours. Two to four control respirometers containing only water were included with each experiment. The oxygen levels in the controls never changed more than 0.01 $\mu\text{l/hr}$. Oxygen uptake was calculated for each respirometer and expressed as μl of oxygen/egg/hr, using the tables of Carpenter (1966). The sample size ranged from three to five eggs for each

developmental stage investigated. Respiration rates were corrected for the water volume displaced by the egg in the respirometer (mean egg volume = 14 mm^3).

Although rinsing in seawater eliminated the larger biota present on the chorion (primarily harpacticoid copepods), protozoa and/or bacteria were assumed to be present (Braum 1978). In order to estimate only the embryo respiration, it was necessary to either kill the epibiota or estimate its fraction of the total egg respiration and correct for it. Since any chemical agent or physical shock used to eliminate the epibiota could potentially alter the metabolic activity of the embryo, the latter approach was employed. Chorions were carefully dissected from three eggs in each experimental lot. These three chorions were then placed in a single respirometer. The mean oxygen uptake for a single chorion was calculated and subsequently subtracted from the oxygen uptake of the entire egg, yielding an estimate of embryo respiration.

Effects of Hypoxia on Embryo Mortality and Development

The purpose of this experiment was to assess the effect of hypoxia on embryos incubating in an egg mass. The mass used in this experiment had a unique configuration resembling a large, uniform loaf of bread (approx. $46 \times 19 \times 12$ cm). This facilitated dissecting it into four pieces of

about equal size and configuration, three of which (911, 915 and 922 g) were utilized in the experiment. The egg mass was collected at a low current site; when sectioned, a central core of apparently dead embryos was readily visible. Dissection of the embryos confirmed the mortalities. Additionally, differential embryo development was evident from the peripheral surface inward, the peripheral embryos (stage 11) being the most advanced with stage 8 embryos adjacent to the necrotic core. The extent of embryo mortality was estimated by slicing off a thin cross-section of the mass and weighing it; the dead core was then excised and weighed. The ratio of core to total was 16.1:109.1 g, or approximately 15% mortality. Since the core did not extend the entire length of the mass, the end piece contained fewer dead embryos than the other two pieces used in the experiment. Examination of the fourth discarded piece (the opposite end of the mass) revealed that the core terminated approximately one-third the distance from the extreme end of the nest. Based on this, embryo mortality in the end piece utilized in the experiment was estimated at 10%.

Each of the three pieces were weighed and then placed in separate circular fiberglass tanks similar to the one described previously, (Figure 3), with the lucite partition removed. Egg masses were secured by wedging them between the sidewall and a rock. Each nest piece was fitted with a

stainless steel needle (sample port) at its center. Current velocity was adjusted daily to maintain interstitial oxygen at near the test levels of 20%, 50%, and 85% saturation. Raw seawater was used in this investigation (temperature = 8.0-9.5°C, salinity = 28.5-29.0‰, oxygen = 84-91% saturation or 5.63-6.05 ml O₂/liter H₂O).

Tanks were inspected daily for newly hatched larvae (a 0.5 mm screen prevented larval escapement), and the standard lengths of a subsample of larvae (n=30) were measured using an ocular micrometer. To assure that no larvae older than 24 hours were included in daily measurements, all larvae were removed from the tanks daily.

After hatching had ceased, the remaining dead eggs were weighed. The ratio of the weight of remaining dead eggs to the initial egg mass weight (corrected for initial weight of dead eggs) was used to estimate mortality.

RESULTS

Interstitial Oxygen and Embryo Mortality; the Spawning Ground

In Dabob Bay nests, interstitial oxygen levels ranged from 2%-35% saturation; with a mean value of 16% (Table 2). Water samples were extracted from egg masses at Dabob during tidal ebb or flood (at vertical fluctuation >2.0 m) when interstitial oxygen levels are probably near peak levels. Oxygen in ambient water at nest sites ranged from 85 to 87% saturation. All 13 nests examined at Dabob Bay exhibited retarded embryo development at the interior with the more advanced stages present at the periphery. Ten of these (77%) incurred mortalities at the interior. The other three egg masses were either transported to the laboratory for experimentation or could not be relocated for further sampling.

The nature of the mortality observed in these masses was quite distinct. A localized concentration of dead eggs was first evident at the innermost portion of the mass or near the substrate contact surface; no viable eggs occurred within the necrotic region of the egg mass. Among the remaining viable eggs, a developmental gradient was apparent; the most advanced embryos resided near the peripheral surface and the most retarded were adjacent to

Egg mass identi- fication	Date Sam- pled	Oxygen % sat. (ml O ₂ · l ⁻¹)		Condition of interior embryos	
		Ambient H ₂ O	Inter- stitial	Develop- mentally retarded	Percent Mortality
DABOB BAY					
†PP1	3/5/80	87(6.24)	3(0.21)	+	†35
PP2	2/6/80	85(6.05)	35(2.49)	+	5
†PP3	"	"	20(1.42)	+	0
†PP3	3/5/80	87(6.26)	2(0.14)	+	†90
PP4	1/25/80			+	†40
†PP5	2/6/80			+	0
†PP6	1/16/81			+	†95
†PP7	3/4/81			+	†90
†PP9	3/4/81			+	†30
†WDF3	12/26/79			+	0
†WW1	3/5/80	87(6.25)	21(1.51)	+	0
†WW1	3/17/80			+	15
†WW12	3/5/80	87(6.25)	15(1.08)	+	90
†WW12	3/17/80			+	†90
†WW2	1/16/81			+	0
†WW3	3/4/81			+	†5
Mean		86(6.18)	16(1.14)		†59
SAN JUAN					
†NCA	3/26/80	88(6.01)	62(4.23)	-	†0
*†NC2	2/17/80	92(6.33)	63(4.33)	-	0
†NC3	"	"	59(4.06)	-	0
*†NC4	"	"	85(5.85)	-	0
†NC11	4/20/80	95(6.34)	79(5.27)	-	0
TIX	3/25/80	88(6.01)		+	†0
†TI3	"	"	43(2.94)	-	†0
†TI7	"	"		-	0
†HI1	2/16/80	87(5.99)	82(5.64)	-	0
HI2	"	"	53(3.65)	-	0
HI3	"	"	85(5.85)	-	†0
†HI4	"	"	80(5.50)	-	0
HI5	"	"		-	0
†HI6	"	"		-	†0
†HI7	"	"		-	†0
Mean		89(6.11)	69(4.73)		†0

* Interior embryos of these egg masses were not examined.

† Mortality estimates for egg masses near hatching; peripheral embryos were \geq stage 12.

† Guardian male present at nest.

Table 2. Interstitial oxygen levels and condition of embryos contained in egg masses at Dabob Bay and San Juan Island spawning areas. Oxygen measurements were taken during tidal excursions (>2 m vertical change) in Dabob Bay, while San Juan measurements were made only during slack tide. Differing oxygen concentrations at identical percent saturation readings is a consequence of slight variations in temperature ($\leq 2^{\circ}\text{C}$) and salinity ($\leq 2^{\circ}/\text{oo}$) between sampling periods.

the necrotic core. As the egg mass developed, the necrotic region expanded toward the periphery.

To estimate the extent of mortality incurred over the course of development, a number of egg masses were sampled close to hatching. Only nests possessing peripheral embryos \geq stage 12 were used for these estimates; these are indicated in Table 2. Of course, mortality could continue to progress until hatching at stage 14, therefore, these are necessarily conservative estimates. Embryo mortality in eight nests near hatching at Dabob Bay ranged from 5-95% (mean = 59%).

Interstitial oxygen in 10 nests at the San Juan nesting areas ranged from 43-85% saturation (mean = 69%), see Table 2. Oxygen in ambient water at nest sites ranged from 86 to 95% saturation. Since tidal current velocities are extremely high in this region, diving could only be conducted during the slack water periods. Consequently, unlike the Dabob Bay samples, oxygen measurements necessarily had to be made when interstitial saturation would be expected to be at its lowest levels. Cores of 13 egg masses (7 at Henry Is., 3 at North Cove, and 3 at Turn Is.) were examined. Only one of the 13 contained developmentally retarded embryos at its interior. This nest was located at Turn Island; stage 12 embryos were observed at the periphery with progressively retarded stages evident inward, until at a maximum depth of 19 cm

only stage 9 embryos were present. Six of the nests were close to hatching, but no localized concentrations of dead eggs, resembling the mortality observed in Dabob Bay nests, were observed in any egg mass (Table 2).

Interstitial Environment; Laboratory

To determine the effect of water current velocity on egg mass ventilation, interstitial oxygen was measured in three egg masses at increasing current velocities. At least 15 minutes elapsed between current velocity increases and oxygen measurement, allowing ample time for oxygen to stabilize. Prior to extraction of the "zero" velocity sample, each nest was situated in an open system with no direct current for two hours.

Even though the masses differ considerably in size and configuration, interstitial oxygen responds similarly to increasing current velocity (Figure 4). 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 oxygen level as the smaller ones. With some allowance for variation due to mass size, current velocities less than 10-15 cm/sec provide inadequate ventilation to maintain interstitial oxygen near that of the ambient water.

In order to assess the effect of current velocity on the distribution of oxygen within a mass, three sampling

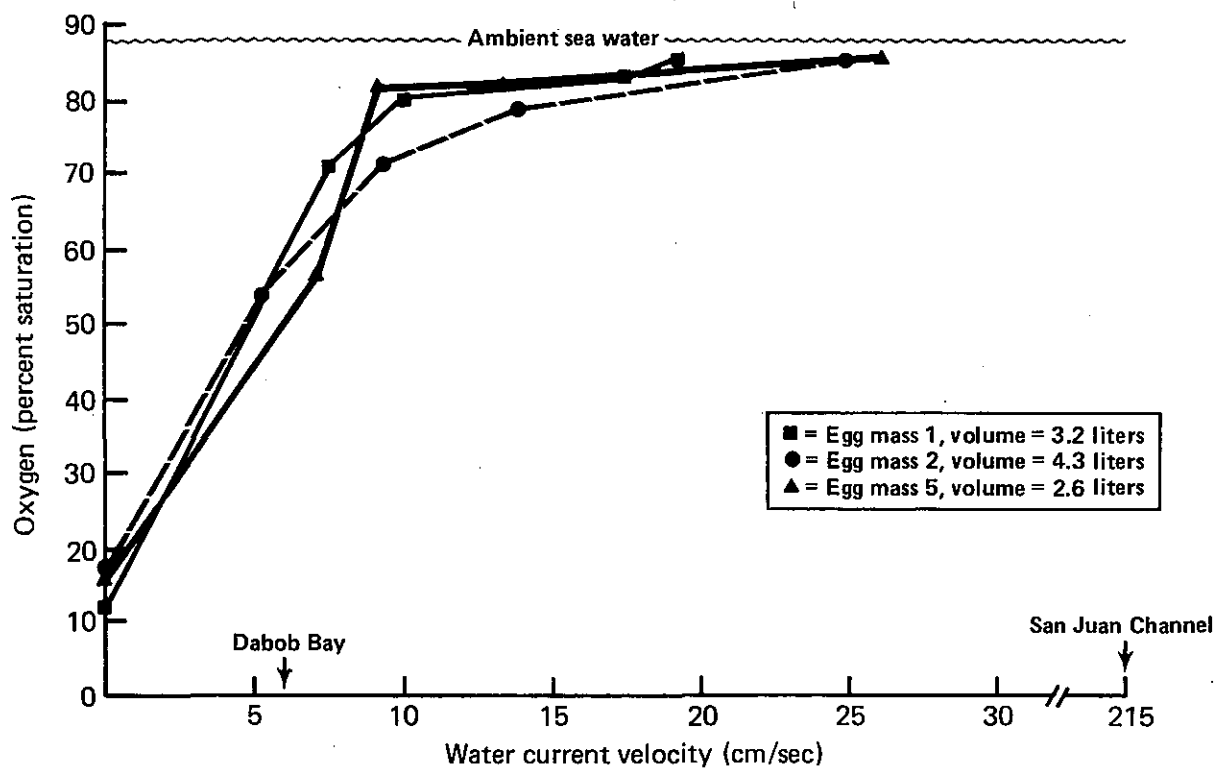


Figure 4. Interstitial oxygen at the center of three egg masses. Samples were taken at increasing current velocities at least fifteen minutes after current adjustment. Ambient water properties; oxygen = 86-87% saturation, temperature = 8.5-9.0°C, salinity = 28.5‰. Arrows indicate maximum tidal current velocity during a three meter tide fluctuation at Dabob Bay and San Juan Channel.

ports were imbedded along the longitudinal axis at the center of mass 5 (Figure 5). Water samples were extracted simultaneously from the three ports at various water velocities. At velocities less than 7.5 cm/sec, oxygen was not distributed evenly throughout the mass (Figure 6). The foremost portion, facing the current, had higher oxygen levels than either the hindmost or center portions. At velocities near 10 cm/sec, interstitial oxygen was nearly uniform along the central core of the mass, and only 5-10% saturation lower than the ambient water.

