ASPECTS OF THE ECOLOGY AND PHYSIOLOGY OF A MARINE CHIRONOMID LARVA, PARACLUNIO ALASKENSIS COQUILLETT

by

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ABSTRACT

The ecological and physiological adaptations of P. alaskensis larvae to the intertidal habitat have been assessed by examining the effects on larvae of environmental factors normally considered to limit the distribution of organisms spatially and temporally in the midlittoral zone.

Desiccation factors play an important role in limiting P. alaskensis larvae because, as shown by experimentation, larvae, when placed in concentrated seawater and in air, are susceptible to evaporation of water through the cuticle. Therefore, larvae must locate in moist areas.

Salinity changes, often thought to be the key barrier to the colonization of the intertidal habitat by insects, do not limit larval distribution. The hemolymph consists of 246 mM Na^+, 126 mM Cl^-, 14 mM Mg^{++} and 530 mOsm osmotic pressure. The composition and ratio of ions changed very little ($\Delta P_i/\Delta P_o = 0$) when larvae were acclimated to freshwater. Larvae regulate both hypo- and hyperosmotically. Water regulation curves indicate that, in salinities expected in the intertidal zone, the volume of the body is well regulated.

A permeable cuticle does not allow larvae to regulate the hemolymph concentration of ions and water as efficiently in high salinities as do larvae of species having an impermeable cuticle. A drinking rate was demonstrated. The respiration rate in seawater at 15° C was determined to be 0.032 µl O_2/mg per hour.

Larvae supercool to -11° C and, therefore, are well adapted to potential freezing temperatures in the intertidal habitat. The necessity of a suitable substrate has been demonstrated. The ecology of this species and particularly the adaptations of the life cycle to seasonal changes in the environment have been discussed.

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Acknowledgements

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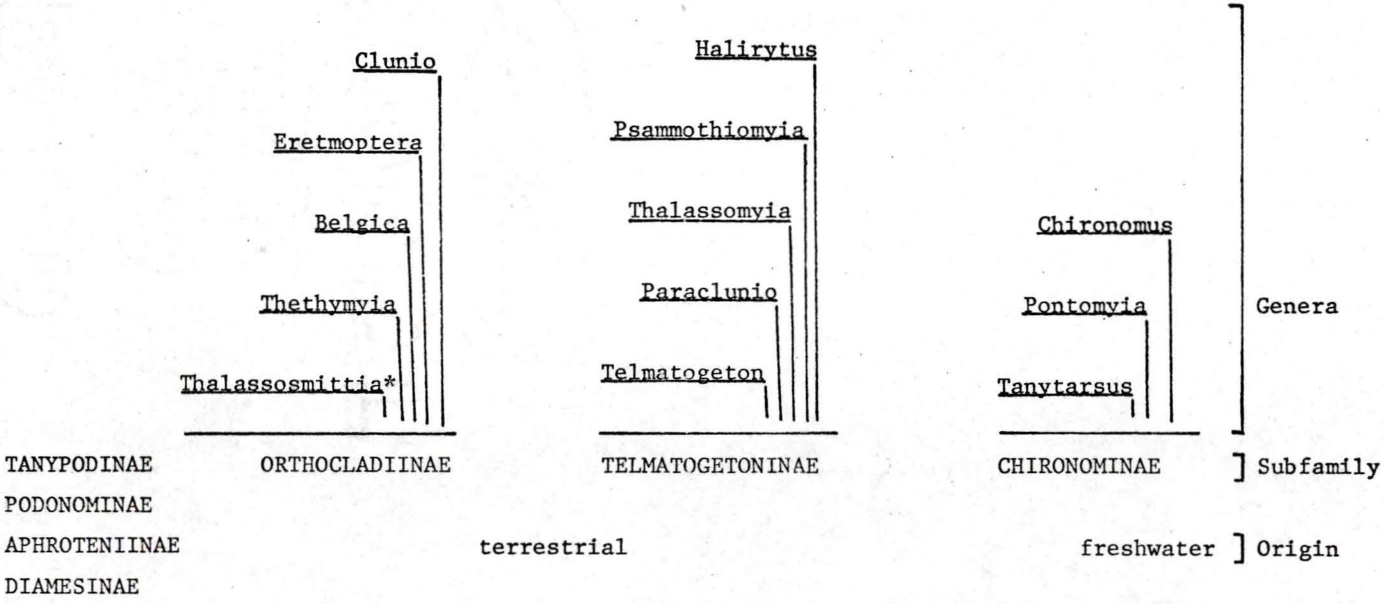
INTRODUCTION

Chironomidae is a family of the order Diptera consisting of small flies commonly called non-biting midges. Among the many types of environments which the Diptera inhabit, several families, and particularly the chironomids, have been most successful in penetrating into the littoral zone. The larval form of intertidal species inhabits areas of rocky and coral shores throughout the world in extremely high densities (Neumann, 1976). There is, at present, a total of 13 genera and 60 species of marine chironomids occurring along the coasts of Japan, North America and Europe. Marine chironomids are of polyphyletic origin. They have adapted independently from freshwater or terrestrial habitats to intertidal environments on many occasions (Neumann, 1976).

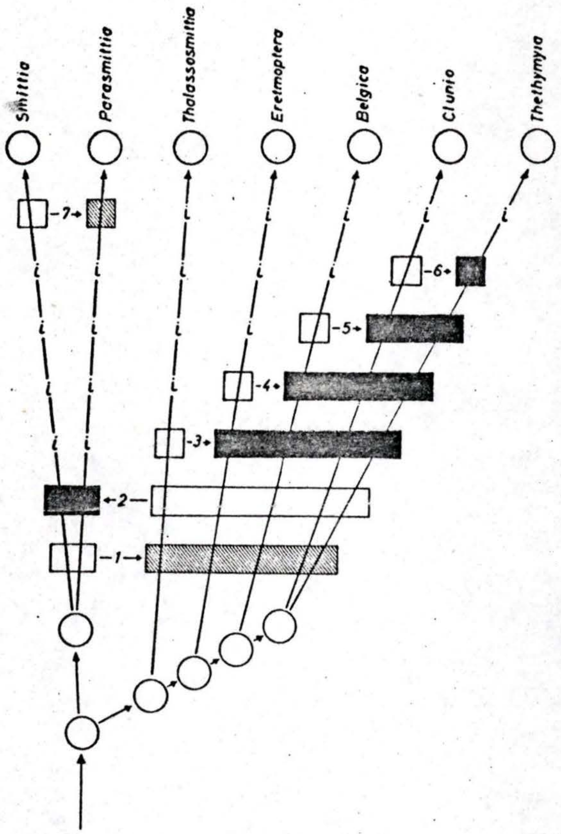
Figure 1 illustrates the three subfamilies of Chironomidae containing marine genera. The origins of only 3 of the 13 genera have been determined. Figure 2 gives a more specific example of the adaptations seen in the Clunio group. Hashimoto (1976) and Neumann (1976) have discussed the evolutionary trends and behavioural adaptations of adult chironomids inhabiting the fringe of the ocean, whereas the main objective of this study is to examine the adaptations of the larval form to the intertidal habitat.

Figure 1. Polyphyletic origins of the intertidal genera of the family Chironomidae (adapted from Neumann (1976))



* = Saunderia, Spaniotoma, Camptocladius sp.

Figure 2. The phylogenetic relationship in the Clunio group of adults (Neumann, 1976).



Phylogenetic relationship in the *Clunio* group The genera are arranged according to seven pairs of characteristics (rectangles). For each pair the white rectangle symbolizes the primitive (plesiomorphic) state and the dark one the derivate (apomorphic) state. 1. limnic or terrestrial habitat ⟶ marine habitat; 2. larvae with a pair of anal pseudopods ⟶ larvae with ventral torus; 3. female imago with intact wings ⟶ brachypterous or apterous female, male imago with normal hypopygium ⟶ hypopygium inversum; 4. female with halteres ⟶ female without halteres; 5. palps of the imago four-membered ⟶ one-membered; 6. male with intact wings ⟶ male with reduced wings; 7. wings without macrotrichia ⟶ wings with macrotrichia; *i* marks an indifferent state of the character concerned.

Natural History

The species examined in this paper is included in the subfamily Telmatogetoninae (Fig. 1). Paraclunio alaskensis Coquillet was first noted in the Pacific Northwest by Saunders (1928) and is typical of a marine chironomid, inhabiting rocky shore areas from southern California to Alaska. Four life stages are evident; egg, larva, pupa and adult (Fig. 3). The eggs are laid in a gelatinous mass on a rock or algal substrate selected by the adult. The eggs develop into larvae which live in the intertidal zone for at least three months. The larvae often build tubes in association with specific algae, on the surface of rock, among barnacles or in crevices. Larvae have been observed to reach maximum densities of 1,613 per ft^2 in early fall (Morley and Ring, 1972b). Their diet consists of algae and diatoms. They are, in turn, fed on by mites, sculpins (Morley and Ring, 1972b) and young salmon (Annan, 1958), although many aspects of the food web have not yet been investigated. The larvae develop through four instars (typical of Chironomids, Oliver (1971)) and then pupate in tubes which they have built on the surface of rocks in the intertidal zone. The adult emerges from the pupa to disperse, mate and lay eggs at low tide. As the tide floods, the adults recede up the shore and eventually die. In laboratory culture, adults do not survive longer than 3 days, presumably because they do not have a functional digestive system. Total developmental time of Paraclunio alaskensis from egg to adult is 204 days at 10°C (Morley and Ring, 1972b).

Marine chironomid larvae may be economically important as pollution indicator species near outfall areas (Cheeseman, 1972), as a food source for young salmon in the Pacific Northwest (Annan, 1958) and as a food source for aquaculture farming of fish and prawns in Hawaii (personal observation).

Bardach, Ryther and McLarney (1972) cite further examples of the use of chironomids in marine aquaculture. The larvae have a high food quotient (weight of food/increase in weight of fish = 2.3-4.4) and are 60% protein by dry weight. In Israel and Taiwan, larvae are raised in trays and fed as a food supplement to marine mullets at a rate of 0.5 kg per day per tray. It is hypothesized that the larvae "may contain some sort of growth enhancing substance which is extraordinarily effective on cold-blooded vertebrates".

Environmental Factors and Habitat

The diversity and number of insect species inhabiting the ocean is low relative to other environments. There are many factors common to freshwater, terrestrial and marine environments to which a species must adapt e.g. high winds, desiccating conditions, temperature extremes and low oxygen tensions. One of the unique features of the midlittoral zone, which is not common to freshwater or terrestrial environments, is salinity. Mechanisms must have evolved to facilitate survival of insect species in a saline environment and these will, theoretically, differ from freshwater or terrestrial counterparts. In addition, several of the above factors, in combination, may form a microenvironment within the algal strands to which a limited number of insect species have succeeded in adapting.

Objectives

The main objective of this study, as stated previously, is to examine the adaptations of the larval form to the intertidal zone. Two aspects of the investigation have been emphasized: the first, to study closely the environmental factors and habitat surrounding P. alaskensis larvae and their responses to these factors, and the second, to examine under laboratory conditions the responses of larvae to ranges in salinity, oxygen levels,

Figure 3

The life stages of <u>Paraclunio alaskensis</u>

a. Larva: lateral view
b. Larva: dorsal view
c. Larva: dissection, ventral view
d. Pupa: ventral view
e. Adult: Lateral view ♀