An experiment was conducted to investigate the effect of slack water conditions, on the rate of oxygen depletion in an egg mass. Two masses were flushed at current velocities ≥ 40 cm/sec for two hours prior to cessation of current, and a time-zero water sample was extracted from the center of the mass. Interstitial oxygen at the center of the egg masses was then monitored for two hours. Oxygen decreased rapidly for the first 40-60 minutes (Figure 7). Beyond this time, the oxygen level stabilized, fluctuating between 8 and 14% saturation. Since there was no water movement, this level was probably maintained by diffusion.

At current velocities which provide only limited ventilation an oxygen gradient was evident from the periphery inward. Oxygen levels were measured in mass 5 at depths of 2 and 8 cm from the periphery and were 75% saturation and 56% saturation, respectively, at 7.5 cm/sec.

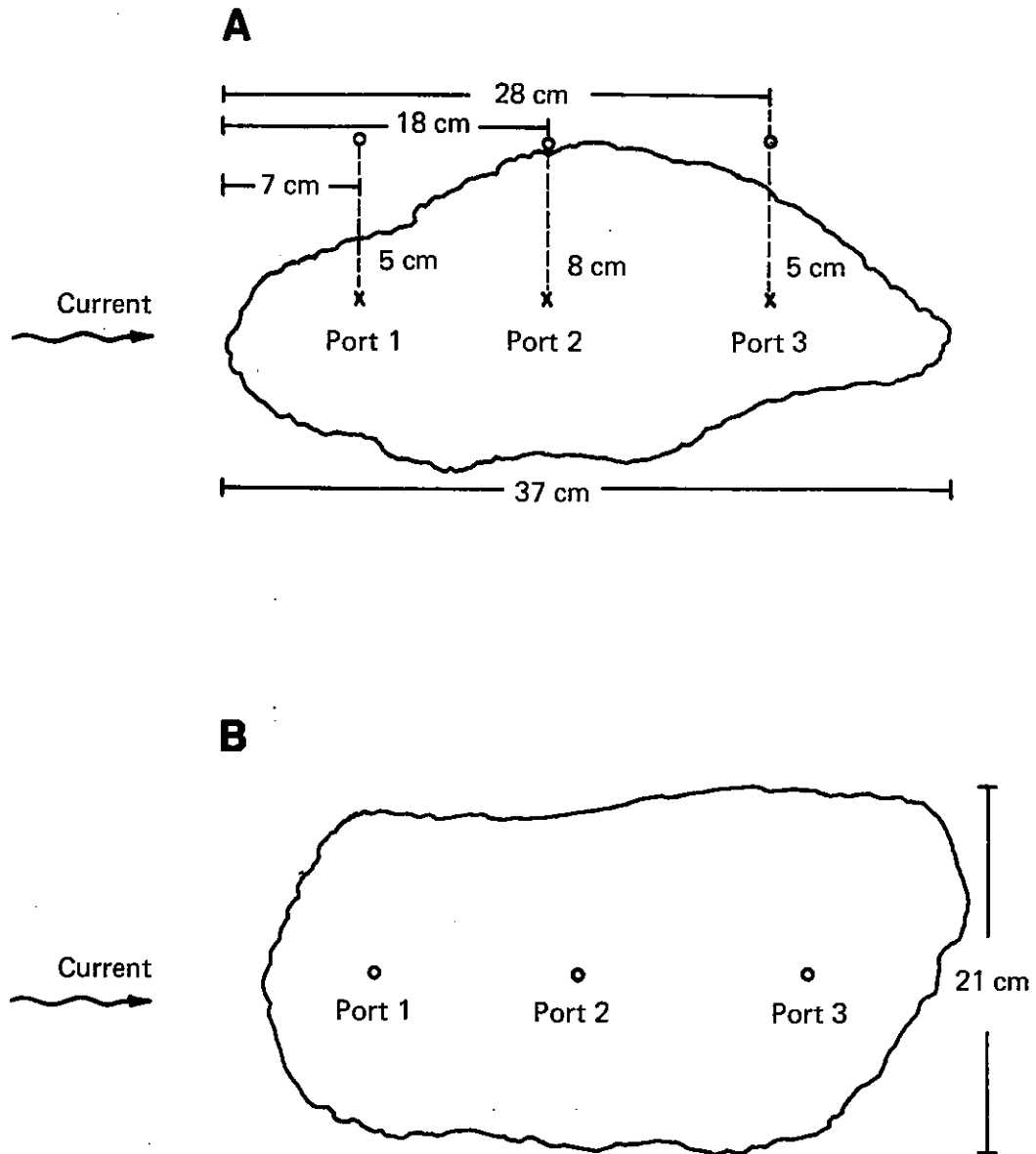


Figure 5. Diagram of egg mass 5 (volume = 2.6 liters, weight = 2.9 kg). Positions of sampling ports are identified. Aspect: A) lengthwise cross-section at center of egg mass. "x" denotes approximate position of needle apertures. B) dorsal surface of egg mass.

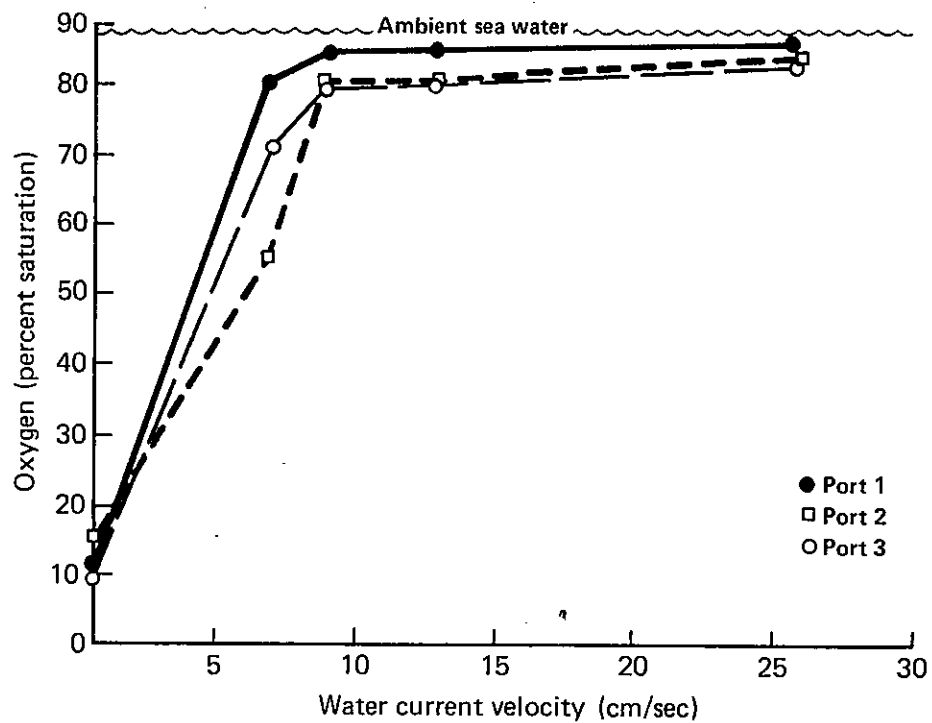


Figure 6. Interstitial oxygen at three positions along the length of egg mass #5 at increasing current velocities. Water samples were extracted from near the center of the mass at least fifteen minutes after current velocity had been altered.

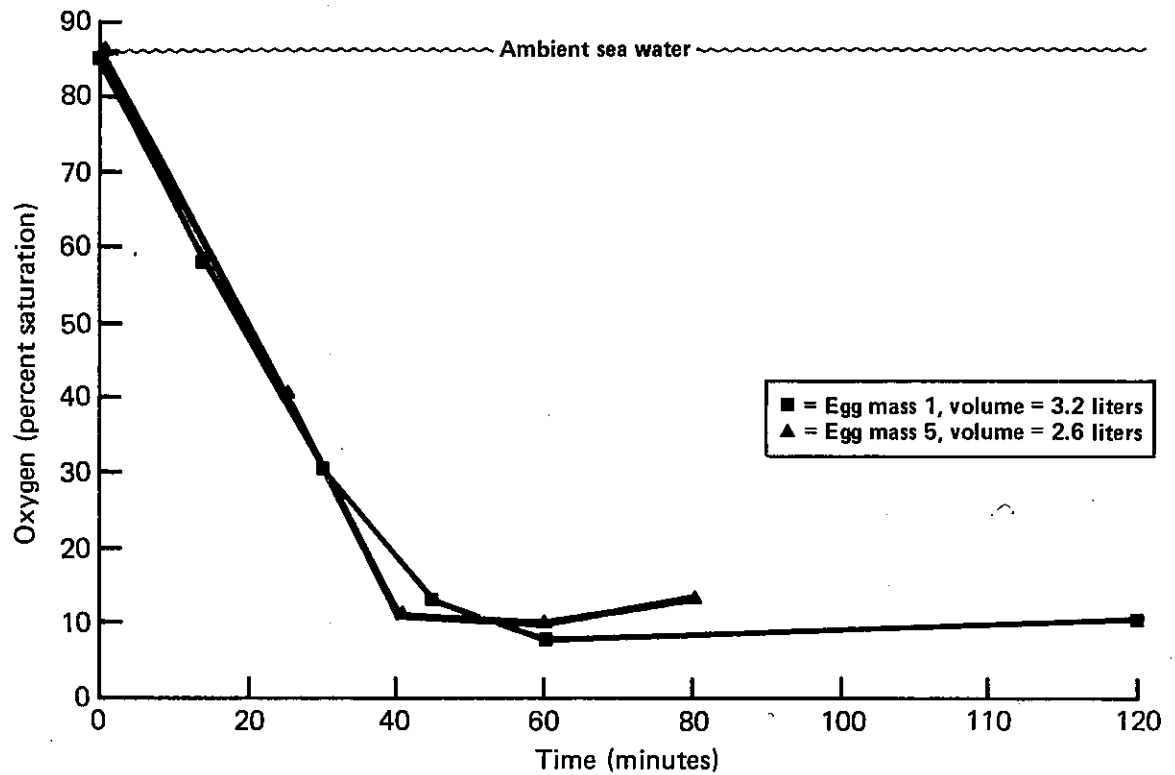


Figure 7. Interstitial oxygen depletion at the center of two egg masses, following cessation of current at time zero. Masses were flushed for two hours at velocities in excess of 40 cm/sec prior to extraction of the time zero sample.

Following a two-hour slack water period, oxygen saturation was 12% at 2 cm and 8% at 8 cm. Therefore, embryos situated progressively further from the interior of the mass experience higher oxygen levels during limiting current conditions.

Interstitial ammonia and pH might change substantially during slack tides due to metabolite production from embryos and the associated interstitial fauna. 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 1 (volume = 3.2 liters, weight = 3.7 kg). The egg mass was exposed to a current velocity in excess of 40 cm/sec for 2 hr prior to cessation of the current to assure ample flushing of the nest.

Oxygen decreased for 45 minutes, then leveled off at 8-13% saturation. PH remained relatively stable for the first hour then decreased to 7.05 at 2 hours (Figure 8). Total ammonia increased for 2 hours with no indication of an asymptotic limit. Un-ionized ammonia (NH_3) concentrations increased steadily to 6 ppb the first hour (Table 3), 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 (not an unrealistic situation based on observations in Dabob Bay nests), all three factors reached their extreme levels at

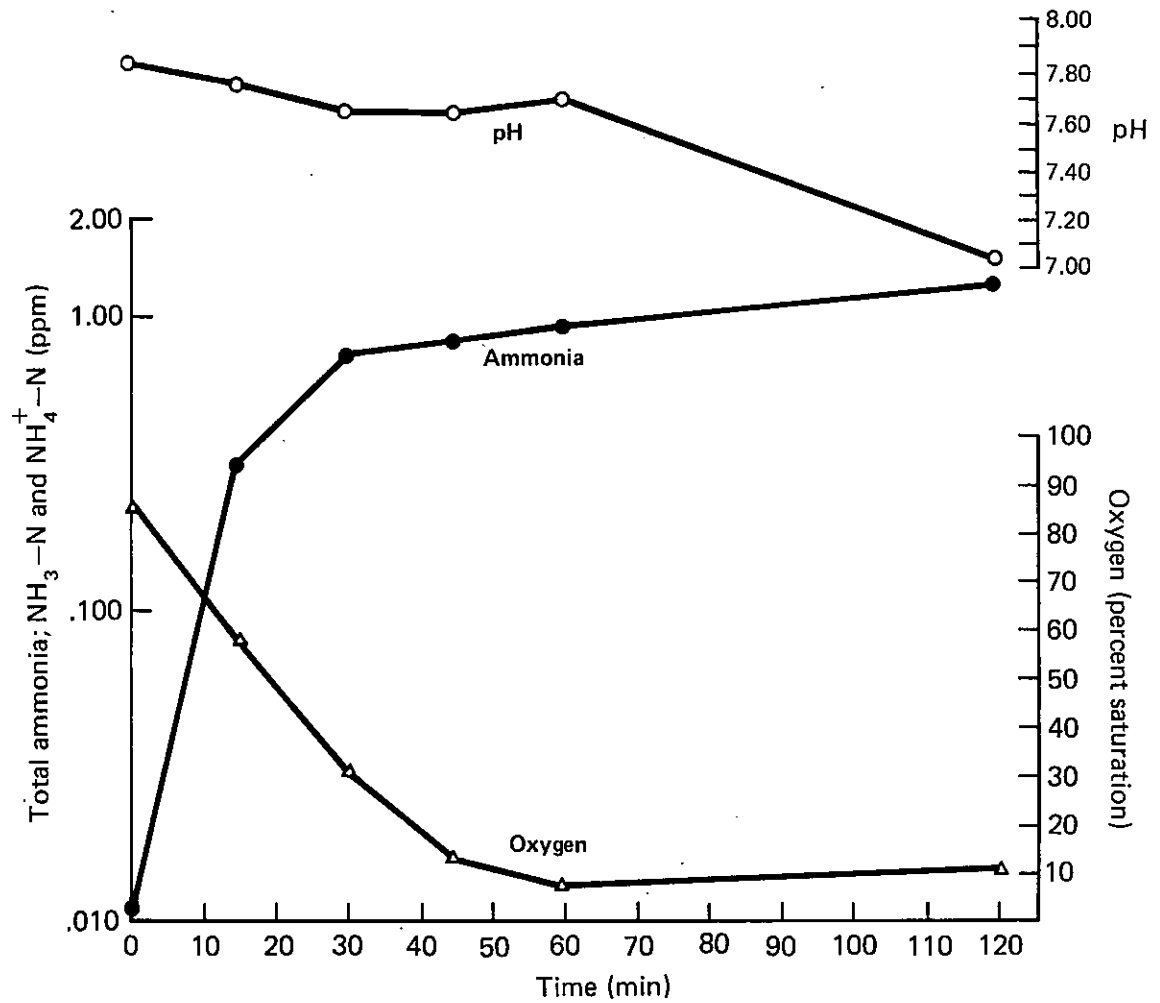


Figure 8. Interstitial ammonia, pH and oxygen of egg mass #1 (volume = 3.2 liters, weight = 3.7 kg) monitored for 2 hrs following cessation of current (>40 cm/sec). Water samples were extracted from a single port near the center of the mass.

Time from cessation of current (min)	Oxygen (% sat.)	pH	Total ammonia - N (ppm)	NH ₃ - N (ppb)
0	86	7.85	.01	.1
15	57	7.75	.32	2.5
30	31	7.65	.68	4.3
45	13	7.65	.80	5.0
60	8	7.70	.87	6.1
120	12	7.05	1.18	1.9

Table 3. Interstitial oxygen, pH, total ammonia -N and NH₃-N of egg mass 1 (volume = 3.2 liters, weight = 3.7 kg) monitored for two hours following cessation of current at time zero. NH₃ values were calculated and rounded to the nearest 0.1 ppb. Ambient water properties: temperature = 9.0°C, salinity = 29‰, oxygen = 87% saturation, total ammonia = 0.02 ppm, pH = 7.90.

the end of the exposure period: oxygen = 4% saturation, pH = 6.60, total ammonia = 7.14 ppm (the NH_3 fraction = 4 ppb), see Table 4. Clearly, the continued decrease in pH serves to prevent the NH_3 fraction, the commonly regarded toxic molecule (Spotte 1979), from attaining appreciable concentrations.

Embryo Tolerance to Hypoxia and Ammonia

To determine the sensitivity of embryos to hypoxia, developmentally advanced embryos were exposed to six oxygen levels (2, 6, 12, 22, 50 and 85% saturation) and data were collected at 4, 24, 48 and 96 hours. Extensive and at times rapid mortality was observed in the 96 hr oxygen bioassay. Control (85% oxygen saturation) mortality never exceeded 5% in 96 hours (5,760 min), see Figure 9, but as early as four hours (240 minutes), mortalities were observed in both the 2 and 6% oxygen saturation treatments. By 48 hours (2,880 minutes) mortalities reached 95% at 2% saturation, 75% at 6% saturation, and 30% at 12% saturation. By 96 hours (5,760 minutes), mortalities at oxygen levels \leq 22% saturation were \geq 60%, with evidence that mortalities at 50% saturation were starting to occur.

Median lethal concentration (LC_{50}) and median lethal time (LT_{50}) values (Sprague 1969) with 95% confidence intervals were calculated using a logit analysis program (Table 5). The LC_{50} estimates at 48 and 96 hr were 9.4 and

Time from cessation of current (hr)	Oxygen (% sat.)	pH	Total ammonia -N (ppm)	NH ₃ -N (ppb)
0	87	7.90	.03	.3
19.5	9	6.85	1.06	1.1
22.0	10	6.95	1.59	2.0
47.5	5	6.85	2.53	2.5
70.0	4	6.60	7.14	4.0

Table 4. Interstitial oxygen, pH, and ammonia monitored over a 70 hour period during which egg mass WA4 was not exposed to direct water current. Water samples were extracted from a single port at the center of the mass. The egg mass was flushed for two hours with water velocity in excess of 40 cm/sec prior to cessation of the current and extraction of the time zero water samples. Ambient water properties: temperature = 9°C, salinity = 29‰, oxygen = 87% saturation, total ammonia = .03 ppm, pH = 7.90. Egg mass volume = 1 liter, weight = 1.2 kg.

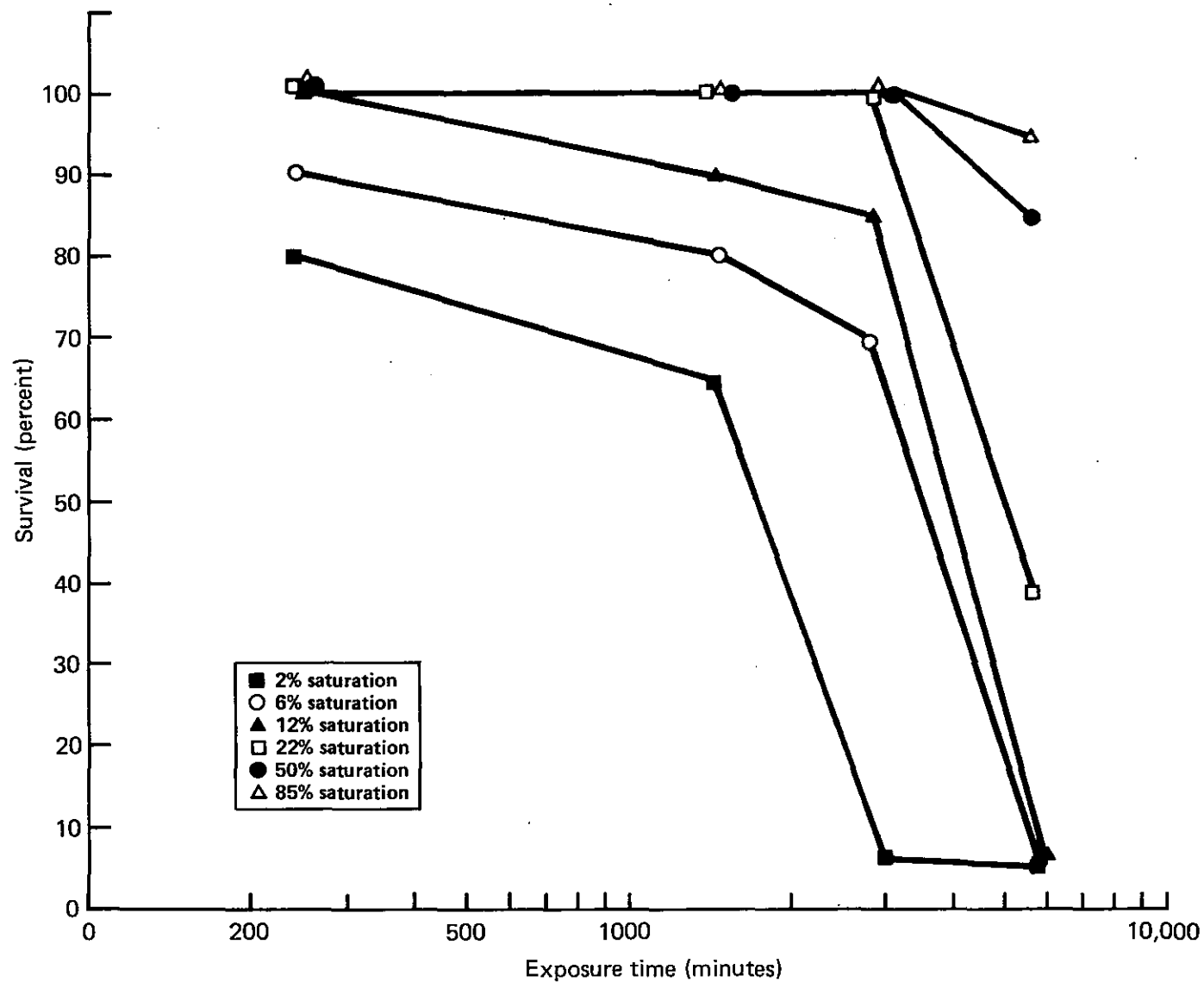


Figure 9. Mean percent survival for stage 10 embryos at four time periods and six oxygen levels. Two replicates per treatment; n = 10-12 embryos per replicate. Ambient controls were held at 85% saturation.

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 ¹	85.0	-----

¹The LT₅₀ value for 22% saturation was estimated by log-probit plot.

Table 5. Oxygen LC₅₀ and LT₅₀ values and 95% confidence intervals for stage 10-11 embryos. Values were calculated with a logit analysis program written by Russ Kappenman, Bio-metrics, Northwest and Alaska Fisheries Center, Seattle, WA.

32.6% saturation, respectively. LT_{50} values at 2, 6, 12 and 22% saturation were 26.2, 40.1, 62.6 and 85.0 hr, respectively. 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 indicate that oxygen levels in nests at Dabob Bay (Table 2) are certainly low enough to be lethal to developmentally advanced embryos. Oxygen in some nests near San Juan Island also drops to potentially lethal level, but typically this condition lasts for only a brief period during the tide change, by no means long enough to induce mortalities.

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) I conclude that the mortalities were chance events and not attributable to ammonia. This indicates that ammonia concentrations did not reach lethal concentrations in poorly ventilated nests.