Figure 3

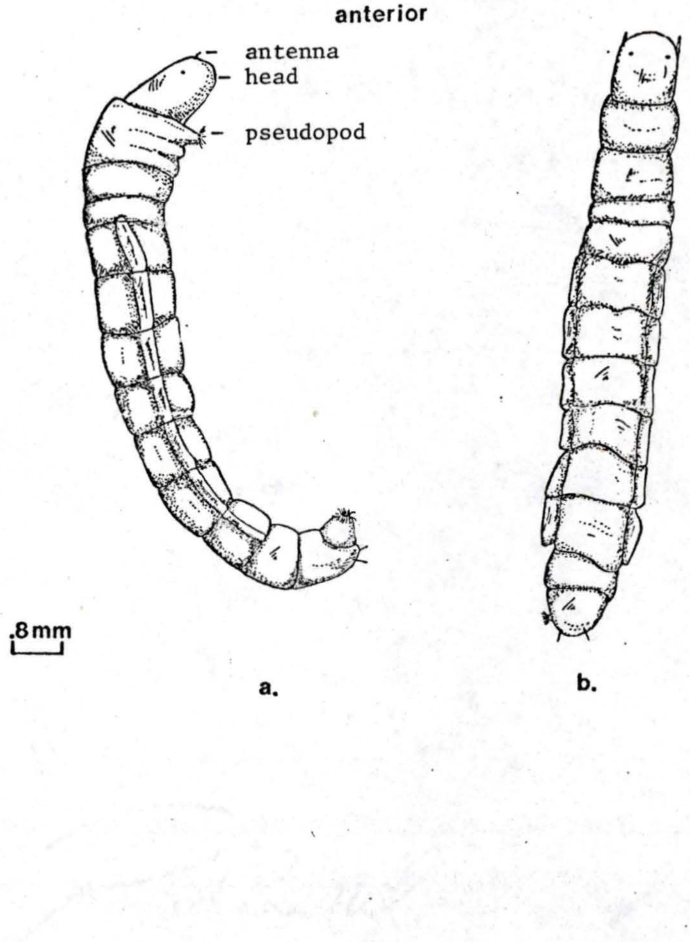


Figure 3

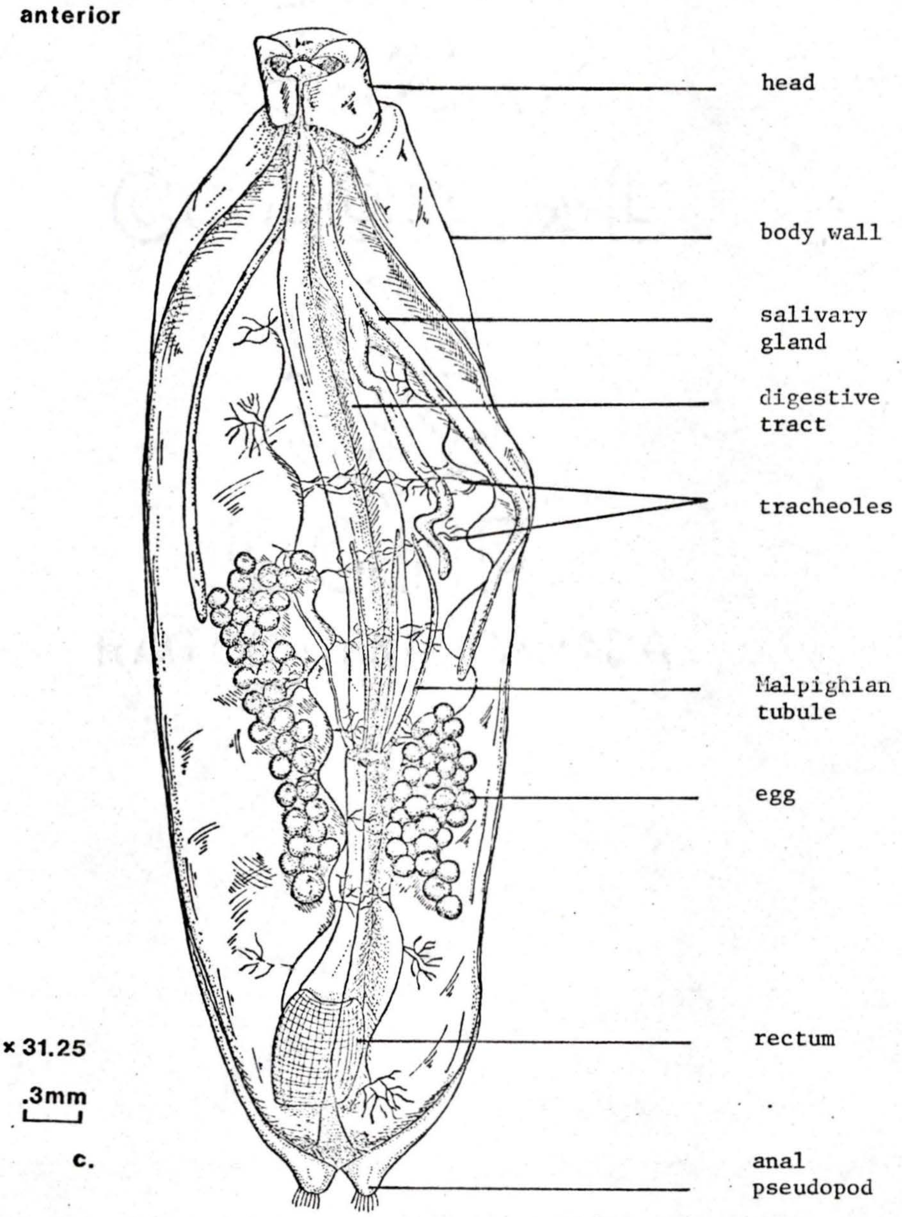
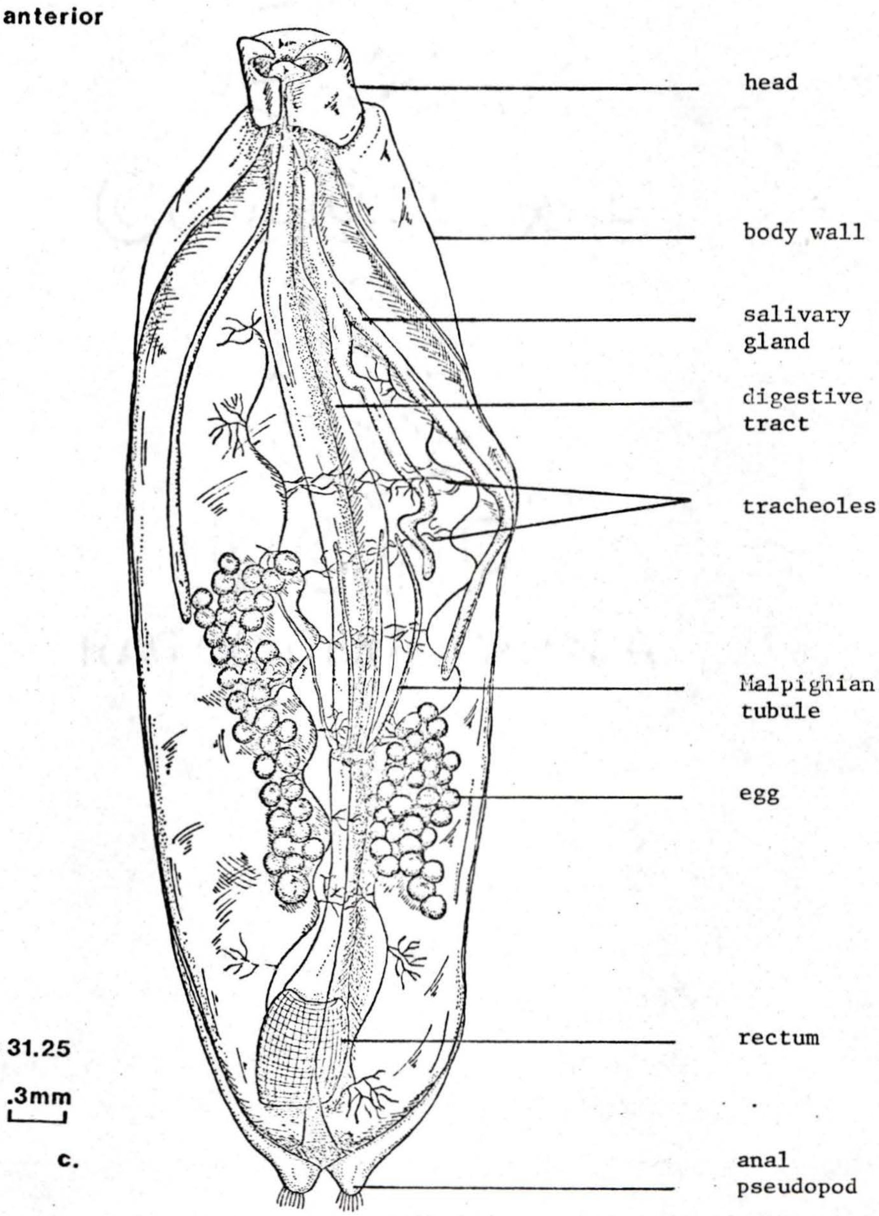

anterior
head
body wall
salivary gland
digestive tract
tracheoles
Malpighian tubule
egg
rectum
× 31.25
.3mm
c.
anal pseudopod

Figure 3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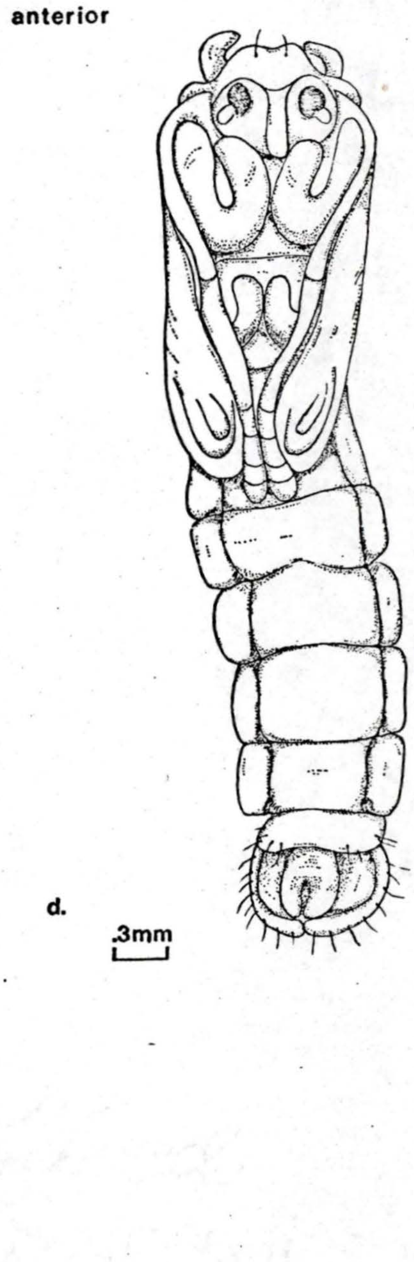
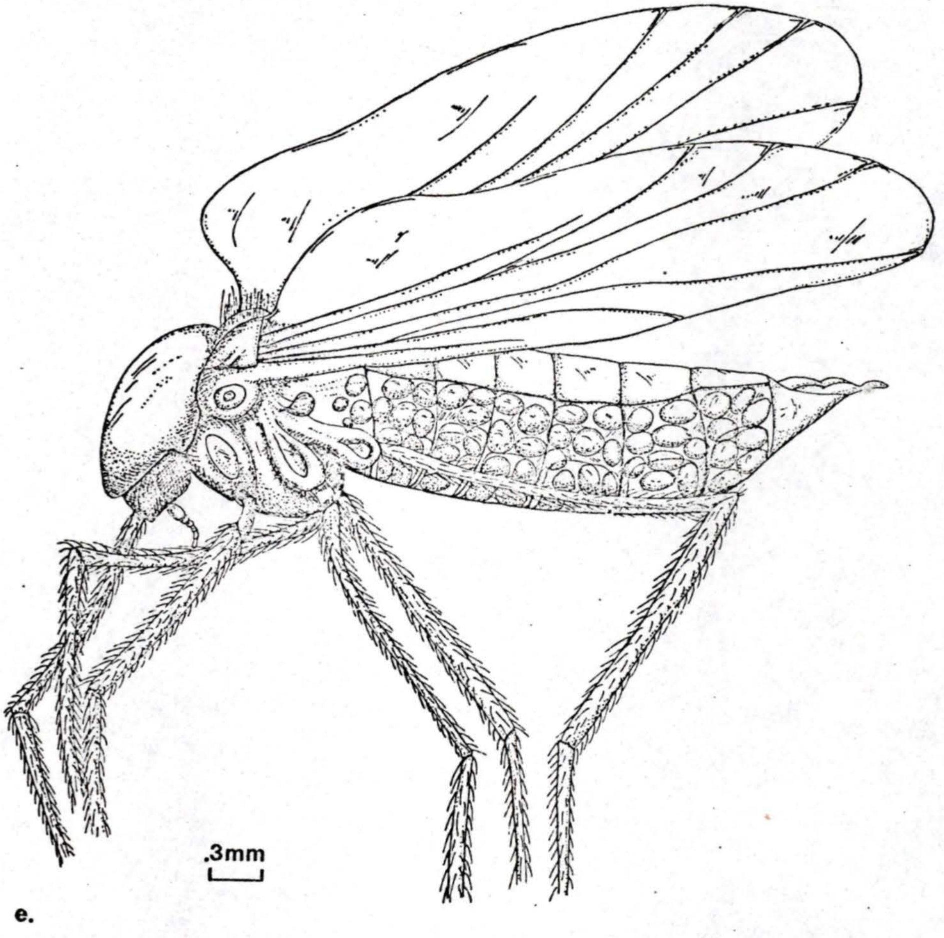


Figure 3



e.

desiccation factors and temperatures known to occur in their natural environment. The results of this thesis should indicate the degree of regulatory ability which *P. alaskensis* larvae possess over a range of fluctuating environmental conditions. The degree of regulatory ability may ultimately be responsible for the survival of this species in the intertidal zone.

MATERIALS AND METHODS

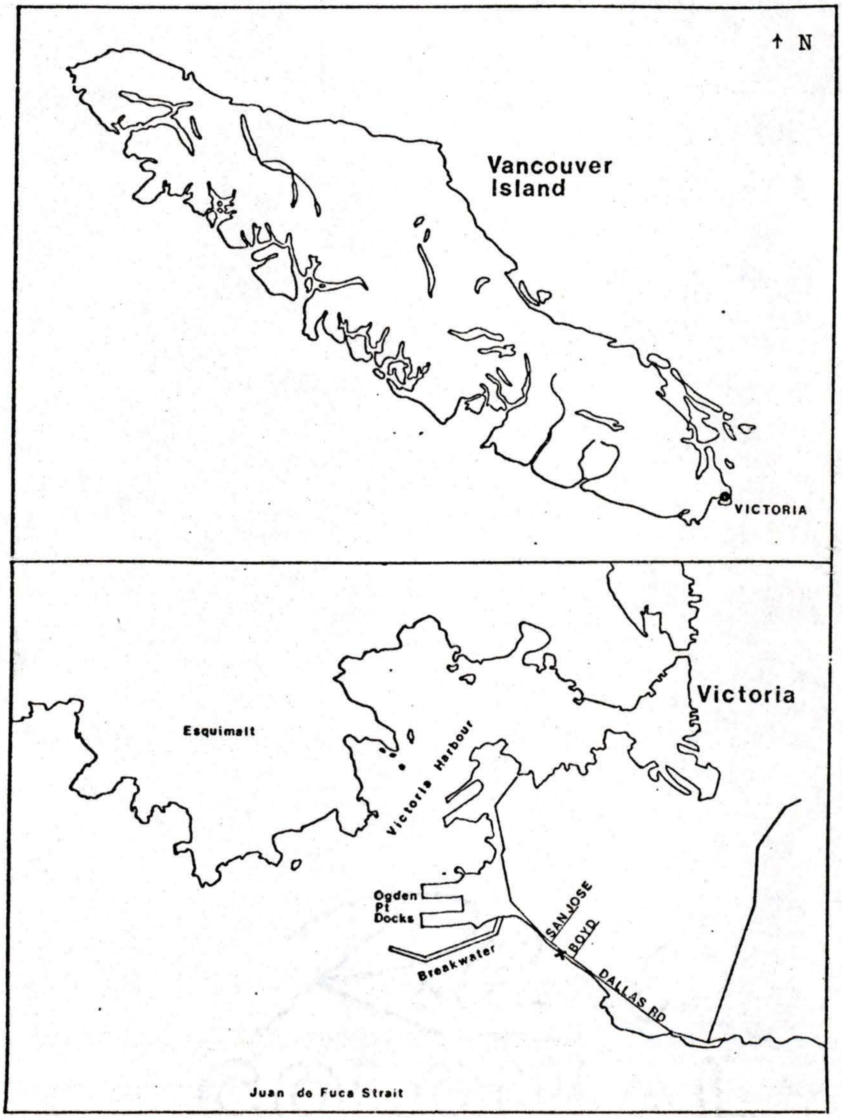
I Field Observations

All observations were recorded at the seawall at Ogden Point in Victoria, B.C. (Fig. 4 & 5). It was built prior to WWI and the large precast concrete blocks form a convenient grid on a vertical wall from which observations can be made. Embedded in the blocks at intervals are drain pipes. Excess water which collects behind the wall runs out these pipes, preventing a build-up of hydrostatic pressure against the wall (Goldy, 1980). The mouth of each pipe is 10 cm in diameter and lies flush with the wall. Figure 6 details the positions of the blocks and drain pipes.

All collecting trips over the four years of this study were recorded in the form of a logbook (Appendix A). The densities of dominant species of invertebrates and algae were approximated in the field by examining random 2.5 cm^2 quadrats along the wall. The data were not quantified statistically but an overall impression of seasonal changes in populations was obtained. A detailed statistical analysis of P. alaskensis larvae has been completed by Morley and Ring (1972b) at the Ogden Point Seawall and has been used for comparative purposes in this study.

In order to determine the possible underlying causes of population fluctuations, meteorological data for the immediate vicinity were obtained from the Gonzales Weather Station operated by the Canadian Federal Government. The factors recorded for 1975-1978 inclusive were hours of sunshine, cm of precipitation, temperature (^{o}C) and wind velocity (km/hr). The field observations are described in the results section under three headings: The Seawall, Organisms and their Cycles, and Meteorological Factors. Subsequent physiological experiments conducted in this study were designed to test the ability of P. alaskensis larvae to withstand environmental extremes known to occur in the field.