Embryo Respiration

Mean embryo oxygen uptake at near ambient oxygen levels (respirometers were filled with water at 87-89% saturation) increased from 0.087 to 0.225 $\mu l/hr$ for developmental stages 4 to 14, respectively (Figure 10). At

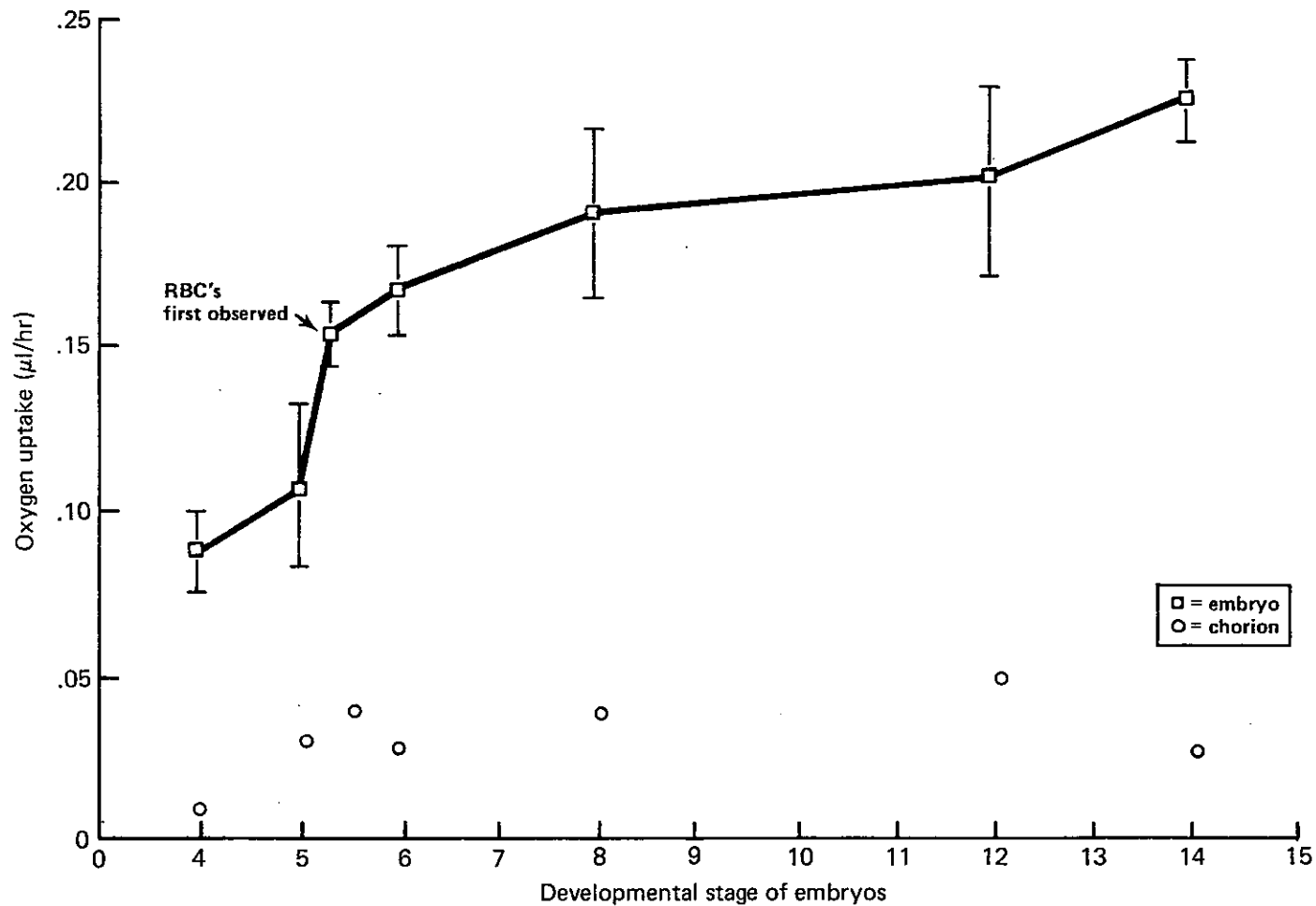


Figure 10. Mean oxygen uptake (μl/hr) + one standard deviation (vertical lines), of stage 4 to 14 embryos, n = 3 to 5. Respirometers were filled with sea water at 87-89% saturation. Estimates of mean chorion respiration for each lot of eggs are indicated by circles.

the end of experimentation oxygen in respirometers ranged from 63.5 to 83% saturation, with a mean value of 71%. Red blood cells (RBC's) were first observed during stage 5. Stage 5 embryos possessing RBC's exhibited significantly higher oxygen uptake ($0.154 \mu\text{l/hr}$) than those stage 5 embryos lacking them ($0.106 \mu\text{l/hr}$; $t = 3.07$, where $t_{0.05,4} = 2.78$).

Mean oxygen consumption for chorions ranged from 0.010 to $0.053 \mu\text{l/hr}$ (Figure 10), accounting for 10 to 24 percent of the oxygen utilized by an entire egg. Presumably, oxygen consumption of the chorion is primarily due to epibiotic contamination of the egg surface (Braum 1978). However, Hamor and Garside (1973) report chorionic respiration in Salmo salar eggs and attribute it to the presence of oxidative enzymes in the chorion.

To determine the effect of hypoxia on embryo respiration, oxygen uptake of early (stage 6), intermediate (stage 8,9) and advanced (stage 12) embryos was measured at three oxygen levels. Mean oxygen levels in respirometers over the course of experimentation and mean initial and final oxygen levels = 77% (87 to 66), 47% (51 to 42) and 20% (22 to 18.5) saturation, see Figure 11. Data was analyzed using two-way analysis of variance (Sokal and Rohlf 1969). Sample size ranged from 3-5 embryos per treatment. Since the analysis requires equal sample size, it was necessary to reduce all samples to a size of three

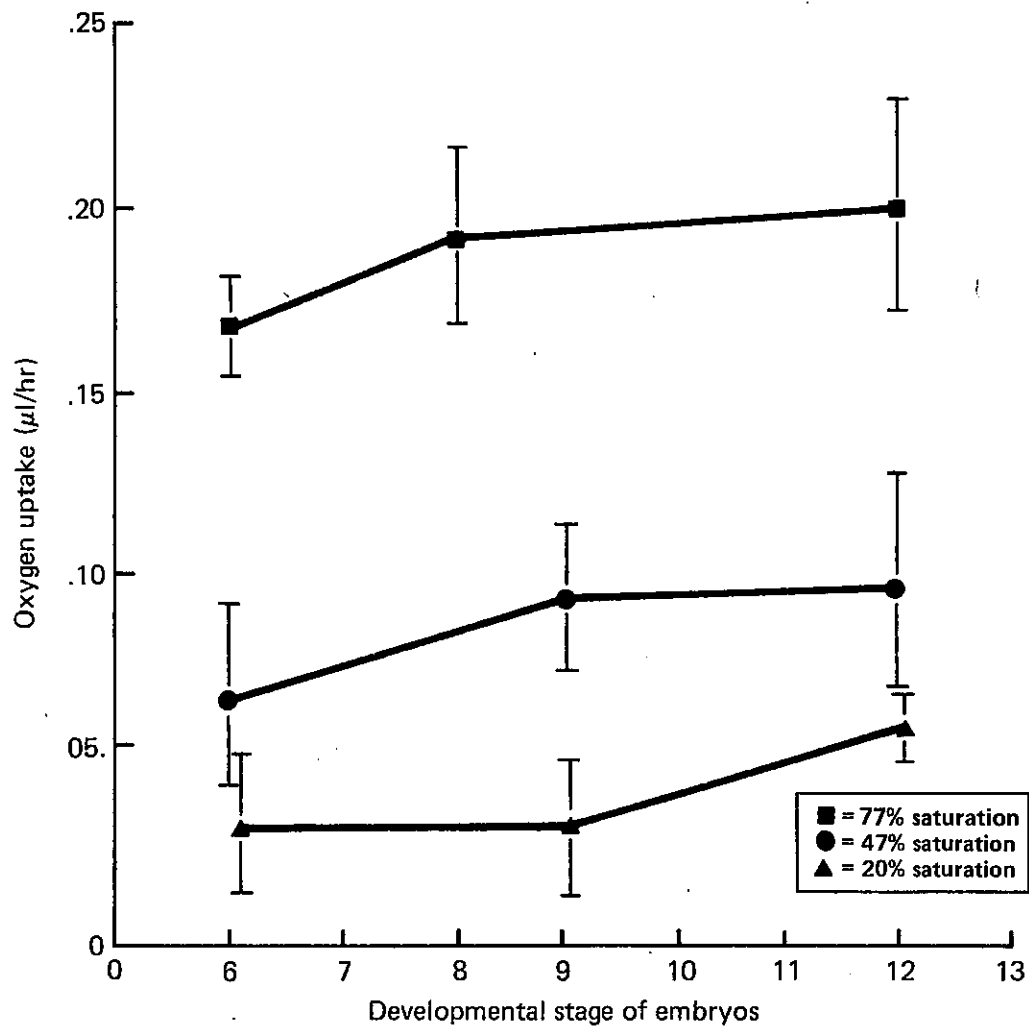


Figure 11. Mean oxygen uptake ($\mu\text{l/hr}$) of embryos, + one standard deviation (vertical lines), at different developmental stages (6, 8 or 9, and 12) and three oxygen levels. Oxygen levels indicated are the mean saturation in respirometers over the course of the experimental period. The 77% saturation treatment represents the ambient condition. $n = 3-5$ embryos.

by randomly selecting individuals within a treatment (Sokal and Rohlf 1969). Both oxygen level and embryonic stage significantly altered the respiration rates ($p < 0.01$), but together had no interactive effect (Table 6). A Newman-Keuls multiple range test (Zar 1974) was employed to test the hypotheses: (1) $H_0 : \mu_e = \mu_i = \mu_a$ (where e = early, i = intermediate and a = advanced embryos) and (2) $H_0 : \mu_{20\%} = \mu_{47\%} = \mu_{80\%}$, Table 7. The first hypothesis was rejected ($p < 0.05$) concluding that $\mu_e = \mu_i \neq \mu_a$, i.e. oxygen uptake increases significantly as the embryos develop from stage 9 to 12, but the increase from early to intermediate stage embryos does not appear to be significant. Hypotheses 2 was also rejected ($p < 0.05$), indicating that $\mu_{20\%} \neq \mu_{47\%} \neq \mu_{80\%}$, i.e. oxygen uptake decreased significantly with increasing hypoxia for each oxygen level tested.

Bradycardia

During hypoxia, bradycardia accompanied the reduction in embryo respiration. Heartbeat frequencies were recorded at four exposure periods (4, 24, 48, and 96 hours) for six oxygen levels (2, 6, 12, 22, 50, and 85% saturation, the latter representing the ambient control). Data were analyzed using analysis of covariance (Zar 1974), see Table A-3. Time and heartbeat rate were the independent and dependent variables, respectively.

Source of variation	df	SS	MS	F
Treatments	8	.1050	.01313	
Developmental stage	2	.0065	.00325	5.91 *
Oxygen level	2	.0982	.04910	89.27 **
Stage X Oxygen	4	.0003	.00008	0.15 ns
Residual	18	.0098	.00055	
Total	26	.1148		
				* ; .005 < p < .01
				** ; p < .001

Table 6. Results of two-way Anova: effects of developmental stage and oxygen level on embryo respiration.

Comparison	p	q	q.05,18,p
Developmental stages			
early vs. intermediate	2	1.538	2.971
early vs. advanced	3	4.744	3.609 *
intermediate vs. advanced	2	3.205	2.971 *
Oxygen levels			
20% vs. 47%	2	5.026	2.971 *
47% vs. 80%	2	12.564	2.971 *
20% vs. 80%	Do not test		
* = significant			

Table 7. Results from Newman-Keuls multiple range test (Zar 1974): effects of developmental stage and oxygen level on embryo respiration.

Heartbeat rates ranged from 0.5 to 2.9 beats/sec; the mean rate in the control treatment was 2.6 beats/sec. Regression elevations of the oxygen treatments were significantly different ($F = 35.35$, $p < 0.001$), therefore, a Newman-Keuls multiple comparison test was employed to delineate the differences. Results are summarized:

<u>6%</u>	<u>2%</u>	<u>12%</u>	<u>22%</u>	<u>50%</u>	<u>85%</u>
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No significant ($p = 0.05$) differences could be detected among oxygen treatments underscored by a common line. Treatments are arranged in order of increasing elevation from left to right. Clearly, all hypoxial conditions resulted in a bradycardial response when compared to the control. The 6% treatment appears to have the most pronounced effect on heartbeat, instead of the expected 2% treatment. A number of explanations can account for this discrepancy: The effects of 2% and 6% saturation are so similar as to be indistinguishable; there is an apparent physiological lower limit of heartbeat rate (~ 0.5 beats/sec) that will effect the regressions; the variation due to relatively small sample sizes masks real differences among treatments. Even though significant differences among treatments could not be demonstrated (the outlying 6% excluded), there was a trend for increasingly severe

hypoxia to progressively depress heartbeat. The heartbeat rate of embryos at ambient oxygen levels (85% = control) did not decline significantly ($F = .84$, where $F_{0.05, 1, 15} = 4.54$), during the 96 hour exposure period. However, the pooled slope^{1/} of the five hypoxial treatments varied significantly from zero ($F = 13.14$, $p < 0.001$ at 1,71 d.f.), demonstrating that the intensity of bradycardia increases with increased exposure time.

Effects of Hypoxia on Embryo Mortality and Development

Three pieces from a common egg mass, incubated at different current velocities, each hatched sequentially from the periphery inward over a two-week period. Interstitial oxygen levels and embryo survival were lower at the reduced current velocities (Table 8). Mortality was 6% at nominal oxygen saturation, 48% at 50% saturation and 93% at 20% saturation. Note that these mortality estimates have been adjusted for pre-experimental mortalities occurring while this egg mass was still in the field, as detailed in the Materials and Methods section.

The effects of oxygen level and time of hatch (begin vs. end) on larval length were tested by two-way analysis

^{1/} Since no difference could be detected among the slopes ($p = 0.1$), they were pooled.

Oxygen level (% saturation)		% mortality	Time in days from start of experiment to		Mean standard length at hatch \pm 1 s.d.	
nominal	range		begin hatch	end hatch	begin hatch	end hatch
85	77-91	6	9	22	11.12 \pm .36	10.25 \pm .67
50	29-76	48	9	23	11.05 \pm .35	9.43 \pm .72
20	4-22	93	9	22	10.91 \pm .40	9.89 \pm .45

Table 8. Mortality, hatching time and mean standard length (\pm 1 s.d.) of newly hatched larvae (n=30) for three pieces of a common egg mass incubated at three different interstitial oxygen regimes.

of variance (Table 9). Both main effects (oxygen level and time of hatch) significantly ($p < 0.001$) influenced larval size at hatch and significant interaction was demonstrated ($p < 0.005$). A Newman-Keuls multiple range test (Table 10) indicated that in all oxygen treatments, the first larvae to hatch (those near the periphery) were significantly ($p = 0.05$) larger than the last hatching larvae from the interior of the nest pieces. The first larvae to hatch (those near the periphery) were the same length in all three treatments. However, there was significant size disparity among the last larvae to emerge in the three treatments (Table 10). Late hatching larvae in the 50% saturation treatment appear to be smaller than expected when compared with the other two treatments. This accounts for the significant interaction detected in the two-way Anova. Although the interaction restricts further interpretations, one fact is clear: late hatching larvae in the 85% treatment are larger than those in either of the other two hypoxial treatments. Since sufficiently low oxygen can decrease the size of larvae at hatch (Shumway 1964, Brannon 1965, Alderdice and Forrester 1971, Carlson and Siefert 1974, Alderdice and Rosenthal 1976, Hempel 1979), embryos in the 85% treatment probably avoided the further stunting experienced by the survivors at the lower oxygen levels.

Source of variation	df	SS	MS	F
Treatments	5	72.73		
time of hatch (begin vs. end)	1	61.91	61.91	238.12 **
Oxygen level	2	6.11	3.06	11.77 **
interaction	2	4.71	2.36	9.08 *
Residual	174	45.84	.26	
Total	179	1118.57		

** $p < .001$

* $.001 < p < .005$

Table 9. Results of two-way Anova: effects of time of hatch (begin vs. end) and interstitial oxygen on the standard length of newly hatched larvae.

Comparison	p	q	$q_{.05, \infty, p}$
Beginning of hatching period			
20% vs. 85%	3	2.258	3.314
End of hatching period			
50% vs. 85%	3	8.82	3.314 *
50% vs. 20%	2	4.95	2.772 *
20% vs. 85%	2	3.87	2.772 *
Begin vs. End			
50% (B) vs. 50% (E)	5	17.42	3.858 *
20% (B) vs. 20% (E)	3	10.97	3.314 *
85% (B) vs. 85% (E)	4	9.35	3.683 *
* = significant			

Table 10. Results from Newman-Keuls multiple range test (Zar 1974): effects of hatching time (beginning vs. end of the protracted hatching period) and interstitial oxygen levels on the standard length of newly hatched larvae.

In all three treatments, hatching commenced and terminated on nearly the same day. Therefore, the degree of retardation established within the egg mass while incubating in the field was not altered by the oxygen regimes experienced in the lab.

Development of embryos in the center of the egg mass had been retarded, apparently, by hypoxic conditions while the nest was in the field; however, hypoxic conditions experienced by stage 8 (the developmental stage of embryos adjacent to the dead central core when the experiment was initiated) and older embryos in the lab did not further retard development, although size at hatching was reduced.

Nest Depth Distribution

During the 1979-80 and 1980-81 seasons nest depths ($n = 50$) at the San Juan nesting sites ranged from -1.8 to -21.9 m. At Dabob Bay, nests ($n = 16$) were deposited from +1.0 to -10.4 m (Figure 12). Depths are relative to mean low low water (NOAA Tide Tables, 1979; 1980). One loose egg mass located at Pulali Point was assumed to have been dislodged by wave action and was recorded as intertidal spawning; it is included in the 0.0 to +1.0 depth interval. Nests are significantly shallower in Dabob Bay ($\chi^2 = 35.62$; $p < 0.005$ at 6 d.f.); nest depths were combined into four meter intervals from +4.0 to -24.0 m for the Chi-square test.

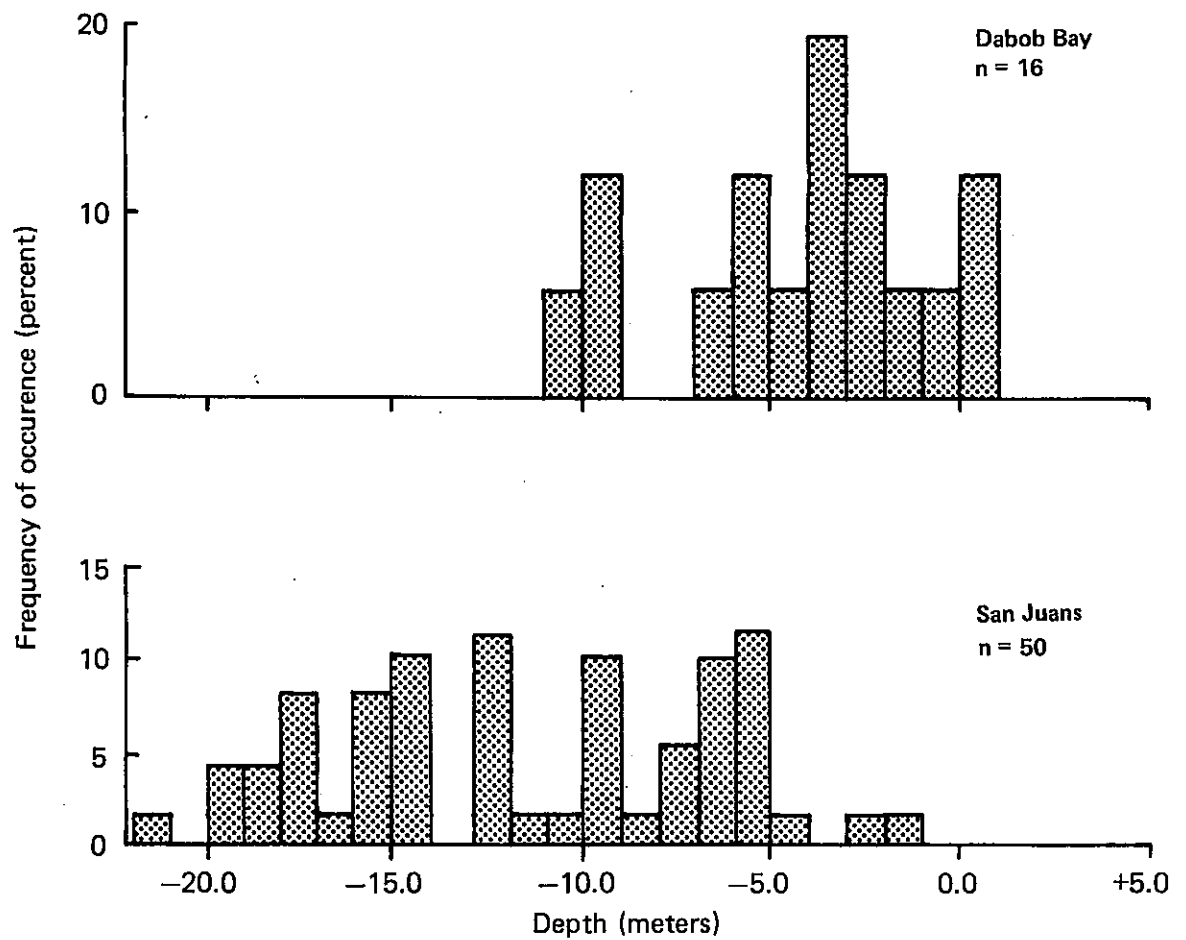


Figure 12. Depth distribution of lingcod nests in Dabob Bay and the San Juan nesting areas; 1979-80 and 1980-81 spawning seasons. Depths are adjusted to mean low low water.

Predation on Nests

In situ nest predation did not appear to be an extensive source of egg mortality at any nesting area. Fish were never observed feeding on nests, whether guarded by male lingcod or not. However, on one occasion a small sculpin proximal to an unguarded nest dropped a few eggs from its mouth, indicating that fishes will prey upon unguarded nests.

Certain invertebrates were effective egg predators. Small gastropods, Amphissa columbiana and Calliostoma ligatum were the most common predators. Three other larger invertebrates were observed foraging on nests: Strongylocentrotus franciscanus, Scyra acutifrons and Pisaster ochraceus. But only one incidence of predation for each of these species was observed during the spawning seasons. Whether or not the seastar was an effective predator is uncertain; its stomach was everted over a portion of an unguarded nest, but no evidence of digestion was apparent.

Pierced or sheared chorions still adhering to the peripheral surface of an egg mass were evidence of gastropod and urchin predatory activity, respectively. Typically, the peripheral layer damaged by gastropods was not extensive (less than 2 cm thick). The extent of urchin damage was difficult to assess, since the feeding method tends to completely remove eggs.

A variety of other organisms were commonly observed on or near nests (pandalid shrimp, brachyuran and anomuran crabs, teleosts of the families Cottidae, Pholidae and Stichaeidae), but their role as predators is uncertain.

Twelve of fifteen nests examined at the San Juan nesting areas exhibited evidence of predation. Two of the three masses not displaying evidence of predation were recently deposited. Eleven of the nests were guarded and nine of those showed evidence of predation (pierced, empty chorions). A chi-square test comparing guardian presence and evidence of predation demonstrated that guardian males were ineffective in preventing predation by small gastropods ($X^2 = 4.68$; $X^2_{(0.05, 1)} = 7.815$).

Nest predation was not an important source of egg mortality in Dabob Bay. Only three of fourteen nests examined had any evidence of predation, and two of those had already incurred substantial asphyxiation mortality near 90%. Guardian males were repeatedly absent at three nests; a small male abandoned one nest when approached on the first dive and was not observed on three subsequent dives. I assumed these nests were unguarded for a substantial part of their development, yet none exhibited evidence of predation (undisturbed diatom mats on the surface of these masses indicated that eggs had not been torn from them by predators).

DISCUSSION

Agents Causing Mortality

The investigations reported herein indicate that some spawning grounds utilized by lingcod are unsuitable for proper embryo development and survival due to inadequate current velocities. In Dabob Bay, where the mean interstitial oxygen within nests was 16% air saturation during the tidal excursions, all of the 13 nests examined contained developmentally retarded embryos at the interior, and at least 59% of all embryos died in situ before hatching (Table 2). At San Juan nesting sites, where the mean interstitial oxygen level was 69% under slack tide conditions, only one of 13 nests contained retarded embryos and no mortality of the type occurring in Dabob Bay was observed. Laboratory investigations indicated that current velocities near 10-15 cm/sec are necessary to maintain the interior oxygen levels of egg masses to within 10-15% of ambient water (Figure 4) and to provide relatively uniform oxygen levels throughout a given mass (Figure 6). In Dabob Bay, tidal current velocities are typically below these values; the maximum midchannel current velocity at a 3 m tidal exchange is approximately 6 cm/sec, while for similar tide height changes in San Juan Channel the velocity is 215 cm/sec. Tide height ranges greater than 3 m occur during spring tides in this region (Holbrook et al. 1980) and are

not common events during the spawning season: consequently, typical velocities for the areas are somewhat lower. The reader should note that these velocities may not actually occur at individual nest sites. However, oxygen levels observed in nests (Table 2 and Figure 6) suggest that these velocities are representative of the general current conditions which prevail at nest sites.