Figure 4. Location of Victoria and Ogden Point seawall (x).



II Collecting and Rearing

All larvae used in the following experiments were from a population of P. alaskensis living on a 30 m section of seawall at Ogden Point breakwater in Victoria (Fig. 5). This population was extremely abundant during the months of July to November and least abundant during the winter storm months of January to March. For example, Morley and Ring (1972b) found mean densities of P. alaskensis larvae as high as 9/1.25 in^2 in the months of September 1969 and as low as 0/1.25 in^2 in March 1970. All experiments were, therefore, restricted to "summer" populations of larvae, eliminating both variability between larvae of different seasons and the collecting problem.

Several attempts made to establish laboratory stock breeding cultures were of limited success. Developmental time was long (204 days at 10°C), cultures required constant control of numerous variables, and adults rarely emerged or mated successfully (Morley and Ring, 1972b). For specific culture methods which were attempted see Appendix B.

Only larvae larger than 5 mm yielded sufficient quantities of hemolymph. This precluded any possibility of sampling Saunderia spp., another intertidal chironomid genus whose larvae inhabit a similar niche. As well as being larger, Paraclunio larvae differ noticeably from Saunderia in the shape of the head capsule. On occasion, the mouthparts were examined to confirm identifications as described by Morley and Ring (1972a).

Head capsule widths are used to determine the instar stage of larvae (Hashimoto, 1976), but were not taken in this study. To be consistent among experiments, only large larvae were collected (~3-7 mg; ~5-9 mm). Larvae too close to pupation were easily recognized by the translucence of the integument and the development of large, white fat bodies.

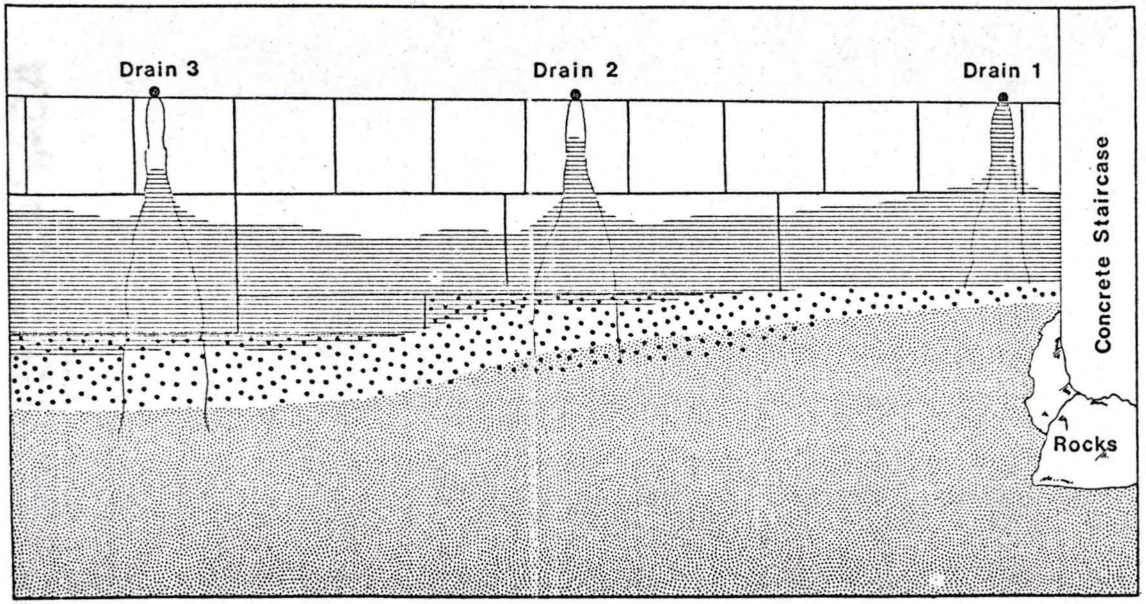
Figure 5.

Study site at Ogden Point seawall

KEY:

□ = Concrete Blocks
⁘ = Concrete and Pebble Mix
▤ = Algal Zone
▒ = Sand
∩ = Drain Openings and Runoff Zones

Figure 5



These were not collected or used in experiments. Therefore, in the following experiments, late instar larvae (either third or fourth stages) were used.

The length of each larva was determined under a dissecting microscope. The larva was placed on graph paper (1x1 mm) and gently arranged with a clear, plexiglass millimeter ruler so that the larva was straight and extended. The length could then be checked both on the graph paper and on the ruler.

Larvae were collected from the seawall and were transported in a flask of fresh seawater. The larvae were reared for experiments in large glass bowls of seawater each with an air supply. Fresh aquarium seawater was added periodically but not within 2 days of an experiment. Cotton batton and algae served as a substrate. The bowls were kept in an environment chamber maintained at 15°C. The chamber was illuminated by two 20 watt cool-white fluorescent tubes providing a photoperiod of 12D:12L.

III Ionic Regulation: Na^+, Mg^{++}, Cl^- and Osmolarity

The first series of experiments determined the levels of sodium, magnesium, chloride and osmolarity of the hemolymph of larvae reared in freshwater and seawater thereby establishing the degree of osmo-regulatory ability which P. alaskensis possesses. Larvae collected and maintained in the manner described above were transferred to bowls of either tapwater or fresh aquarium seawater. The bowls contained cotton battingsubstrate and an air supply. The larvae were left to acclimate, unfed, for a minimum of 2 days at 15°C.

Hemolymph was extracted from larvae after they had been blotted dry and placed on parafilm. Under a dissecting scope, a small incision was made on the mid-lateral surface using minute dissecting scissors. The scissors were quickly withdrawn to prevent capillary action of the fluid along the blades of the scissors. A 5 μl capillary was held near the incision so that the hemolymph was drawn up rapidly. The capillary was premeasured to ensure that it was exactly 70 mm long. Any part of the capillary filled with hemolymph could then be measured and its volume calculated. All sampling was done rapidly to prevent water loss from the sample. Samples visibly contaminated by gut contents or salivary glands were discarded. The hemolymph collected was added to a small vial of DDD H_2O which was then tightly sealed. The sampling procedure continued until 5 µl had been collected which required an average of 24 larvae. The vial of sample was diluted to 10 ml with DDD H_2O. From this, six readings could be made on a Varian Techtron 1100 atomic absorption spectrophotometer for sodium and magnesium. Standards of known concentration were run first for each ion.

The measurement of chloride and osmolarity levels did not require pooled samples. The hemolymph from a larva was collected in the manner described above. Chloride concentrations were measured according to the electrometric method of Ramsay, Brown and Croghan (1955). Osmolarity was determined using a nanoliter osmometer (Clifton Technical Physics Ltd.) for hemolymph (Frick & Sauer, 1973) and a Westcor 5100B vapour pressure osmometer for media of large quantities such as seawater, drain water and rearing solutions.

IV Osmotic Regulation

a. Per cent Body Water

The total water content of whole larvae was calculated for larvae collected during low tides from September to December. The wet weight of freshly collected larvae was recorded on a Cahn G2 Electrobalance (accurate to 0.001 g). The larvae were desiccated in a Virtis freeze-dryer (Model 10-100) for 48 hours and then reweighed.

b. Water Regulation

The tolerance of *P. alaskensis* larvae to a range of salinities was tested and the changes in body weight (signifying water regulation) recorded. Groups of larvae, unfed and acclimated to Victoria seawater at 15°C for two days, were weighed and transferred to glass bowls filled with pre-cooled solutions of either tap water, seawater, 150% seawater, or Ca^{++}-free seawater. At time intervals up to 72 hours, larvae were reweighed on the Cahn G2 electrobalance. Before each weighing larvae were carefully blotted dry. Each weighing was done as quickly as possible since evaporation of water from the surface of larvae could, over time, be observed to alter an initial reading significantly. The bowls were maintained at 15°C throughout the experiment. In Experiment B the temperature dropped to 4°C after 2 hours which had a noticeable effect on the rate of water loss.

Concentrated seawater was made by evaporating normal seawater to one half of its original volume. Ca^{++}-free seawater consisted of the following components in deionized double distilled water:

25.50g/1 NaCl, 4.66 $MgCl_2 \cdot 6H_2O$, 6.29g/1 $MgSO_4 \cdot 7H_2O$, 0.67g/1 KCl, 0.18 $NaHCO_3$. Calcium-free seawater is known to interfere with osmoregulation (Krogh, 1939).

V Supercooling

The supercooling points of whole larvae were determined using a Bailey Cryo-thermometer (Model BAT-5) and a Cole-Parmer Electronic Cryo-Bath (Model 3851). Larvae were held in place on the thermocouple tip with silicone grease.

VI Respiration Rate

The rate of respiration was determined using a IL 500 Blood Gas Analyzer. Larvae, collected and maintained in the manner described above, were placed in 10 ml syringes with filtered seawater and cotton batting. The syringes were kept in a 10°C water bath under a light proof container. Care was taken not to disturb the larvae in any way. The syringes were sealed with plungers, ensuring that the fluid level was exactly 10 ml and that there were no trapped air bubbles. The syringes could, at specified intervals of time, be screwed into the vent of the gas analyzer and a 2 ml sample of water injected past the $_{p}O_2$ and $_{p}CO_2$ electrodes. Gentle inversion of the syringe is recommended in order to mix evenly the gases in water before injecting a sample. Consideration must be given to the fact that each time a sample is injected, less seawater, and therefore oxygen, is available to the larvae. After several injections, the rate of respiration may be affected in some way by this. Also, the volume of sample injected was not always 2 ml due to the fact that air bubbles were often created on the electrodes when the sample was injected. The bubbles had to be flushed out and this used extra sampling fluid. But, overall, the basic respiration rate could be determined and the response of larvae to low O_2 conditions could be observed with repeated accuracy.

VII Labelling

Low levels of glucose, similar to those found in the ocean, were used in the following experiments with _P. alaskensis_ larvae. The results will then indicate the ability of _P. alaskensis_ larvae to take up and use dissolved organic matter (DOM) (Chapman, 1975). No ligatures to the mouth or anus were applied therefore the richness of label in specific organs cannot be traced necessarily to the origin of entry but can indicate certain selective absorption processes by these organs.*

a. Autoradiography

P. alaskensis larvae were exposed to d-glucose-^3H (specific activity 8.45 Ci/mM) in 0.45μ filtered (Millipore) seawater for 24-hour periods in 500 ml glass bottles at densities of 2 larvae per bottle. The concentration of glucose used was 4.6×10^{-6}M. Following exposure, larvae were rinsed in

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* Labelling experiments were conducted in 1975 by P. Chapman, research assistant for Dr. R.A. Ring.

filtered seawater and fixed in hot Bouin's solution. Fixed larvae were dehydrated with t-butyl alcohol, embedded in Paraplast and sectioned serially at 8μ. Autoradiographs were prepared by dipping sections in liquid Kodak NTB 3 Nuclear Emulsion. Sections were stored desiccated at 15°C and developed after 14 days, an optimum exposure time determined empirically. Sections were then stained through the emulsion with Eosin and Mayer's hemalum. Grains were counted with a square-graticule ocular. At 100x magnification, each ocular square represented $100\mu^2$. Labelling intensity was based on the mean grain counts for representative squares.

b. Isotope Counts

The rate of uptake of radioactive glucose was determined from isotope counts on whole larvae. By measuring the initial linear increase in whole body activity, the drinking rate of larvae can be determined. The following methods have been adapted from Chapman (1975) to apply to experimental procedures with P. alaskensis.

Larvae were maintained in 0.45μ (Millipore) filtered seawater in the dark for no more than two days prior to use. It was found that measurements of radioactivity in larvae (body counts) were not affected by bacterial counts. Experimental systems consisted of 0.45μ membrane (Millipore) filtered seawater, rinsed P. alaskensis larvae, antibiotics and ^{14}C-d-glucose (specific activity 53.7 mCi/mM) at a concentration of 4.6×10^{-8}M. Controls consisted of 1) unfiltered seawater and live larvae without glucose and served to monitor background radioactivity, and 2) filtered seawater, antibiotics, glucose and larvae killed by immersion in liquid nitrogen. Both controls served to differentiate between surface contamination by radioactivity and uptake of ^{14}C-d-glucose, and all results were corrected for these controls. Body counts were conducted by removing larvae from the bottles at the end of the experimentation, rinsing with distilled water and freeze-drying.

Dried larvae were then weighed, added to 2 ml of Protosol (New England Nuclear) in scintillation vials and solubilizing at 60°C for 36 hours. Fifteen ml of fluor (5 g PPO dissolved in 1 l of toluene) was added to the vials and the vials were then stored in darkness for 21 days to avoid chemiluminescence. Quenching was corrected for on an individual basis by the addition of 10^4 dpm toluene-^{14}C (specific activity 4.15×10^5 dpm/ml) to each scintillation vial and recounting. Radioactivity was measured by means of a Beckman liquid scintillation system, the efficiency of which was 95±3% as determined by argon-toluene standards.

RESULTS

I Field Observations

a. The Seawall

The seawall, as stated previously, is concrete. The surface of the wall has a rough texture and the fusions of the blocks of concrete form many crevices. The wall is completely vertical, without steps, so that little change in exposure of any kind occurs along the wall.

The runoff from the drains has a noticeable effect on the fauna and flora of the seawall. At low tide, only the areas directly beneath the pipes are wet. Consequently, organisms collect under the drains in higher densities and at higher positions relative to the organisms on the rest of the wall. During times when larvae are absent from most of the seawall, the areas below the drains continue to have a high density of larvae ($\sim 1/2.5\text{cm}^2$). Referring to Figure 5 , it can be seen that only Drain #1 supports algal growth up to the mouth. Drains #2 and #3 have a slick growth of ooze, presumably diatoms and bacteria, directly beneath the mouth, followed by an algal layer. The water supply from the drains is constant throughout the year and supports a stable population of algae and invertebrates, unlike other areas of the wall. The water flow is slight, providing no more than a thin surface film (~1mm thick) which flows downward due to gravity. At no point does the water run fast enough to create drips from protrusions. The water collected from the mouth of drain pipes, after having passed through the soil behind the wall, was of a higher osmolarity than freshwater but lower than seawater. The osmolarity of water collected from a drain varied over time and from drain to drain (Table 6).

b. Organisms and their Cycles

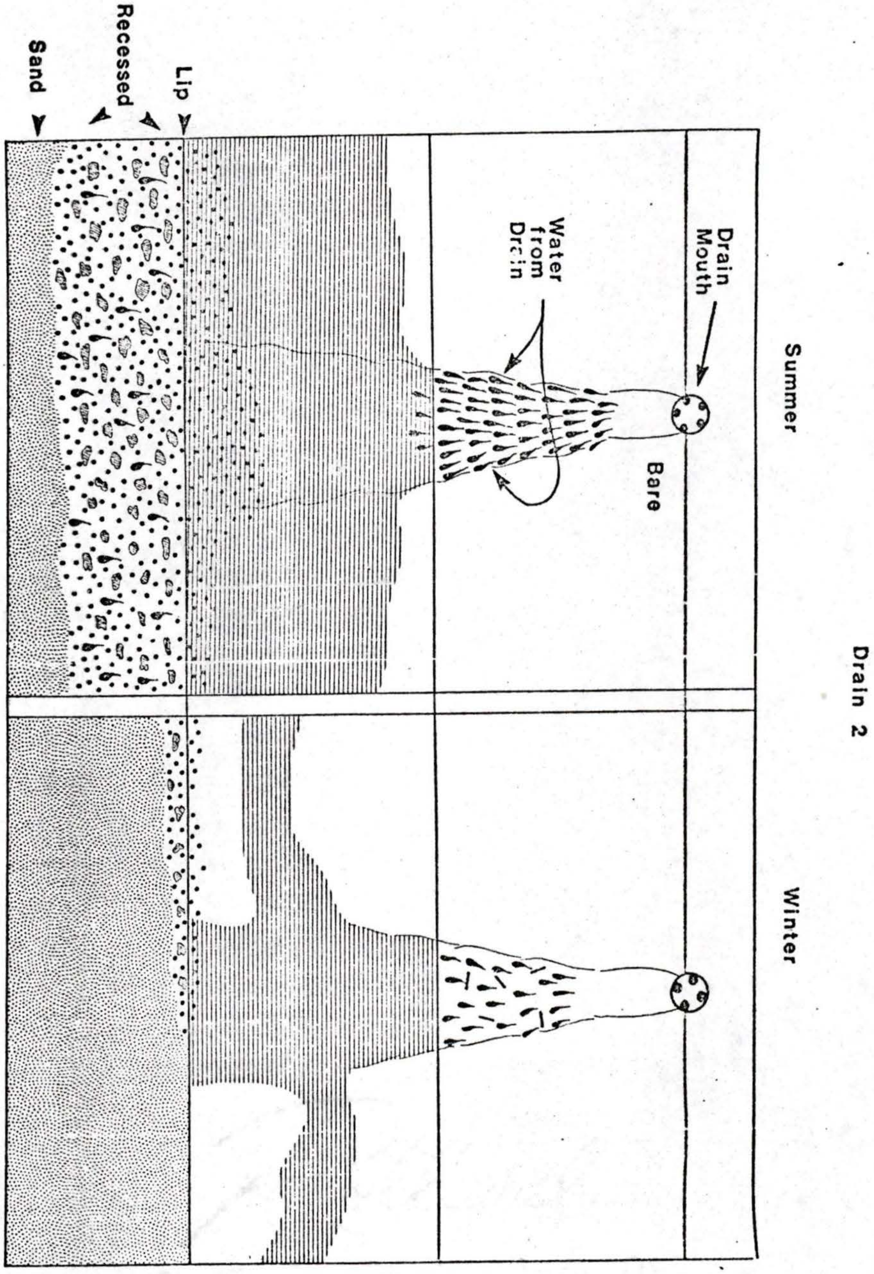
The seaweeds most commonly found on the wall were two greens, *Bangia fuscopurpurea* and *Enteromorpha linza* (Fig. 6). Occasionally, small clumps

Figure 6. Changes in the positions of organisms observed during one season at Drain # 2.

KEY:

▲ = **Limpets** (in drain mouth)
▨ = **Concrete and Pebble Mix**
⁙ = **Barnacles**
▤ = **Enteromorpha sp. and Bangia sp.**
🌢 = **Ulvoid sp.**
▒ = **Sand**
□ = **Concrete**
— = **Larval Tubes**

Figure 6.


Drain 2
Summer
Winter
Drain Mouth
Water from Drain
Bare
Lip
Recessed
Sand

of unidentified reds and browns could be observed. The cyclic nature of the green algal zone over the year is dramatic. In early fall (August-September) the strands are densely packed forming a continuous felt-like mat along the length of the seawall. Particles of sand become trapped in holdfasts of algae providing a layer into which organisms burrow. By the end of the winter months (February-March), only a diffuse algal band remains. The strands are short and have a dried appearance. Bare rock is visible between the strands and they no longer hold sand. Between summer and winter, the algal band shifts to a higher position on the wall. Also, the sand beach builds up in the winter so that the algal band appears to be at the same height. Figure 6 details the changes observed at Drain #2 over one season.

The species of invertebrates present on the seawall are listed in Table 1 along with the location and periods of abundance of each population.

Swallows were observed flying close to the seawall during the months of August and September. They would dart up and down along the length of the green algal band probably feeding on flying insects, such as P. alaskensis adults.

Lastly, the field observations of P. alaskensis larvae have been very useful in the understanding of adaptations of larvae to the intertidal habitat. From these observations, a more detailed description of the life cycle of P. alaskensis can be hypothesized and this will be analyzed in the discussion.

c. Meteorological Factors

The seawall is exposed to a range of temperatures over the year. The amount of rainfall and hours of sunshine are also highly variable. Monthly averages for each factor are shown in Graph 1 for the four years of this study (1975-1978).

Table 1. Diversity and Cycles of Organisms found on Ogden Point Seawall

Species	Location on seawall	Periods of abundance
Insecta -		
Diptera:		
Dicranomyia(= *Limonia*) *signipennis*	algal band spin cases in upper zone	April - October, larvae; August-October, adults.
Coleoptera:		
	algal & barnacle zones free crawling on surface	infrequently found, no cycles observed
Arachnida -	algal band free crawling on surface	August-October eggs and adults
Crustacea -		
Balanus sp.	below algal band continuing to the beach	adults consistently abundant, summer blooms of immatures
Ligia sp.	on slime below drains	<4 per drain no cycles observed
small amphipods	on slime below drains	<6 per drain no cycles observed
Gastropoda -		
Acmaea sp.	in crevices, in and around mouth of drains, down to edge of algal band	<12 per drain no cycles observed
Nemertea	among barnacles	infrequent, no cycles observed

SUBTIDAL SURVEY

Crustacea -

- amphipods
- dungeness crab
- hermit crabs
- barnacles

Vertebrates -

- fish school
- sculpins (clinging to the wall)

Mollusca -

- periwinkles
- whelks
- limpets

Firstly, as the temperatures indicate, freezing can be expected for a number of days in the winter months. In the results (section IV), the supercooling ability of larvae collected in the late summer through to early winter months is recorded.

Secondly, the amount of precipitation can be expected to affect the salinity of the environment surrounding larvae living on the seawall. Precipitation is greatest during winter months. During the six hours of low tide, larvae are exposed to a predominantly freshwater situation during heavy downfalls. Osmotic and ionic regulation abilities, important in the survival of larvae, are tested for in this thesis (Results section II).

The third meteorological factor recorded is the number of hours of sunshine. The greatest number of hours of exposure to the sun would be experienced by larvae during summer months. In addition, low tides in the summer occur during the day. The desiccating effect of the sun can be clearly viewed on a hot August day at low tide. The algae are warm and are beginning to dry because of exposure to the sun. Larvae do survive the exposure period and Section IV of the results confirms the ability of *P. alaskensis* larvae to withstand desiccation.

Another environmental factor, wave action, presents a strong force during storms and, in conjunction with wind, could easily remove larvae from the rocks and sweep flying adults away from breeding areas. Both larvae and adults have specific adaptations to prevent being swept away. The changes found in the marine chironomid adult as compared with its freshwater counterpart have been reviewed by Hashimoto (1976). Possible larval adaptations will be analyzed in the discussion section of this paper.

Graph 1

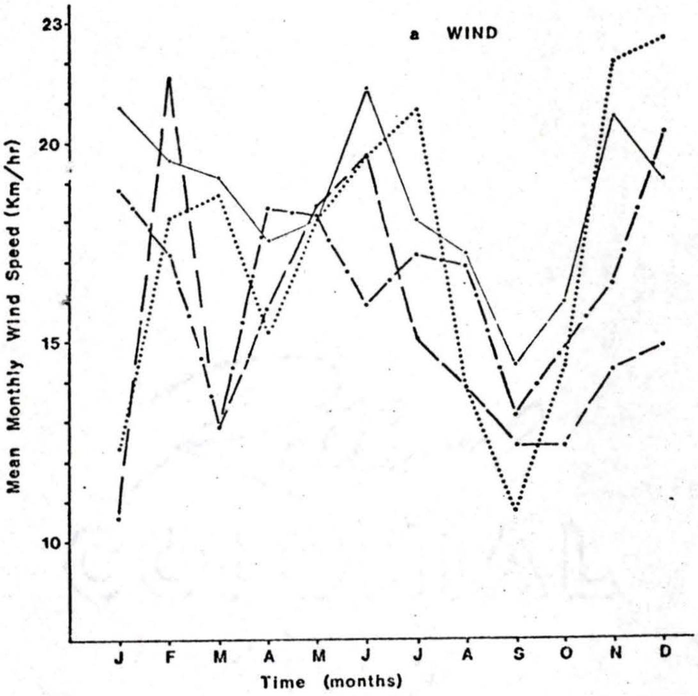
Seasonal cycles during 1975-1978 of:

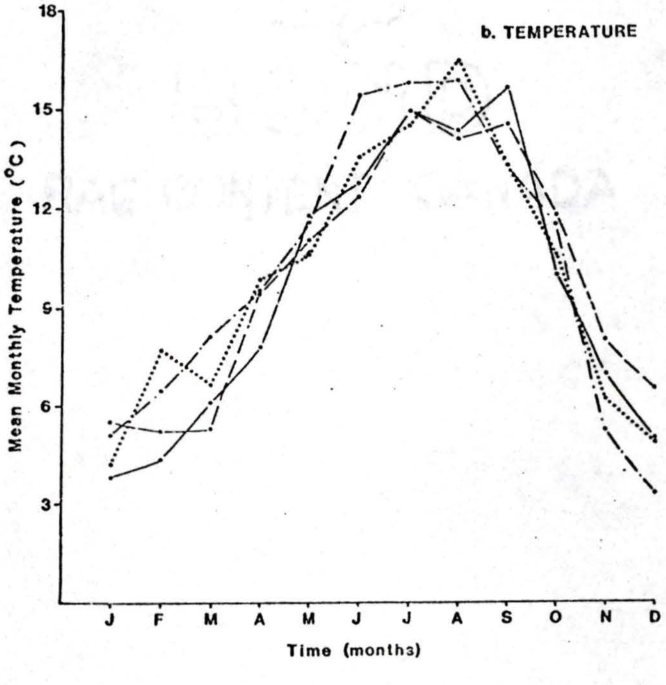
a. wind
b. air temperature
c. precipitation
d. sunshine
e. larval densities of
Paraclunio alaskensis
and
Saunderia spp. (1969-70).

KEY:

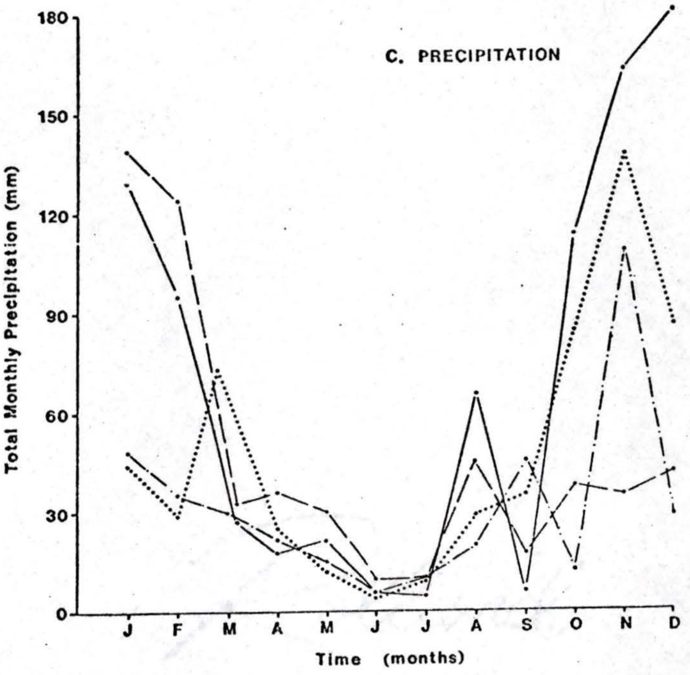
________ 1975
_ . _._ 1976
.... 1977
_ _ _ _ 1978

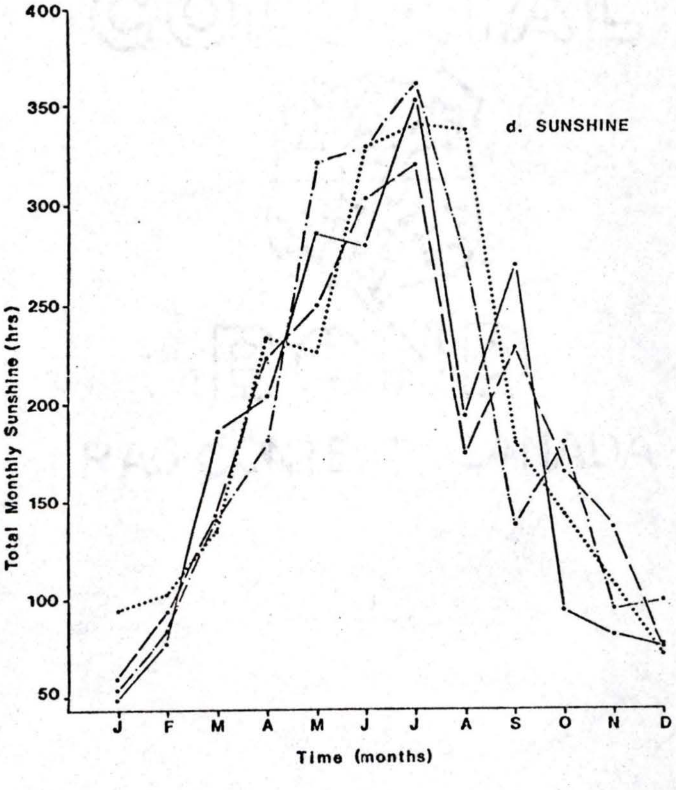
Graph 1


a WIND
Mean Monthly Wind Speed (Km/hr)
23
20
15
10
J F M A M J J A S O N D
Time (months)