Several investigators (Wickett 1954, Coble 1961, Phillips and Campbell 1961, McNeil 1966) demonstrated that hypoxic conditions observed in salmon redds were a result of inadequate water circulation and that embryo survival was positively correlated with dissolved oxygen. Taylor (1971) and Hempel (1971) observed that embryo mortality in herring spawn increased with the thickness of the egg mat. They suggested that either hypoxia or metabolite accumulation could have caused the mortalities. In this study laboratory investigations indicated that hypoxia caused the embryo mortalities observed in Dabob Bay. Oxygen bioassays demonstrated that at approximately 33% oxygen saturation slightly lower than the highest interstitial level of 35% saturation observed in Dabob Bay nests, 50% of advanced stage embryos died in 96 hours (Table 5). At oxygen levels near 16% saturation (the mean value in Dabob Bay nests), 50% of the embryos would have died sometime between 63 and 85 hours (Table 5). The mean interstitial oxygen level in San Juan nests was 69%

saturation, with a low value of 43% saturation. These levels occurred during slack tide and probably were of short duration (approximately 1-2 hr), given the rapid current velocities characteristic of the area. Laboratory experiments demonstrated that such short exposures to moderate hypoxia are not severe enough to cause mortalities. To further emphasize the critical role of current velocity on embryo survival, data presented in Table 8 demonstrates that survival is positively correlated with current velocity (as indicated by the interstitial oxygen levels). Furthermore, current velocity treatments which produced interstitial oxygen levels approximating those observed in Dabob Bay (20% and 50% nominal saturation; Table 8), resulted in massive mortality, up to 96%.

Lingcod embryos do not appear to be as resistant to hypoxia as some other temperate species possessing demersal eggs. Although no LT_{50} could be estimated, advanced embryos were starting to die after 96 hr exposures to 50% oxygen saturation (Figure 9). During a seven day exposure the LD_{50} for developmentally advanced chum salmon eggs was 1.0-1.4 ppm approximately 9-12% saturation, (Alderdice et al. 1958). Pacific cod eggs were tolerant of reduced dissolved oxygen to levels of 2 to 3 ppm, approximately 16-30% saturation (Alderdice and Forrester 1971). Similarly, Braum (1973) found that another marine species,

Clupea harengus, required at least 20% oxygen saturation for any embryo survival. Unfortunately, factors such as the duration of exposure period to hypoxia, the developmental stage of the eggs assayed and methods used by investigators to generate mortality estimates are not standardized and consequently not equatable; thus comparisons between species must be cautiously addressed. However, demersal eggs in general tend to be more resistant to hypoxia than their pelagic counterparts (DeCiechowski 1965).

Oxygen uptake by an aquatic organism is a function of both the partial pressure of oxygen and the velocity of water at the respiratory surface (Wickett 1954, Daykin 1966). In an effort to both standardize experiments and simulate conditions that probably occur within inadequately ventilated masses, both bioassay and respirometry experiments were conducted in static water. This was based on the assumption that given the slow current velocities commonly prevailing in Dabob Bay, water movement in the interior of egg masses is probably minimal. This assumption was supported by both field and laboratory observations. In the absence of exogenous water movement interstitial oxygen at the center of some egg masses remained in the range of 4 to 14% saturation even during exposures of up to 70 hours (Figure 7 and Table 4). Interstitial oxygen levels in several nests at Dabob Bay

were sufficiently low (PP1 = 3%, PP3 = 2%, WW12 = 15% saturation) during tidal movements (>2.0 m in height) to suggest negligible influence of ventilatory currents in the interior of the nests. Presumably, these levels are maintained by diffusion, however, Obrien et al. (1978) described a mechanism of natural convection produced at the surface of salmon eggs. Briefly, they contended that metabolic wastes (ammonia and carbon dioxide) excreted from the egg produce a solution at the surface of the chorion which is denser than ambient water. The denser solution sinks creating a weak convection current (approximately 0.02 cm/sec). Whether such a mechanism exists in the marine environment and could be active in lingcod egg masses is uncertain and remains to be demonstrated.

Ammonia within egg masses did not increase to toxic levels. Bioassays (96 hr) with total ammonia-N concentrations up to 50 ppm (444 ppb as $\text{NH}_3\text{-N}$) did not kill advanced embryos. This level is far in excess of the extremes, 7.14 ppm total ammonia-N and 6 ppb as $\text{NH}_3\text{-N}$ (Table 3), which accumulated in nonventilated egg masses at periods up to 70 hours (Table 4). Teleost eggs in general tend to be quite resistant to ammonia. Burkhalter and Kaya (1977) exposed Salmo gairdneri eggs to $\text{NH}_3\text{-N}$ concentrations up to 370 ppb throughout the incubation period; they observed no differential egg mortality between high and low concentrations. Rice and Stokes (1975) demonstrated that

fertilized eggs and alevins of the same species could survive 24-hr exposures to un-ionized ammonia levels of 3.58 ppm. Similarly, eyed eggs of another salmonid, O. gorbuscha, proved to be resistant to NH_3 , incurring no mortalities during a 96 hr exposure to concentrations up to 1,500 ppb (Rice and Bailey 1980a). Typically, the NH_3 species is considered to be the form toxic to aquatic animals (Spotte 1979), however Armstrong et al. (1978) showed that the ionized form (NH_4^+) may be significantly toxic to Macrobrachium rosenbergii larvae at low pH conditions. Their LC_{50} values for total ammonia at pH 6.83 (the lowest treatment) ranged from 90 ppm at 144 hr to >200 ppm at 12 hr. These are extremely high concentrations, far greater than those which accumulated within unventilated lingcod nests observed in the laboratory, and far in excess of intragravel ammonia reported in pink salmon redds (0.24 ppm total ammonia; 0.1 ppb as NH_3) by Rice and Bailey (1980 b). Within an egg mass, ammonia occurs primarily as the ionized species (Tables 3 and 4); the fraction existing in this form increases through time due to the concomitant decrease in pH (Figure 8). Consequently, accumulation of the commonly recognized more toxic species (NH_3) is prevented.

A pH range of 6.5 to 8.5 is considered to be safe for most marine organisms (EPA 1973). Wedemeyer et al. (1976) suggests a pH range of 6.7 to 8.6 for holding fishes in

captivity. The pH levels observed in lingcod egg masses (6.65-7.90) during this investigation are in accordance with these commonly accepted safe limits. However, acidic conditions can reduce the affinity of hemoglobin for oxygen, due to the Bohr Effect (McCauley 1971). Often the reduction in environmental pH is primarily a consequence of increased carbon dioxide levels (Alderdice and Wickett 1958, Spotte 1979) produced by respiring organisms. Thus, the acidic conditions which develop in poorly ventilated egg masses (Tables 3 and 4, Figure 8) may further limit the respiratory ability of embryos already residing in a hypoxial environment.

Nest Site Selection

Since some water movement is necessary to adequately ventilate a concentration of demersal fish eggs, it is plausible that this may be an important stimulus for lingcod spawning site selection. Keenleyside (1979) contends that accelerating water flow seems to be a strong stimulus for salmonid nest site selection. Two cottids, Enophrys bison and Hemilepidotus hemilepidotus, spawn adhesive egg masses in or near the intertidal zone where they are most exposed to water movement (DeMartini 1978, DeMartini and Patten 1979). In areas such as Dabob Bay where tidal currents are weak, lingcod may be expected to select sites in shallower water, where surface waves and

vertical tide changes create surging, oscillating water movements. This is precisely what the depth distribution of nests suggests (Figure 12): nest sites in Dabob Bay are significantly shallower ($p \leq 0.005$) than those in the San Juans, with some intertidal spawning occurring. Another observer has reported intertidal lingcod nests in Dabob Bay. During the 1978-79 spawning season, five intertidal nests were observed at Pulali Point (Ray Buckley, Washington Dept. of Fisheries, pers. comm.). The nests were deposited on cobble-oyster shell substrate, not on high relief rocky substrate where nests are usually spawned (Jewell 1968, Low and Beamish 1978, LaRiviere et al. in press).

Mark LaRiviere (pers. comm.), a Sea Grant investigator, has conducted spawning ground surveys near San Juan Island for the past 3 years (through 1981), but did not observe any intertidal spawning. The shallowest recorded deposition was 1.8 m below mean low low water. Several investigators (Low and Beamish 1978, LaRiviere et al. in press) contend that rocky crevices and caves in areas of suitable water movement are prime requirements for lingcod nesting. Clearly, this is not always the case, as witnessed in Dabob Bay. Lingcod do spawn in areas lacking high relief rocky substrate (usually quite shallow), and as I hypothesize, do so in an effort to provide adequate nest ventilation.

According to Sverdrup et al. (1942), surface wave action is capable of generating water movement at depth according to the formula:

$$V = \frac{2\pi}{T} a e^{-2\pi(z/L)}$$

Where a = wave amplitude, V = velocity of water at depth Z , L = wave length, and T = wave period. Assuming that the maximum wave height experienced during storm conditions approaches 2 m (based on personal observation), the shortest wave length possible is 14 m and the typical period would be 6 seconds (Sverdrup et al. 1942). Under these conditions, the depth at which appreciable water movement could occur is near 10 m (water velocity = 1.2 cm/sec at 10 m), the lower limit of nest deposition at Dabob Bay nest areas.

During the winter spawning season, surface water in Dabob Bay can at times be cooler and less saline than subsurface water. This was especially apparent during the winter of 1979-80 (Table A-6). On February 2, 1980, surface water was 7.5°C and 24.0‰, while at a depth of 10 m it was 9.0°C and 29‰; the oxygen solubility at these two depths is 7.18 and 6.72 ml/liter, respectively (Carpenter 1966). This suggests that lingcod could be selecting shallow water spawning sites on the basis of elevated levels of dissolved oxygen. However, during the

course of the 1978-79 spawning season water temperature and salinity showed little variation with depth (Table A-6), yet 5 intertidal nests were observed at Pulali Point in Dabob Bay (Ray Buckley, Wash. Dept. Fisheries, pers. comm.). Thus field observations do not appear to support the explanation that nest sites are selected with regard to dissolved oxygen levels, rather on the basis of water movement.

There may be more obscure benefits resulting from intertidal spawning. DeMartini (1978) suggests that intertidal spawning of Enophrys bison may be beneficial to male foraging. The observed egg deposition at an average depth of +0.1 m above mean low low water would permit temporary egg desertion by the guardian male for foraging, while the spawn was exposed during low tides. Whether or not guarding male lingcod would take such an opportunity to forage is uncertain. Probably a fish as large as the lingcod can easily survive without feeding during the few weeks of nest guarding. DeMartini (1978) further suggests that intertidal deposition affords the eggs a temporal and spatial escape from certain fish predators. This implies that the guardian male of this species is not an effective deterrent to these predators, and that egg predation during tidal exposure is unimportant. In the case of the lingcod, this is probably not a realistic consideration since the

male guardian appears to be aggressive towards most fish predators (Jewell 1968, Low and Beamish 1978).

There may also be certain deleterious consequences of intertidal spawning. Dessication and thermal stress are considered important sources of mortality for eggs of surf smelt Hypomesus pretiosus (Penttila 1978) and herring, C. harengus pallasii (Outram and Humphreys 1974, Barton and Steinhoff 1980), both of which are intertidal spawners. Lingcod spawn during the winter months when the lowest tides occur at night and temperatures often fall below freezing, therefore, dessication and heat stress are not likely problems. However, egg masses exposed to reduced air temperatures may incur substantial mortalities depending on the temperature and duration of exposure. Predation by terrestrial animals at low tide is another possible source of embryo mortality. Gulls and diving ducks are important predators of herring spawn. Outram and Humphreys (1974) estimate that herring egg mortality from both physical factors and predation can reach 80%. In addition to birds, mammals such as river otters, raccoons, and skunks may be effective egg mass predators, especially since masses are most likely to be exposed at night when these nocturnal animals are actively foraging.

The question as to why lingcod spawn in Dabob Bay instead of locating more suitable habitat elsewhere remains to be addressed. Tagging studies indicate the species is

basically sedentary as an adult, only rarely moving more than a few kilometers from the original tagging sites (Hart 1943, Chatwin 1956, Reeves 1966, Miller and Geibel 1973, Mathews et al. 1979), and consequently, would not be expected to undertake extensive spawning migrations. There is, however, evidence from catch data to suggest that females may well execute vertical spawning migrations from the deep basins onto shallow reefs (Miller and Geibel 1973). Given their sedentary nature, it seems plausible that spawning adults would seek out the best available nest sites in the immediate locale, which according to the hypothesis developed in this paper, is selected primarily on the basis of water movement. An alternative explanation is that Dabob Bay contains a genetically isolated population, whose propensity for shallow water spawning is an adaptation to local conditions in the fjord. However, considering the dispersal abilities of the epipelagic larval-juvenile stage, which can be 3-4 months in duration (Phillips and Barraclough 1977), this does not appear likely.