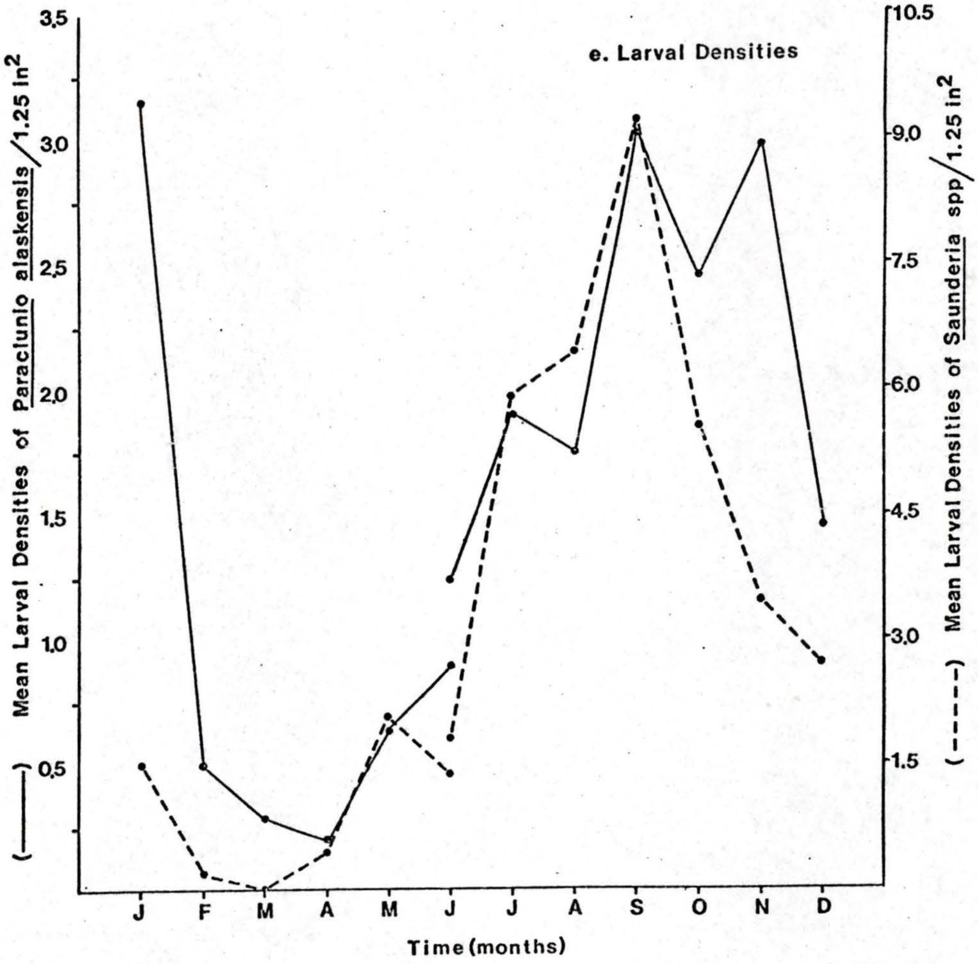

b. TEMPERATURE
Mean Monthly Temperature (°C)
18
15
12
9
6
3
J F M A M J J A S O N D
Time (months)

Graph 1


C. PRECIPITATION
Total Monthly Precipitation (mm)
180
150
120
90
60
30
0
J
F
M
A
M
J
J
A
S
O
N
D
Time (months)


d. SUNSHINE
Total Monthly Sunshine (hrs)
400
350
300
250
200
150
100
50
J
F
M
A
M
J
J
A
S
O
N
D
Time (months)

Graph 1



(Morley and Ring, 1972b)

II Ionic Regulation

a. Hemolymph

Sodium ions accounted for most of the cation contribution to the hemolymph osmotic pressure (Tables 2 - 5). Larvae are capable of regulating the concentrations of Na^+, Mg^{++} and Cl^- over a range of salinities from freshwater (20 mOsm) to full strength seawater (approximately 900 mOsm). In each instance, the levels of ions in the hemolymph were maintained hyposmotically in seawater and hyperosmotically in freshwater as Graph 2 illustrates.

Larvae survive in freshwater for the same length of time (several weeks) as larvae reared in seawater under comparable conditions (15°C, steady supply of oxygen, no food) but appeared to be unable to moult or pupate. No other signs of stress were evident.

The line joining the points indicating hemolymph levels has been estimated to be rectilinear, although, with further measurements, deviations might result. The levels of Na^+, Mg^{++}, and Cl^- in the hemolymph of larvae kept in freshwater and seawater were 101 mM, 8 mM, 61 m-eq/1 and 246 mM, 14 mM and 127 m-eq/1, respectively. Ionic levels for seawater and freshwater rearing solutions are also listed in Table 6 .

b. Osmolarity

Over a range of 878 mOsm, the larvae of P. alaskensis could regulate the hemolymph ionic concentration to within 107 mOsm. Table 5 shows that, in a freshwater solution (19 mOsm), hemolymph osmolarity of larvae was 417 mOsm whereas in a seawater solution (897 mOsm) hemolymph osmolarity of larvae rose slightly to 524 mOsm. The slope of the regulation line is shown in Graph 2.

Table 2. Concentration of sodium ions in hemolymph of *P. alaskensis* reared in freshwater (15 mM NaCl) and in seawater (450 mM NaCl).

Sample		n	µg/ml of sample	dilution factor (x)	µg/ml hemolymph	mM
Seawater larvae	1	29	2.53	2000	5060	220
	2	19	2.07	2666	6745	293
	3	20	3.20	2000	6400	278
	4	40	2.25	2000	4500	195
						$\overline{X}$ = 246.5 ± 23.2
Freshwater larvae		18	2.20	2000	4400	191

Table 3. Concentration of magnesium ions in hemolymph of *P. alaskensis* reared in freshwater (15 mM NaCl) and in seawater (450 mM NaCl).

Sample		n	µg/ml of sample	dilution factor (x)	µg/ml hemolymph	mM
Seawater larvae	1	15	.185	1923	356	15
	2	26	.130	2000	260	11
	3	20	.160	2336	379	15
	4	40	.200	2000	400	16
						$\overline{X}$ = 14.2 ± 1.1
Freshwater larvae		18	.100	2000	200	8

Table 4. Concentration of chloride ions in hemolymph of *P. alaskensis* larvae reared in freshwater (0.1 m-eq./L Cl^-) and seawater (500 m-eq./L Cl^-).

	m-eq./L Cl^- in hemolymph	
Sample	of larvae in freshwater	of larvae in seawater
1	69.51	121.95
2	107.52	134.19
3	89.74	94.72
4	64.28	112.99
5	51.28	102.08
6	65.85	139.03
7	67.92	154.86
8	37.00	145.16
9	56.00	125.00
10	72.00	141.02
11	28.50	139.03
12	21.20	110.00
$\overline{X}$	60.90 ± 7.07	126.67 ± 5.37

Table 5. Osmolarity of hemolymph of *P. alaskensis* larvae reared in freshwater (19 mOsm) and seawater (897 mOsm).

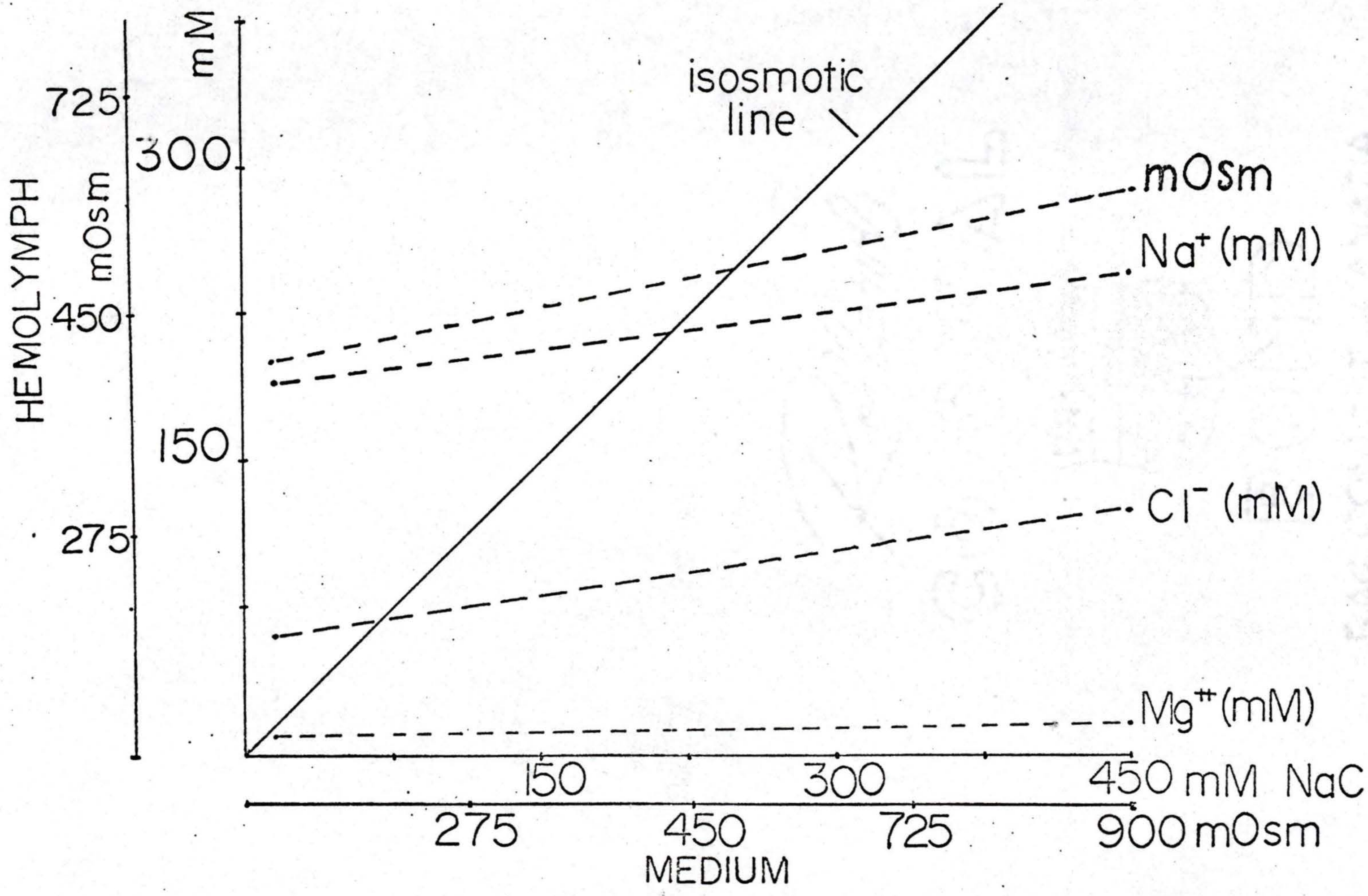
	Osmolarity of hemolymph in mOsm / L	
Sample	larvae in freshwater	larvae in seawater
1	451	645
2	519	683
3	521	625
4	527	744
5	370	583
6	370	640
7	370	640
8	370	583
9	390	473
10	415	473
11	400	732
12	310	553
13		629
14		500
15		624
16		516
17		452
18		430
19		450
20		440
21		450
22		430
23		345
24		325
25		305
$\overline{X}$	417.7 ± 20.2	530.6 ± 24.8

Table 6. Osmolarity of solutions used to rear larvae and solutions from habitats of larvae

	Osmolarity of solutions (mOsm/L)				
				Drains[a]	
Sample	Freshwater	Seawater	#1	#2	#3
1	16	891	233	404	381
2	23	895	238	411	371
3	24	896	238	407	369
4	13	895	232	412	384
5	21	897	230	411	373
6	22	900	261	530	
7	22	896	251	533	
8	16	903	251	531	
9	19	903	249	533	
10	16	897	249	530	
11		887	254		
12		887			
13		905			
		898			
$\overline{X}$	19.4	897.3	268.6	470.2	376.5
s.e. ±	1.2	1.4	3.1	20.4	2.9

[a] see Figure 5

Graph 2. Osmoregulation curves for P. alaskensis larvae: Na^+, Mg^{++}, $Cl^-$and osmolarity.



III Osmotic Regulation

a. % Body Water

The larvae used in dry/wet weight studies were collected during a five month period. The months covered a spectrum of conditions in the intertidal zone from hot, sunny early fall months when larvae densely populate the shore to cold, rainy winter months when densities are low. Also, the tide is lowest during the day in the fall and gradually shifts to the night throughout winter. P. alaskensis larvae averaged 71.7% water over the five month period. There is no statistically significant difference between months (Table 7 Graph 3).

b. Water Regulation

The ability of P. alaskensis larvae to survive and regulate in a wide range of salinities is excellent. All larvae in both freshwater (FW) and seawater (SW) at 15°C survived for the full 48 hours of the experiment and, afterwards, continued to remain healthy for several weeks. Larvae were observed to moult and pupate in seawater but not in freshwater. In concentrated seawater (Conc. SW), larvae also survived for 48 hours, but 25% mortality occurred after 72 hours. In Ca^{++}-free seawater (Ca^{++}-free), all larvae were alive after 48 hours but were noticeably flaccid. Mortality rates reached 100% after 92 hours in Ca^{++}-free seawater.

The ability of P. alaskensis larvae to withstand desiccation in air is poor. Larvae decreasing their weight by more than 50% could not survive. Mortality in Experiment A after 48 hours was 50% and in Experiment B after 24 hours was 66%.The higher value experienced in Experiment B was due to an experimental error of a lowering of the temperature. Larvae lost water when exposed to air at a rate of 0.152 mg/hr.

Graph 4 indicates the weight change averaged from groups of larvae (n = 8,6 or 4) weighed at specified time intervals. Table 8 summarizes

Table 7. % Water Content of whole *P. alaskensis* larvae

Date	n	Wet weight (mg) $\bar{X}$	Dry weight (mg) $\bar{X}$	% water content $\bar{X}$ ± S.E.
Sept 1/77	21	5.34	1.27	75.9 ± 0.87
Sept 17/77	24	3.51	1.00	69.5 ± 2.43
Oct 26/77	27	2.79	0.87	68.4 ± 1.79
Dec 10/77	14	1.30	0.38	69.1 ± 2.87
Jan 7/78	30	2.64	0.11	74.5 ± 1.05
Jan 13/78	22	1.87	0.86	72.8 ± 2.20
				$\bar{\bar{X}}$ = 71.7

A single classification analysis of variance test, using percent water content values, showed that no statistically significant difference existed among groups. The value, Fs, was calculated to be 1.064 and $F_{.05(5,126)}$ is equal to 2.29 (Sokal and Rohlf, 1969).

Graph 3. Wet weight, dry weight and % water of P. alaskensis larvae collected from late summer (1977) to winter (1978).