Adaptations and Responses to Inadequate Ventilation

The hemoglobin-bearing erythrocytes which first appear in stage 5 embryos (Figure 10) may play a more detailed role in embryo survival other than the obvious enhancement of oxygen transport. Hemoglobin may also function as an

oxygen store, which would be readily available to the embryo during short-term hypoxia at slack tide. Hemoglobin is known to serve an oxygen storage function in burrowing arenicolid polychaetes. It has been suggested that they utilize their hemoglobin as an oxygen reserve during low tide, when oxygen tensions in the burrow and surrounding sand decline to low levels (Barnes 1974).

It is probable that decreased respiration also helps to assure survival during short-term hypoxia. The rate of oxygen consumption by embryos is dependent on dissolved oxygen, at least over the range of oxygen levels tested (Table 6). This metabolic adjustment would enable embryos to survive hypoxial conditions which otherwise might be deleterious if the metabolic rate remained elevated. Mangum (1970) observed that burrowing polychaetes decrease their oxygen consumption as environmental hypoxia intensifies. She suggested that during periods of water stagnation and accompanying hypoxia, both decreased respiration and the presence of hemoglobin enables the worms to survive until environmental oxygen can be replenished.

Decreased heart rate in response to hypoxia has been observed in adults of several fish species (Garey 1962, Randall and Shelton 1963, Holeyton and Randall 1967) as well as Salmo gairdneri embryos (Holeyton 1971). However, bradycardia during hypoxia is typically accompanied by a

decrease in peripheral circulation. Restricted peripheral blood circulation provides a greater quantity of oxygenated blood to vital organs, such as the heart and brain. Both grunion (Atherinidae) and flying fishes (Exocoetidae) display these bradycardial responses when out of water (McCauley 1971). Fish embryos and larvae often use epidermal body surfaces as sites of oxygen exchange (Krogh 1941, DeSilva and Tytler 1973, Weihs 1980). Whether decreased peripheral circulation occurs during hypoxia for these early life history stages is uncertain.

Individual eggs within a poorly ventilated egg mass do not all experience water of identical oxygen content (Figure 6). Generally, embryos situated progressively closer to the peripheral, exposed surface experience higher oxygen levels than those at the interior. Consequently, the interiormost embryos (often those in contact with the substrate) will display the most depressed metabolic rates, with embryos residing progressively nearer the periphery displaying increasingly greater oxygen consumption. Since hypoxia can retard the development of incubating fish eggs and protract the incubation period (Brannon 1965, Oseid and Smith 1971, Carlson and Siefert 1974, Siefert and Spoor 1974), peripheral embryos should develop faster than those at the interior. This was supported by field observations: Dabob nests consistently exhibited developmentally retarded embryos at their interior, while San Juan nests typically

contained embryos which were developmentally synchronized (Table 2). The faster development and higher oxygen consumption of peripheral embryos relative to interior ones serves to further reduce oxygen levels in the interior. As the mass matures, more oxygen is removed from the ventilating currents transversing its interior, intensifying the hypoxia. The ultimate consequences are two: (1) the condition is so severe that some embryos die forming a necrotic core which expands until the remaining viable embryos hatch, or (2) hypoxia does not reach lethal levels, but the hatch is protracted, commencing at the periphery and advancing inward with the entire nest eventually hatching. The first condition is illustrated for three pieces of a common egg mass (Table 8) that hatched sequentially from the periphery inward over a two week period. I never observed the second condition in the field, but both Wilby (1937) and Jewell (1968) described protracted hatches of lingcod nests (up to 7 days duration) and did not mention observing residual dead eggs.

Typically, egg masses deposited in high current areas (e.g. San Juan sites) can be characterized as containing developmentally synchronized embryos throughout (Table 2). It is plausible that these embryos would hatch in a synchronous fashion, although I have not observed such an event in the wild. However, Low and Beamish (1978) reported that nests at their high-current study sites

usually hatched completely in less than 24 hr. Likewise, LaRiviere et al. (in press) described the relative speed with which advanced nests disappeared from high current velocity sites. Although some nests were dislodged, by the swift currents, their observations suggest that the hatching process is relatively rapid. In the laboratory, I have incubated nest pieces removed from high current areas in the San Juans, taking care to provide adequate ventilation. Typically, these pieces hatch quickly with waves of larvae emerging for a few hours and most stragglers hatching within 24-48 hr. Jeffrey Marliave (pers. comm.) at the Vancouver Aquarium has described similar rapid hatches from lingcod nest pieces which he incubated in swift currents. If, in fact, the synchronous hatching of egg masses is typical at most high current spawning sites (as the evidence suggests), it presents some interesting ecological considerations concerning the larvae, especially when contrasted with the protracted hatching of egg masses in low current regions (Table 8). A synchronous hatch could be disadvantageous. Dense concentrations of emerging larvae may attract predators. Low and Beamish (1978) observed a school of seaperch gathered about a hatching nest; they consumed all emerging larvae during a 10-minute observation. In the San Juan Archipelago, schooling planktivorous rockfishes, e.g. Sebastes melanops and S. flavidus, are abundant (Moulton

1977). Predation could be especially acute during periods of slow tidal flows or slack tide when pelagic rockfishes could easily congregate about hatching nests. Furthermore, it is precisely during such tidal conditions when peak hatching might be expected. Alderdice et al. (1958) demonstrated that a reduction in environmental dissolved oxygen induced hatching of developmentally advanced O. keta embryos at a rate dependent on the degree of hypoxia. Conversely, a potential advantage of a synchronous hatch may be that mass emergence of larvae would overwhelm predators, assuring the escape of some. Life history strategies of certain insects support this explanation. Lloyd and Dybas (1966) discussed the phenomenon of cicada mass emergence after an extended (13 or 17 yr) larval phase. They suggested that the large emergence numbers synchronized to hatch precisely during specific years acts to swamp predators. Larvae emerging from a nest over a protracted period of several days (Jewell 1968) to two weeks (Table 8) may benefit by being inconspicuous to predators; on the other hand, they could not benefit from a swamping mechanism.

Presumably, the oxygen consumption estimated for chorions (Figure 10) is primarily due to epibiotic contamination (Braum 1978). However, Hamor and Garside (1973) reported chorionic respiration in Salmo salar eggs and attributed it to the presence of oxidative enzymes in

the chorion. Whether or not chorionic oxidative enzymes occur universally is uncertain. In any event, at least a portion of the observed respiration is due to epibiota. This epibiotic demand (as much as 24%, if oxidative enzymes are of minor importance) could potentially reduce interior oxygen to deleterious levels in a poorly ventilated nest. This suggestion is supported by observations of dungeness crab (Cancer magister) eggs. Fisher and Wickham (1976) suggested that epibiotic fouling (cyanophytes and bacteria) on crab eggs may restrict gaseous exchange and/or consume oxygen necessary for developing embryos causing the egg mortalities they observed. Direct causality has not been demonstrated, but the number of dead eggs in an egg mass was positively correlated with increased epibiotic fouling (Fisher 1976).

Larval Size; Ecological Considerations

Larvae surviving hypoxia at the nest interior are significantly smaller in length than their siblings residing at the periphery (Table 9). This size differential could potentially affect swimming speed. Rosenthal and Hempel (1973) found that there was approximately a 37% increase in the swimming speed of herring larvae which increased in total length from 8-11 mm to 11-15 mm. Swimming speed is of ecological importance with regard to the frequency of prey encounter, prey

capture efficiency and predator avoidance (Braum 1978). Potential invertebrate predators of epipelagic lingcod larvae might include euphausiids (Theilacker and Lasker 1974), coelenterates and chaetognaths (Fraser 1969), and hyperiid amphipods (Westernhagen and Rosenthal 1976). These predators are on a comparable size scale with the larvae. In these cases, there may be some escape advantage afforded to the larger larvae. Juvenile and adult predatory fish, on the other hand, are at least an order of magnitude larger than their prey and typically sustain cruising speeds of two to three body lengths per second, with much faster bursts possible (Alexander 1967). It, therefore, seems unlikely that any swimming speed differential that might exist between stunted and normal size lingcod larvae would result in significantly different capture efficiency by predatory fishes.

Another important facet of swimming velocity concerns the feeding proficiency of larvae. The consequences of slower swimming speed include a reduced search volume per unit time (Blaxter 1969), a real impediment when prey items are sparse. It also seems reasonable that prey handling ability may also be less efficient for small larvae. Size also affects a larva's ability to endure periods of prey item scarcity. Blaxter and Hempel (1963) demonstrated that the time to starvation and point of no return (May 1974) is reduced for smaller herring larvae. Additionally,

conspecific competition within a cohort can be effected by disparate size composition. Mason (1969) found that coho salmon eggs incubated under hypoxic conditions produced smaller larvae at hatching and that these were at a competitive disadvantage among larger larvae in the cohort.

Auxiliary Ventilation

A number of freshwater and marine fishes are reported to fan eggs (Breder and Rosen 1966). DeMartini (1978) observed egg fanning by the buffalo sculpin, Enophrys bison, which spawns a demersal egg mass in areas similar to those utilized by lingcod. Presumably, this behavior can be instrumental in ventilating egg masses.

Wilby (1937) reported that guardian male lingcod fanned egg masses with their pectoral fins. However, fanning behavior was not reported in three subsequent nesting studies (Jewell 1968, Low and Beamish 1978, and LaRiviere et al. in press), nor did I observe any behavior that might be construed as fanning during this investigation. At Dabob Bay, five of six nests exhibiting low interstitial oxygen (Table 2) had males in attendance immediately preceding sample extraction. Likewise, 11 of 13 nests containing either dead or retarded embryos were guarded by males. It is certainly possible that fanning behavior occurs when observers are absent; but if it does,

the field evidence suggests that it is ineffective in adequately ventilating the nests.

Diatom growths were common on the exposed surfaces of lingcod egg masses, especially in Dabob Bay. DeMartini (1976) suggested that diatom growths on demersal egg masses may provide supplemental oxygen to developing painted greenling (Oxylebius pictus) embryos. This analysis does not appear to be plausible; in fact, diatoms may compound the problem of egg mass hypoxia. Oxygen would only be generated during daylight hours when the light reaction of the photosynthetic process was occurring; at night oxygen would be consumed. Additionally, a diatom-mat could occlude interstices, restricting circulation.

Predation on Nests

The degree of predation on lingcod nests varies considerably at each spawning area. In Dabob Bay predation was rare, only 3 of 15 egg masses examined showed any evidence of predatory activity. However, in the San Juan study areas predation was common, with 12 of 15 nests showing evidence of predator foraging (Appendix 7). Likewise, in the same area LaRiviere et al. (in press) reported frequent predator sightings, with (as noted in this study) the small gastropods Amphissa sp. and Calliostoma sp. being the most common. Low and Beamish (1978) reported that predation on lingcod nests by

invertebrates and fish was common in the Strait of Georgia and constituted a major source of egg mortality. They estimated that 74% of nests in that region did not hatch, primarily as a consequence of nest dislodgment (fast currents were typical at their study sites) and ensuing predation. Apparently some nests were consumed in situ at the site of deposition but it was not clear in their text what this percentage was. LaRiviere et al. (in press) reported similar findings, estimating that about 60% of their study nests were lost to dislodgment by currents and/or predation.

Invertebrates can be effective predators on guarded as well as unguarded nests. Data presented in this investigation indicates that males are ineffective at curtailing predation by invertebrates, especially small gastropods and urchins. Jewell (1968) observed urchins foraging on 13 nests, 11 of which were guarded; five of the 13 were consumed before hatching occurred. Similarly, Low and Beamish (1978) and LaRiviere et al. (in press) noted the relative ineffectiveness of guardian males in preventing foraging by these two groups of invertebrates.

Small fish predators (Jordania zonope and Artedius sp.) have been regularly observed near guarded and unguarded nests (Low and Beamish 1978, LaRiviere et al. in press), but their impact is difficult to assess. Larger fish, e.g. greenlings (Hexagrammos sp.), the sculpin

Scorpaenichthys marmoratus, and various Embiotocidae species, are major egg predators in some areas (Jewell 1968, Low and Beamish 1978), but typically forage only on unguarded nests indicating that males are a deterrent to these predators.