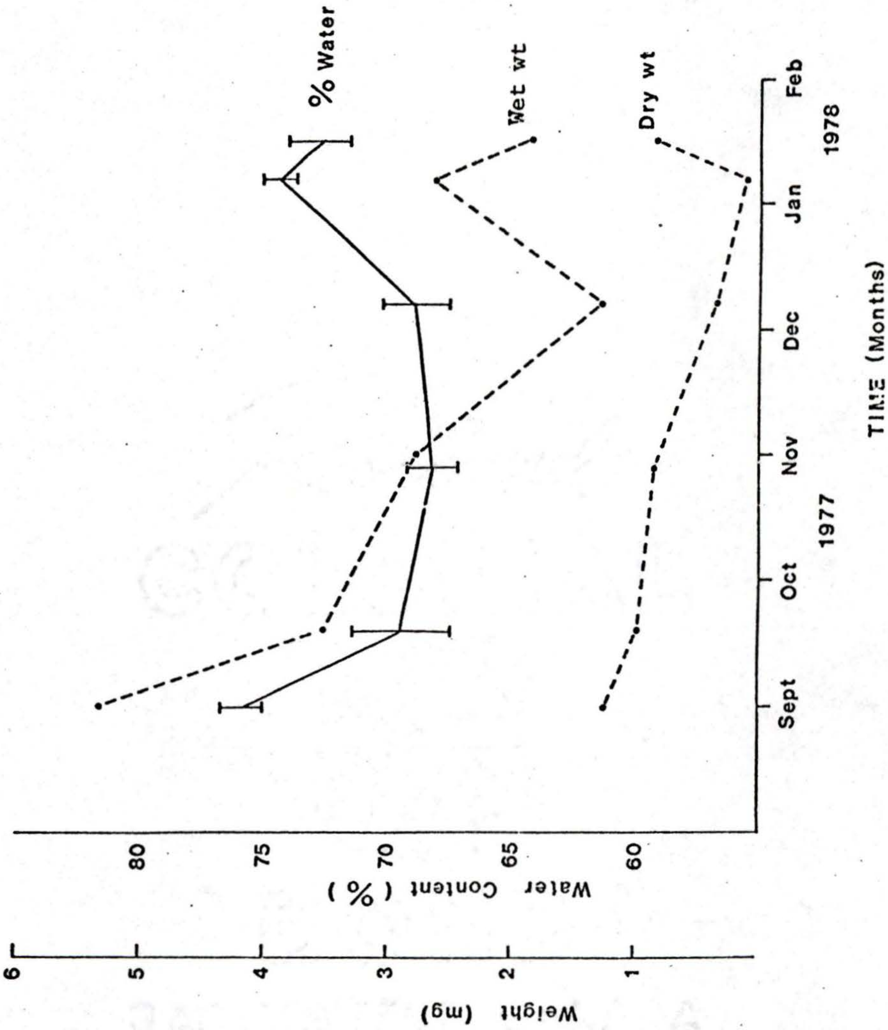
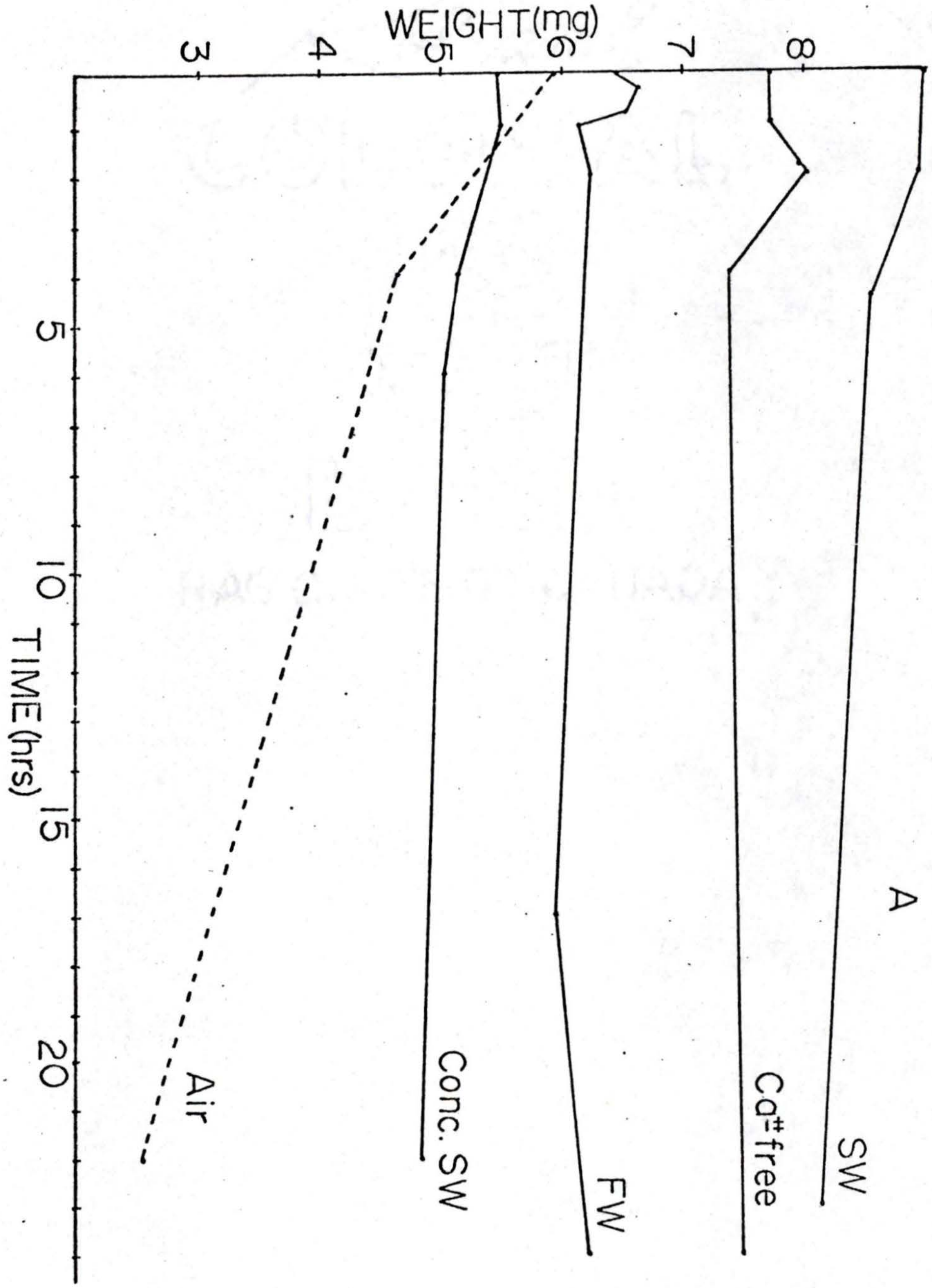


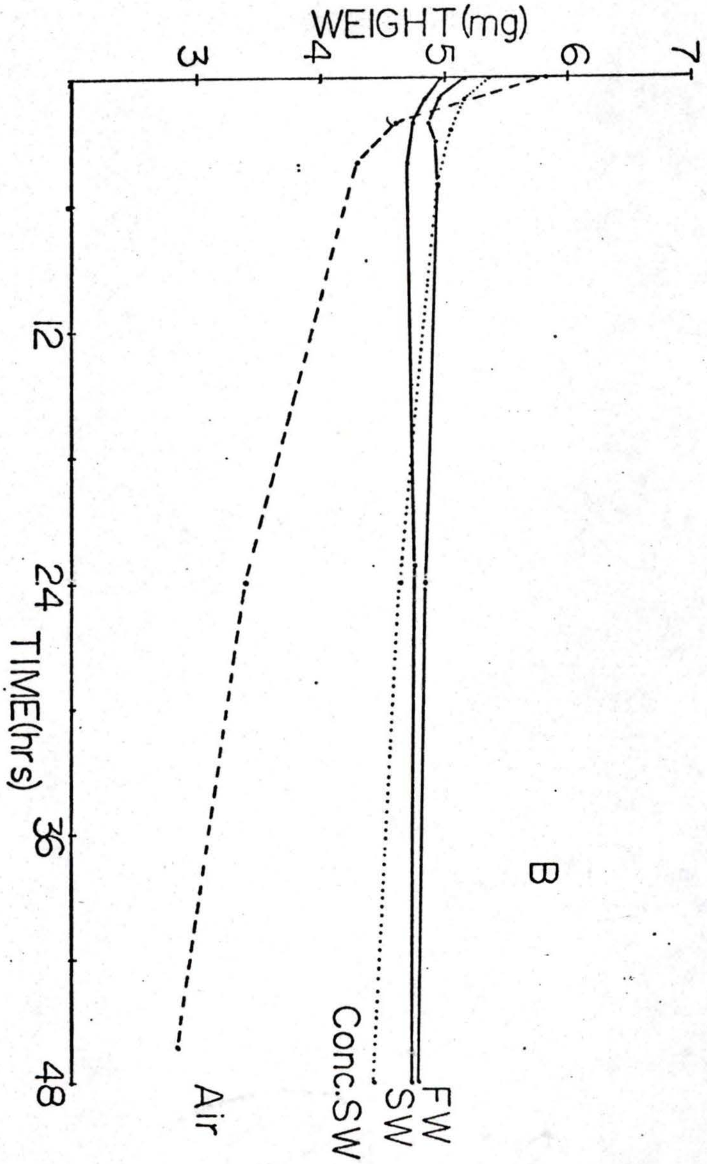
Table 8 . Water Regulation of *P. alaskensis* larvae in various media for 48 hours

		Statistical analysis (Exp. A)				
Solution	% weight lost Exp. A (n=8)	F_s	$F_{(0.5)df}$	Probability	Significant?	% weight lost Exp. B (n=4)
FW	10.47	14.77	1,5=6.61	>0.0121	significant	7.3
SW	0.77	0.06	1,7=5.59	>0.8084	NOT significant	4.8
Conc. SW	15.17	61.04	1,7=5.59	>0.0001	highly significant	16.4
Ca^{++} free	7.72	11.98	1,5=6.61	>0.0180	significant	
Air	56.68	24.84	1,5=6.61	>0.0042	highly significant	50.6
Anova between solutions		3.43	3,24=3.01	>0.032	significant	

Graph 4a. Weight change of P. alaskensis larvae over 24 hours in seawater, freshwater, concentrated seawater, calcium-free seawater and air (Experiment A).



Graph 4b. Weight change of P. alaskensis larvae over 48 hours in seawater, freshwater, concentrated seawater and air (Experiment B).



the data and the results of a two way analysis of variance between and among solutions. The raw data are included in Appendix D .

IV Supercooling

Larvae have an average supercooling temperature of -11.48°C. The temperature measurements were taken from larvae collected over the same time period as those in Section 3 (Water Content). There was no difference in the supercooling points of larvae collected between September and January. The results are summarized in Table 9 and Graph 5. Larvae did not survive freezing.

V Respiration Rate

P. alaskensis larvae do actively respire when submerged in seawater at 15°C (Graph 6 and Appendix F). A respiration rate of 0.032 ± 0.0146 $\mu g O_2$/ mg dry wt./hr. indicates that larvae are not simply tolerating submergence and then resuming active respiration during low tides.

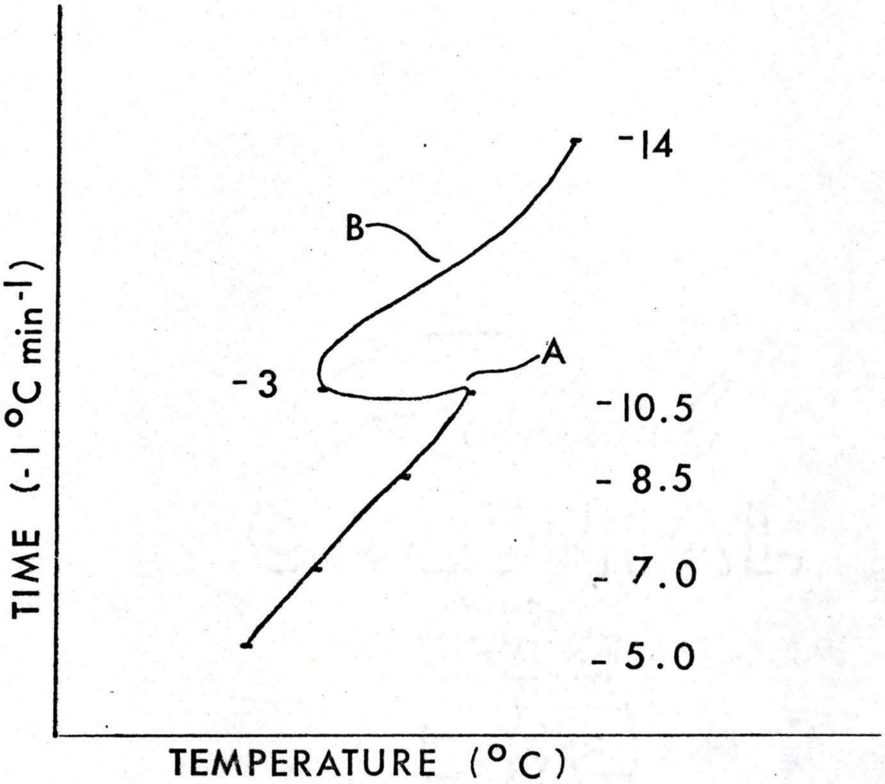
Graph 6 shows the change in pO_2 over time. The slopes of lines A, C, E and F are steeper than those of lines B and D because of the static nature of the sampling procedure. Each time a container is sampled, there is less water left in the container. As a result, the ratio of larvae to available oxygen is increased and, therefore, the rate of O_2 consumption will appear to increase. The respiration rate appears to be higher in those containers A, C, E and F where water samples were taken frequently in a short period of time. The respiration rate of lines B and D is a more accurate measurement since the ratio of larvae to available oxygen was held more constant over a length of time.

The overall respiration rate was calculated for the first injected sample only. Subsequent pO_2 values are shown in Graph 6 but they are not true indicators of an overall respiration rate. A sample of the calculations

Table 9. Supercooling temperatures of whole *P. alaskensis* larvae and water content of larvae expressed as per cent weight change

Date	Larva #	Supercooling Point (^{o}C)	% water content (Wet-dry wt x100)
Sept. 1/77	1	- 8.5	72
	2	- 8.5	74
	3	-10.7	79
	4	-11.5	62
	5	-11.5	78
		$\overline{X}$ = -10.1 ± 0.68	
Sept. 17/77	1	-12.5	65
	2	-14.5	63
	3	-20.0	62
	4	-12	60
	5	-12	73
		$\overline{X}$ = -14.2 ± 1.52	
Oct. 26/77	1	-11.5	62
	2	- 8.5	63
	3	- 7.7	66
	4	-14.5	68
	5	-12.7	58
		$\overline{X}$ = -10.9 ± 1.27	
Dec. 10/77	1	- 8.5	
	2	- 8.5	
	3	-11.5	
	4	- 9.75	
	5	-12.0	
		$\overline{X}$ = -10.1 ± 0.73	
Jan. 7/78	1	-16	77
	2	-11	78
	3	-11.5	83
	4	-10	74
	5	-10	69
		$\overline{X}$ = -11.7 ± 1.11	
Sept. 9/79	1	-13	
	2	-10.5	
	3	-7	
	4	-11.5	
	5	-16	
	6	-14.5	
	7	-11.5	
	8	-12	
		$\overline{X}$ = -12.0 ± 1.61	
		$\overline{\overline{X}}$ = -11.48 ± 1.61	

Graph 5. Supercooling curve of a larva of P.alaskensis.



A = supercooling point

B = freezing plateau

(see page 77 for explanations)

which convert readings of $mmHgO_2$ to $\mu l\ O_2$/mg dry wt./hr. is found in Appendix F .

The use of the Blood Gas Analyzer to measure the respiration rate of small organisms is useful but the static nature of the closed syringe system has many inherent problems.

VI Labelling

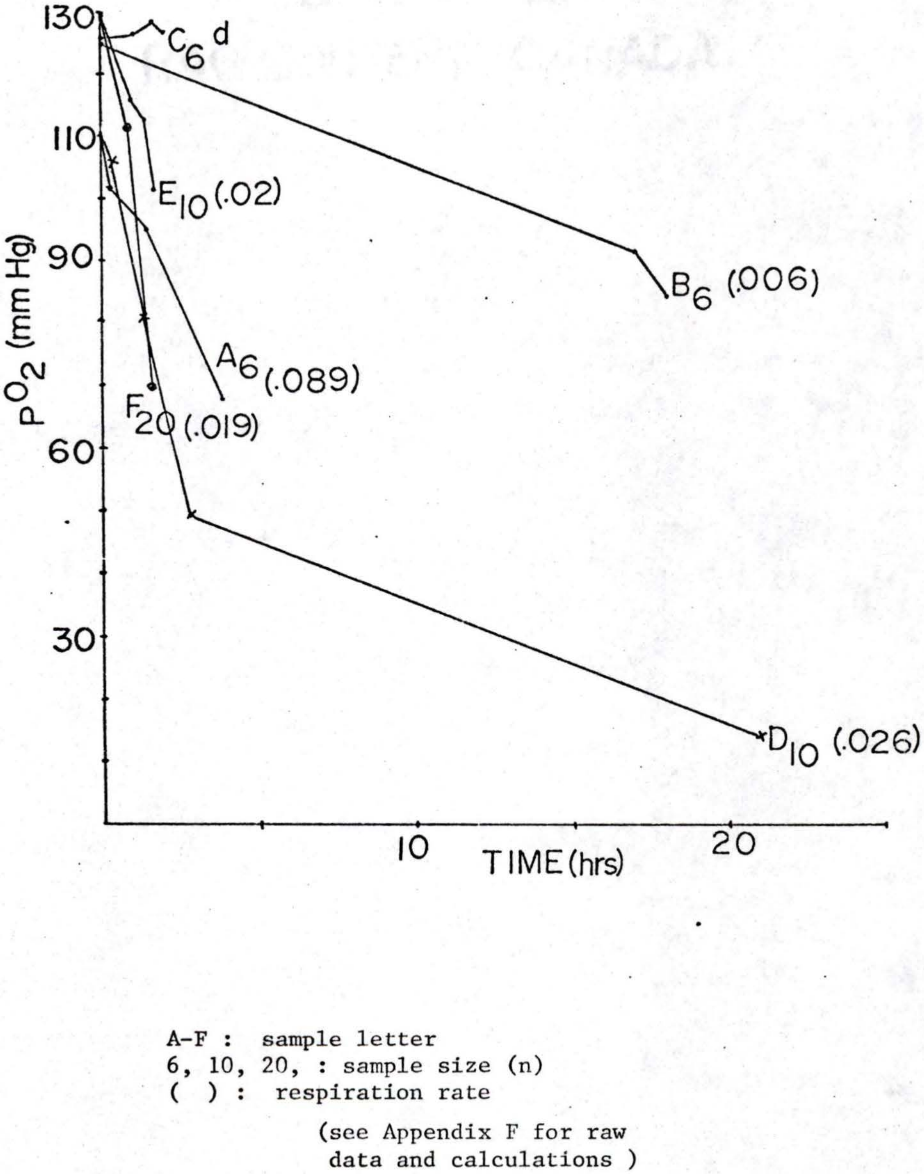
a. Autoradiography

All sections of P. alaskensis larvae showed the presence of silver grains indicating that d-glucose-H^3 had been incorporated into the tissues. Labelled glucose was most concentrated in the muscle and gut tissue. Since the level of glucose in the test solution closely approximates that of sea-water (10^{-8}M), then it can be inferred that P. alaskensis larvae may supplement their nutrient requirements with dissolved organic matter (DOM). Figure 8 illustrates the areas which concentrate glucose.

b. Isotope counts

Larvae take up glucose at a measurable rate (Graph 7). This can be translated into an approximate drinking rate. The concentration of ^{14}C-d-glucose was 4.6×10^{-8} M (specific activity 50.03 mCi/mM). A maximum accumulation of 3190.5 dpm over 24 hours occured. The drinking rate can be calculated to be 26 nl $larva^{-1}hr^{-1}$ (Appendix G). This is assuming a constant rate over the 24 hours. Graph 7 indicates that a slight change in the rate did occur but the reasons for this are unknown. The rate is comparable to that of Aedes taeniorhynchus which drinks 92 nl $larva^{-1}hr^{-1}$ (Bradley & Phillips, 1975).

Graph 6. Respiration rate of <u>P</u>. <u>alaskensis</u> larvae.



A-F : sample letter
6, 10, 20, : sample size (n)
( ) : respiration rate

(see Appendix F for raw data and calculations)

Graph 7

Uptake of radioactive glucose
by *P. alaskensis* larvae

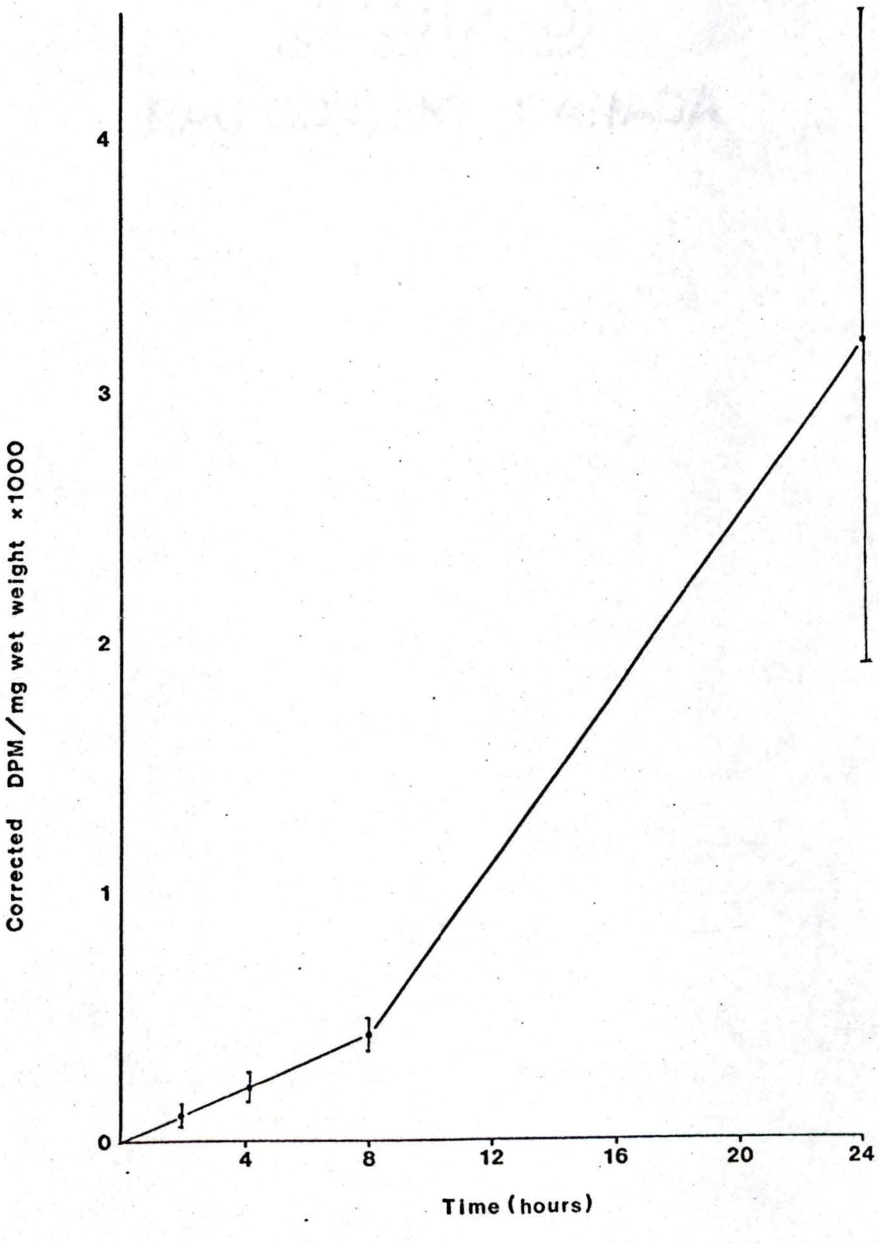
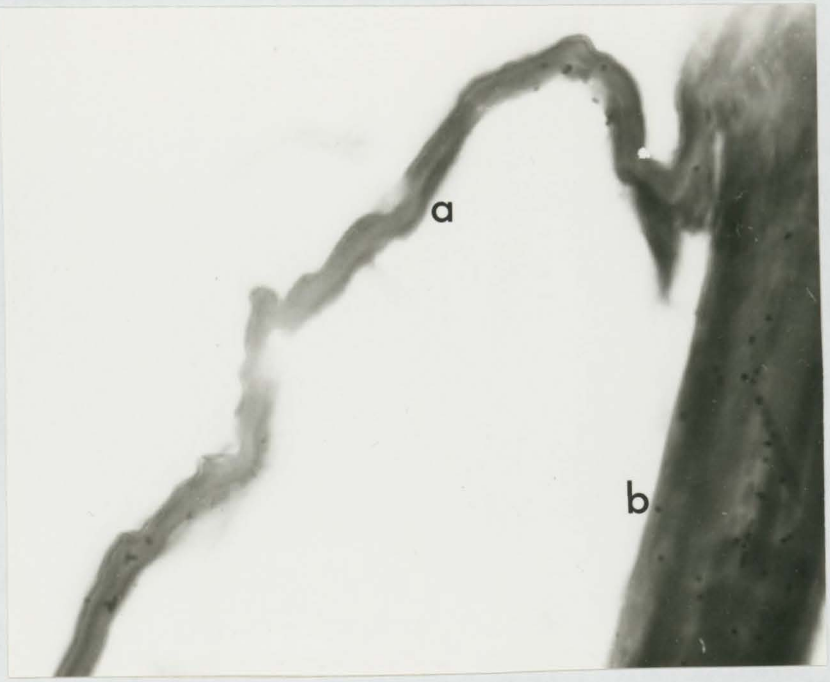

Corrected DPM/mg wet weight ×1000
0
1
2
3
4
4
8
12
16
20
24
Time (hours)

Figure 8

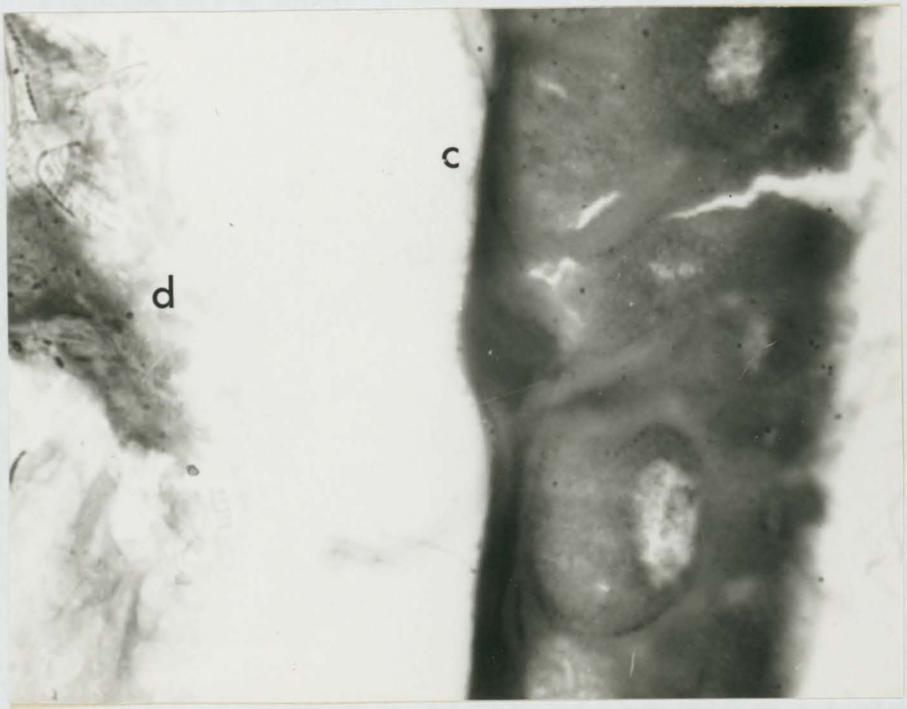
Labelled sections of P.*alaskensis* larvae showing uptake of glucose

a. Section of cuticle (a) and muscle tissue (b).

b. Section of digestive tract wall (c) and contents (d).



a



b

DISCUSSION

Field Observations of P. alaskensis Larvae

Over the past four years, the field observations of P. alaskensis larvae and the surrounding ecosystem have been of great assistance in the understanding of the adaptations of larvae to the intertidal habitat. But, at the same time, the interpretation of these field observations has required a comprehensive understanding of the nature of the intertidal zone itself. Marine ecology is a subject of long standing and many researchers have endeavoured, over the years, to resolve the dispute pertaining to the cause of intertidal zonation, including diversifications which occur spatially and temporally (Carefoot, 1977; Chapman, 1946; Ricketts and Calvin, 1963; Stephenson and Stephenson, 1972; Widdowson, 1959). No single event or factor has been shown clearly to regulate zonation. Numerous factors and their effects on zonation have been reported and, to date, all evidence indicates that it is a combination of these factors which controls zonation. Understandably, the difficulties involved in isolating and testing the effect of a single factor on zonation, or for that matter on a single organism such as P. alaskensis, are considerable. Therefore, the purpose of the following discussion is to determine the combination of factors which appear to be controlling spatial and temporal patterns observed at the seawall and to determine any predominant and limiting factor(s) to which P. alaskensis have adapted which enables them, as an insect, to survive in the littoral zone.

Environmental Factors and the Habitat - A strikingly seasonal event is observed to occur at the Ogden Point seawall. Each winter the algal layer diminishes considerably, losing also its thick, sandy base. Similarly, the larval population declines in that area. In contrast, throughout the year, larvae continue to inhabit the moist areas below the drains and among the barnacles in relatively high abundance. As indicated in Graph 1 , the cyclic nature of variables such as sunshine, temperature and precipitation is obvious. The density of chironomid larvae increases as the temperature and hours of sunshine increase and as the amount of precipitation decreases. These three factors clearly correspond to and are probable causes of changes in larval densities.

More importantly, changes in larval densities appear to be dependent on the changes in the density of the algal layer. Chironomid larvae use the algal zone in many ways; as shelter against desiccation by the sun and from lack of moisture during immersion, as a three-dimensional substrate in which to anchor themselves against the force of waves and as a source of food. In culturing P. alaskensis in the laboratory, several of the above uses were apparent. For example, when cotton batting was added to a culture bowl, larvae built more tubes and survived much longer than they did without a three-dimensional substrate. In the field, larvae are rarely found in Ulva sp. or Monostroma sp. which are too foliose and provide little cover and few niches for tube construction (Morley and Ring, 1972b). Quantified tests would be necessary to prove this observation. Larvae feed preferentially on diatoms (Fig. 8) and can also be reared successfully on algae. Bardach et al (1972) cite an example of the grazing effect of a freshwater population of the chironomid Tendipes longilobus which consumes benthic algae at an estimated rate of 60-90 Kg/day. The effect of P. alaskensis in the intertidal food web has not as yet been quantified. One last use of the algal layer by chironomid larvae, that of preventing desiccation, can be partially substantiated by the fact that larvae desiccate rapidly (0.15 mg/hr) and can survive only a 50% weight loss which will occur within 24 hrs. under normal atmospheric conditions at 15°C. As larvae are found at least 90% of the time within the algal layer or in the moist drain areas, it appears likely that the effect of desiccation is a major determining factor in their distribution on the seawall. The importance of desiccation will also be discussed under the section on respiration.