Although gastropods were the most common nest predators observed during this study, they consume relatively few eggs (primarily at the periphery of the egg mass). However, their foraging activity may promote nest dislodgment. The adhesive secreted with the egg mass cements the eggs to each other, but not the substrate. The extrusion of the mass into crevices and between rocks anchors it in place. Erosion of these protrusions by small gastropods may in time result in dislodgment, especially in areas of strong current. Such a mechanism may explain the extensive nest dislodgment reported for lingcod nests in the Strait of Georgia (Low and Beamish 1978) and the San Juan Channel (LaRiviere et al. in press).

DeMartini (1976) found eggs of Oxylebius pictus (Hexagrammidae) in the stomachs of guarding males of the same species. He interpreted this as evidence for egg cannibalism and suggested that this is an adaptive mechanism that permits the guardian male to sustain himself for the duration of the incubation period without having to leave the nest unguarded to forage. Observations on lingcod (also a hexagrammid) suggest an alternative

explanation: the eggs could have been ingested incidentally while the guarding male was attempting to remove small predators from the egg mass. Both Moulton (1977) and LaRiviere et al. (in press) observed both small gastropods (egg predators) and lingcod eggs in the stomachs of some guardian males. Furthermore, the majority of guarded egg masses in Dabob Bay showed no evidence of predation, including any discontinuity at the periphery that might indicate that a chunk of eggs was removed by an attending male. DeMartini (1976) did not identify items in the greenling stomachs other than the eggs, nor did he discuss what egg predators, if any, were present near or on nests at the time of sampling. This information would be needed to clarify that species' egg consuming behavior.

Egg Mortality

The mean egg mortality of 59% estimated for nests in Dabob Bay is in agreement with presumed nest mortalities reported by Low and Beamish (1978) and LaRiviere et al. (in press) of 74% and 60%, respectively. In the latter investigations, nest dislodgment and predation were the causes of mortality, rather than hypoxia. Whether the mortality estimates discussed here are representative over the range of the species is uncertain. Demersal eggs which are guarded by a parent are often expected to realize high survivorship to hatching (Lagler et al. 1962, Marshall

1966, Hempel 1979). 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 fecundity of this species, further suggests that these high mortality estimates may be reasonable. A large female is capable of producing in excess of 0.5 million eggs (Wilby 1937). Such high fecundities are not typical for teleosts which produce large demersal eggs (Blaxter 1969). But when egg mortality is extensive (as present estimates for lingcod indicate), increased fecundity would probably provide an important adaptive advantage in the overall context of this species' life history.

Certainly the extent of embryo mortality on the spawning grounds is a major concern to agencies which have the responsibility of developing management plans for a particular species. The magnitude of mortality witnessed in Dabob bay, the San Juans (LaRiviere et al. in press) and the Strait of Georgia (Low and Beamish 1978) may be typical for this species. However, more field data are needed to determine whether embryo survival rates are similar in other areas. It is not difficult to identify oceanographic locales within the range of the species where current velocity or wave force would be sufficient to provide adequate nest ventilation but not so extreme as to cause extensive dislodgment. Additionally, concentrations of potential nest predators often vary between spawning areas.

Whether or not lingcod utilize such areas which could be conducive to high egg survival is uncertain; observations are very limited, usually restricted to the shallow (<40 m) nearshore habitat. More expansive surveys, including deeper offshore basins and reefs, which focus not only on numbers of nests but also on the extent of embryo mortality resulting from poor ventilation, predation or some other mechanisms we have yet to identify, are necessary to better assess the role of the egg stage in the recruitment process.

SYNOPSIS

- I. Some lingcod spawning grounds in Puget Sound are unsuitable for proper embryo development due to prevailing weak tidal currents which do not adequately ventilate nests. Hypoxic conditions which develop at the interior of poorly ventilated nests can be severe enough to be lethal to developing embryos.
- II. In a poorly ventilated egg mass embryos at the interior that survive sublethal levels of hypoxia are smaller at hatching and hatch later than siblings residing near the surface of the mass, where water is sufficiently oxygenated. Size and time of hatching may be important to larval survival.
- III. Data presented here suggests that water movement is an important stimulus for nest site selection by lingcod. In Dabob Bay where tidal currents were weak nest deposition occurred in shallow water, where surface waves and vertical tide motion provide water movement. At San Juan spawning

grounds (swift current areas) nests were significantly deeper than those observed in Dabob Bay. Suitable spawning substrate occurred well below the deepest nest observed in Dabob Bay.

- IV. In situ predation on nests was not extensive at any spawning area. The most commonly observed egg predators were small gastropods. Although they consume relatively few eggs their foraging activity may promote nest dislodgement by eroding portions of egg masses which act as anchors. Guardian males are not an effective deterrant against either the gastropods or urchins.
- V. The presence of hemoglobin during most of the embryo development period and the observed reduction in metabolism under hypoxic conditions may afford embryos a survival advantage during short-term exposures to hypoxia which can develop during slack tide.
- VI. Epibiota present on the surface of lingcod eggs can account for as much as 24% of the total oxygen uptake of a single egg (assuming that oxidative enzymes, if present in the chorion, are of minor importance). Epibiota may restrict gaseous

exchange or consume oxygen necessary for developing embryos, of particular concern in poorly ventilated egg masses.

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APPENDIX A: Supplemental Tables

Table A-1.--Mortalities of embryos (stage 10-11) incurred during oxygen bioassays. Two replicates per treatment; sample size is designated in parentheses. Salinity = 28.5 ‰, temperature = 8.7 - 9.5° C.

Exposure time (hr)	Oxygen (% saturation)					Ambient control 85
	2	6	12	22	50	
4	4(10)	0(11)	0(10)	0(10)	0(10)	0(10)
	0(10)	0(10)	0(11)	0(11)	0(10)	0(10)
24	3(10)	3(10)	0(11)	0(12)	0(10)	0(10)
	4(10)	1(10)	1(10)	0(10)	0(10)	0(10)
48	10(10)	9(10)	2(10)	0(10)	0(11)	0(10)
	9(10)	6(10)	4(10)	0(10)	0(12)	0(11)
96	11(11)	10(10)	9(10)	6(10)	2(10)	0(12)
	9(10)	9(10)	9(10)	6(10)	1(10)	1(10)

Table A-2 .--Data used for two-way analysis of variance. Oxygen uptake per embryo·hr⁻¹.

Develop- mental stage of embryos	Mean oxygen (% sat.) in respirometers		
	20	47	77
	.024	.096	.153
6	.011	.054	.164
(early)	.051	.054	.182
	.026	.063	.150
8 and 9	.013	.087	.188
(intermediate)	.048	.111	.210
	.065	.137	.226
12	.056	.115	.178
(advanced)	.065	.069	.212

Table A-3.--Bradycardia regression data used for analysis of covariance. The number of heartbeats per 10-second interval was recorded at four time exposures and six oxygen levels; control = 85% saturation. Notation is according to Zar, 1974.

	x^2	xy	y^2	n	a	b	Resid- ual SS	Resid- ual DF
Regression 85%	15,388	- 300	110	17	24.1	-.0195	104.43	15
Regression 50%	15,953	-1559	437	17	21.5	-.0977	284.62	15
Regression 22%	7,936	- 646	361	18	19.2	-.0814	308.04	16
Regression 12%	8,802	-1045	245	19	17.0	-.1188	120.34	17
Regression 6%	4,712	- 392	73	11	11.9	-.0831	40.86	9
Regression 2%	6,168	- 718	116	8	14.9	-.1164	32.59	6
Pooled							890.88	78
Common	58,960	-4660	1342				973.66	83
Total	62,274	2925	3185				3047.38	88

Table A-4. -- Standard lengths (mm) of larvae hatching from three portions of a common egg mass, each of which was subjected to a different current velocity to produce the appropriate interstitial oxygen level. n = 30.

	Interstitial oxygen (% saturation)					
	20		50		85	
Start of hatching period	11.01	10.75	10.75	10.88	11.39	10.11
	10.62	11.01	11.01	11.01	10.88	11.01
	10.37	10.62	11.14	11.14	11.52	11.26
	10.50	11.26	11.01	11.26	11.01	11.14
	11.14	10.88	11.01	11.52	11.65	10.75
	11.26	10.88	11.14	11.14	11.39	10.75
	11.26	11.26	11.26	11.52	11.26	10.75
	11.39	10.75	11.14	11.39	11.14	11.78
	11.90	11.01	11.01	11.14	11.39	11.01
	11.39	10.50	11.26	10.88	11.26	11.26
	11.01	9.98	11.14	10.88	11.52	10.88
	11.01	11.01	11.01	11.01	11.65	11.14
	10.75	9.98	11.14	11.26	10.88	10.88
	10.75	11.01	10.88	11.01	11.39	10.75
	11.01	11.14	9.47	11.14	11.39	10.50
End of hatching period	9.47	9.98	9.34	9.34	10.37	10.24
	10.37	10.37	9.47	9.22	10.37	10.37
	9.86	10.62	8.83	9.73	10.75	11.01
	9.34	10.62	8.70	10.11	9.73	10.88
	9.22	9.86	9.60	8.83	11.14	10.11
	8.70	10.24	9.98	9.73	10.62	9.09
	9.60	10.50	10.24	9.86	10.50	10.11
	9.86	10.24	7.94	8.96	10.39	10.24
	9.98	9.87	10.11	9.86	9.73	10.24
	9.22	10.34	9.60	7.17	8.96	10.88
	9.73	10.11	9.34	9.86	8.19	9.86
	9.47	9.98	9.98	9.86	10.24	9.86
	9.47	9.73	9.60	8.45	10.88	10.37
	9.98	9.73	10.24	9.73	11.26	9.47
	10.11	10.11	8.96	10.37	11.01	10.50

Table A-5 .--Depth (m) of lingcod nests below MLLW during 1979-80 and 1980-81 spawning seasons.

Dabob Bay			
3.3	5.1	+1.1	4.2
3.3	3.5	>0.0	5.4
9.9	2.7	1.9	0.2
9.3	6.0	2.9	10.4
San Juan Island			
9.8	9.1	18.6	17.4
14.5	10.5	14.7	14.2
7.6	12.0	5.1	6.3
17.1	12.9	16.1	12.0
5.6	11.0	17.7	18.9
15.5	17.4	15.2	5.6
6.7	6.1	19.8	7.6
7.6	14.2	21.9	6.7
12.5	5.9	12.8	8.5
19.5	15.2	12.8	1.8
9.8	15.8	6.1	5.6
9.6	2.4	14.5	
9.0	4.8	5.9	

Table A-6 .--Temperature (°C) and salinity (‰) at two depths in Dabob Bay during the 1978-79 and 1979-80 lingcod spawning seasons. Measurements were taken near Pulali Point at the middle of the bay. Data was provided by Mark Ohman, Dept. of Oceanography, University of Washington.

Date	Temperature (°C)		Salinity (‰)	
	Surface	10 m	Surface	10 m
1-3-79	7.6	7.6	31.5	31.5
1-9-79	6.8	7.9	29.5	30.0
1-24-79	7.3	7.7	30.0	30.0
2-8-79	7.4	7.1	26.0	29.0
2-21-79	6.4	7.0	28.0	29.5
3-6-79	7.5	7.2	27.0	29.5
3-22-79	9.2	7.7	26.5	29.0
11-29-79	<8.0	10.3	28.0	30.0
1-3-80	-	9.0	-	29.0
2-7-80	7.0	8.5	25.0	29.0
2-20-80	7.5	9.0	24.0	29.0
3-6-80	7.5	9.0	24.0	28.5
3-19-80	8.0	8.5	24.5	28.0

Table A-7 .--Incidence of guardian males and predator sightings or evidence of predator activity (pierced, empty chorions) on peripheral surface of egg masses.

Egg mass identifi- cation	Guardian male present	Predator sighting or evidence of foraging
San Juans		
NCA	+	empty chorions, gastropods
NC2	+	" "
NC3	+	" " , gastropods
NC4	+	" "
NC11	+	none
TIX	-	empty chorions
TI3	+	" " , gastropods
TI7	+	" " ,
HI1	+	" " , gastropods
HI2	-	" " ,
HI3	-	empty chorions, "
HI4	+	none
HI5	-	"
HI6	+	empty chorions
HI7	+	" "
Dabob Bay		
PP1	+	none
PP2	-	"
PP3	+	none
PP4	-	"
PP5	+	none
PP6	+	"
PP7	+	empty chorions
PP9	+	none
PP10	-	"
WDF3	+	none
WW1	+	"
WW2	+	none
WW3	+	"
WW4	-	<u>Scyra, Pisaster ochraceus</u>
WW12	+	<u>Strongylocentrotus franciscanus</u>