To return to the interpretation and understanding of environmental factors, one factor, wind, does not appear to be as clearly seasonal as the other factors monitored. An analysis of the wind speed data (Graph 8) ? shows that, consistently, over the four year period, February, July and December were each year one of the six windiest months. August, September and October were consistently one of the six least windiest months. All other months varied randomly over the four years. The density of *P. alaskensis* larvae in 1969-70 were correspondingly high in September, October and November and low in February, March and April. A correlation between wind and larval densities is further substantiated by wind speed data for 1969-70 (not indicated).

The months again could be sorted into consistently high and low wind months corresponding to spring and fall seasons. Several points do tend to support the hypothesis that wind is a determining factor in the abundance of *P. alaskensis* populations. For example, Morley and Ring (1972b) found that of the four types of shore habitats studied, chironomid species were noticeably reduced in number on the most exposed shores. As a winged insect, high wind and wave action would be detrimental to the mating success of the adults, thus decreasing the density of larvae to be found the following months. Similar effects have been documented in freshwater chironomids in Iceland where successful breeding occurs only on "dead calm days" (Lindegaard, 1979). On examining Graph 1, the density of *P. alaskensis* larvae does appear to Pg 29 follow, with an appropriate lag time, the seasonal fluctuations in wind intensity.

After having examined the seasonal nature of several environmental factors and accepting the hypothesis that the larval stage of P. alaskensis is dependant on the seasonal cycles of the algal layer, the question arises as to how the environmental factors affect the algal zone. As with other organisms in the littoral zone, the distribution of algae is not determined by a single factor but by a combination of factors. Widdowson (1959) described the vertical distribution of algae at twenty-six stations extending from Victoria to Port Renfrew. Included in his work are tentative correlations of the observed distribution with conditions monitored in the environment. The final analysis of his thorough investigation is that a combination of environmental factors is responsible for limits in distribution of marine algae. Several previous investigators have attempted to demonstrate the importance of one factor over all others in limiting distribution. Widdowson (1959) reviews the investigations into the effects of such environmental factors as wave exposure, tidal amplitude, exposure to drying, salinity, temperature, and intensity and spectral quality of sunlight. Recent studies also indicate that the settling rates of larval stages of intertidal organisms are strongly correlated with environmental factors and may determine not only the vertical distribution but also the seasonal variations in population densities (Osman, 1977). Field studies of algae continuously monitored over years are rare but noticeable seasonal variations have been observed in the smaller and higher forms of algae (Widdowson, 1959).

Considering specifically the observations of algae at the Ogden Point seawall, wave action in the winter storm months appears to have a scouring effect on the seawall. A winter berm builds up against the wall, raising the lower limits of the algal zone. Normally, in the summer, the algae will grow right to the sand/wall interface. In the winter, the algae, as well as occupying a narrower band, do not extend to the sand/wall interface. This implies that, with the scouring effects of the pounding waves containing sand, the delicate and filamentous greens do not survive. The survival rate of the gametes would also decline. The observations are supported by the fact that Enteromorpha linza is absent from exposed coasts (Widdowson, 1959) and that substrate disturbance has a marked effect on larval settling rates and therefore on the seasonal distribution of organisms (Osman, 1977). Enteromorpha linza is known to tolerate 84% water loss and some freezing (Kanwisher, 1957). Enteromorpha linza also lives indefinitely in freshwater if the bacterial count is kept low (Muenscher, 1915). From this, one would expect a growth of algae in the drain areas throughout the winter if wave action was not having some effect. In calmer areas of Victoria, Enteromorpha linza will survive throughout the winter (Widdowson, 1959; personal observations). Ogden Point is situated in Juan de Fuca Strait (Fig. 4) and can be expected to be exposed to violent winter storms coming from the open ocean. The wave action at other times of the year would be less than that of an unprotected coast.

Population dynamics of P. alaskensis - As stated by Morley and Ring (1972b), little is known about population fluctuations in intertidal chironomid larvae. P. alaskensis appears to be multivoltine due to the fact that all four life stages are present, in varying densities, at any given time during the year. This needs to be quantified more exactly. Adults are most abundant during the fall and early winter (October, November, December)(Saunders, 1928; Morley and Ring, 1972b; personal observation). By mid-winter, the algal layer has diminished due to environmental factors such as wind and lack of sunshine as discussed previously. The decrease in available habitat has a detrimental effect on the survival of larvae hatching from eggs laid during large adult emergences in the fall. If this were not so, one would expect to be monitoring high densities of 2nd instar larvae after approximately three months and mass adult emergences after another 3-4 months. This is based on the fact that total development time for P. alaskensis is 204 days at 10°C (Morley and Ring, 1972b). For example, if adults emerge in September to lay eggs, one would expect emergences of adults again in March. Even if larval development is slower over the winter months, due to cooler temperatures, at least larval densities should be high in March. In fact, larval densities are lowest at this time of year. As the environment becomes more favourable (i.e. more food, shelter and calm for breeding), the survival rate of larvae increases and so does the population density. In short, the population of intertidal chironomids on the seawall is seasonal.

Two types of population fluctuations seem to be occurring throughout the year. One is a large seasonal fluctuation as described above, and the other is a series of small, frequent fluctuations owing to the multivoltine nature of the population. The fact that P. alaskensis recolonizes the algal zone as quickly as it does can be attributed to the small but continual emergence of breeding adults throughout the winter. The adults emerge, via the pupal stage, from larvae inhabiting "overwintering" sites, such as drain areas, crevices and the barnacle zone. These sites are similar to the algal layer in that they offer protection from storms and from the combined effects of cold and desiccation. The larvae in the overwintering sites constitute a "stock" population which enables the species to take advantage of increases in habitat availability under more favourable environmental conditions. Other species (Table 1) may also take advantage of the increasing habitat availability and thus a type of succession can be visualized on the seawall during the late spring months (April, May, June).

Insects in the intertidal zone have the inverse of the problem of dispersal as compared to other intertidal organisms. The adult is the dispersal agent. Consequently, currents, salinity and temperature, as they normally affect invertebrate populations, are not limiting factors in the distribution of an insect population. The ability of P. alaskensis adults to mate successfully and select egg-laying sites, as discussed previously, is highly dependent on more terrestrial-related conditions, such as wind. Dispersal may also, at times, be achieved by the larval form. Evidence of this was found when examining the seawall at low tide in the summer when larval densities were high. Approximately 10 larvae per m^2 could be seen lying on the sand at the base of the wall. Others could be made to drop off by lightly touching them with tweezers, as often happened when trying to collect them. Perhaps larvae are then washed to new areas of the seawall and, of course, perhaps they are lost. Foster and Treherne (1976) have commented on several species inhabiting streams which disperse, in part, by the larval form. Further testing of this hypothesis is required. More probable is the fact that displaced larvae may fall prey more easily to other organisms such as those observed in the subtidal survey (Table 1). High larval counts in the gut analyses of intertidal sculpins (Morley, 1971) and young salmon (Annan, 1958) have been reported. Trimble and Wellington (1979) have also demonstrated that site selection by ovipositing Aedes togoi mosquitoes is dependent on salinity. Tests such as these, applied to P. alaskensis, could assist in further explaining observed population dynamics.

The seasonal changes in the patterns of the tidal cycles must also be taken into account when considering the fluctuations of populations of P. alaskensis. Many marine organisms are known to have reproduction cycles corresponding to lunar cycles (Palmer, 1974). Neumann (1971) has shown that eclosion of an intertidal chironomid species, Clunio marinus, under controlled experimental conditions follows a lunar periodicity. Morley and Ring (1972b) have noted, at low tide, emergences of adults which occur high on the shore, subside and then occur again lower down. The vertical distribution of populations of P. alaskensis on the seawall appears to extend from 0.5 to 1.2 m.

Below this, larvae can be found at 0.6 m on rocks protruding out of the sand. Since no other studies on the vertical distribution of chironomid larvae have been completed, no comparisons can be drawn. Leader (1976) mentions briefly that divers have found marine caddisfly larvae (Philanisus plebeius) subtidally but feels that they were carried there accidentally by currents. Assuming that larvae do not migrate and that larvae develop and pupate at a level on the shore rarely exposed except at extremely low tides (e.g. 0.2 m), then these larvae can emerge as adults only at a tide of an equally low level. Since the lowest tides occur in June, July and August, then mass adult emergences from these areas may contribute to the exponential increase in the larval population in the ensuing months. Again, as environmental factors are favourable during this time, the survival rate of eggs and larvae is high, unlike that which occurs in the winter. Equally low tides occur in November, December and January which would expose the lower populations after approximately the necessary development time of 200 days and allow successful eclosion to occur. But do these adults then survive and mate successfully? Perhaps, with a few calm December nights for breeding, a large percentage of larvae will begin to develop. This could be a possible explanation for the increase in larval density observed in January 1970 (Graph 1) which otherwise remains uninterpreted except on the basis of a particularly clumped sample. The larval population drastically declines after this time due to environmental factors as described earlier. Overall, the population of P. alaskensis appears to be both seasonally determined and synchronized with the tides.

Seasonal variation occurs between summer and winter populations of larvae. Winter larvae are smaller (Morley and Ring, 1972b; personal observation) and were not used in this study for physiological tests because sufficient hemolymph could not be obtained from them. Densities of larvae were low in the winter months, except in drain areas where a thick growth of ooze provided shelter, moisture and some nutrients. Food, in the winter months, probably does not occur in sufficient quantities to support the grazing rate of the population and this may in turn limit the growth rate of individuals. Hashimoto (1968) has thoroughly described seasonal variation of body length in the intertidal chironomid Clunio tsushimensis and also cautions that one has to keep in mind differences in size and pigmentation between summer and winter generations of larvae in taxonomical studies. The growth rate of larvae slows down below a critical temperature of 8-10°C, and pupation, as well as emergence is stopped. A change in photoperiod has no effect and Müller (1970) has named this phenomenon thermal quiesence. P. alaskensis possibly undergoes a form of this phenomenon and this will be discussed in the section on temperature adaptation.

The hatching and settling patterns of 1st instar larvae have not been thoroughly investigated. Upon what limiting factor(s) is their survival dependent? Wave action, temperature and habitat availability are each potentially limiting and experimental work in this area would shed much needed light on the underlying causes of the fluctuating population dynamics of P. alaskensis.

Once larvae have successfully settled, they are well adapted in body form to cope with the intertidal zone. They have well developed pseudopods ringed with hooks (Fig. 3a) unlike species of freshwater chironomid larvae (Oliver, 1971). The hooks readily catch and tangle on any filamentous substance, such as Enteromorpha sp. or cotton batting, making their removal difficult. Later in the life cycle, the larvae build tubes. The tubes do not noticeably vary in size, indicating that early instar stages do not build tubes. It is possible that sufficient anchorage is available in the substrate for the small larvae but that increased size requires better anchorage methods. Late instar larvae feed from the tubes as well as using them to pupate in later on. The larvae use the posterior pseudopods to hold the posterior end of the body in the salivary tube while the anterior end carries out the feeding activities. The head of a feeding larva moves in a vertical motion while it scrapes diatoms and algae from the substrate. Larvae have been observed to reach the anterior and posterior pseudopods with their mouthparts and to clean particles from the hooks. If larvae are touched with a probe, they react violently, twisting and biting at the disturbance. When anchored in a tube and disturbed, they are quick to retract into the tube. Tubes can only be used by one larva because once the larva has pupated, the exuviae are left in the tube upon emergence. An incident of territoriality was clearly observed between two larvae, in which a free-crawling larva approached another larva in a tube and tried to enter into the tube several times. The larva in the tube at the time displayed violent twisting and snapping motions towards the intruder and eventually succeeded in fending off the intruder. This may be an example of territoriality between larvae.

Tubes do not remain on the wall for long once the adult has emerged. This may be further evidence of the scouring action of waves during the winter months which removes tubes built by larvae prior to large fall emergences. The tubes may also be used to assist in the respiration process of submerged larvae, since underwater observations indicated that the tubes were silvery as if filled with air.

Another dipteran of the family Tipulidae also inhabits the midlittoral zone. The population appears to have a pronounced seasonal cycle. A stock population does appear to overwinter in the same areas as P. alaskensis although in very low numbers. Also interesting is the fact that the larva is a typical cyclorrhaphous form, with no pseudopods or hooks and piercing stylet mouthparts. Nothing is known about the ecology of this species.

In summarizing the adaptions of P. alaskensis larvae to the intertidal zone, the key feature of their success can be attributed to their ability to inhabit protected niches in the algae, in ooze, in crevices and between barnacles. From this they can survive, within limits, the effects of both desiccating factors experienced at low tide and wave action. They are limited then in their geographical distribution as determined by wave exposure. They are also limited by the seasonal variation in the algae upon which they depend for shelter. The population dynamics studied indicate that seasonal cycles, correlated with environmental factors, are acting to control the population density. Superimposed on this cycle are small but continuous emergences of adults from stock, overwintering larval populations. The success of P. alaskensis in the intertidal zone can also be attributed to the fact that adults emerge and disperse in small numbers throughout the winter, therefore rapidly recolonizing areas as soon as the habitat can support larvae. This gives the population flexibility which is very necessary in the intertidal system. Adult emergences are also timed by lunar cycles.

Food chains and competition are still far from completely understood, but as underwater studies show, various predators not evident at low tide may be affecting the population at high tide.

Lastly, larvae have well developed anatomical features for anchoring and feeding, such as hooks on the pseudopods and heavy sclerotized jaws for scraping up detritus. Tubes are an important adaptation which serve to protect the larva, anchor the pupa and provide a means of leverage for the emergence of the adult.

Physiological Aspects

A species may adapt to one or more environments by suitably conforming to or regulating one or more of its life processes. Behavioural adaptations, structural variations and life cycle modifications are a few of the types of adjustments which can be found within a species or group of organisms inhabiting a spectrum of environments. To analyze possible adaptations observed in an organism, one must necessarily compare them to other organisms, either of the same species inhabiting other environments or perhaps different, but closely-related species inhabiting the same environment. Broad comparisons between phyla are also valuable in phylogenetic discussions.

The physiological processes investigated in this study of P. alaskensis larvae were selected over others because of the possible significance that these processes may play in adaptations to the intertidal habitat. The significance of these processes is based on both observations made in the field and disputes recurring in the literature.

The physiological processes examined will be discussed under the following headings: (i) Ionic and Osmotic Regulation, (ii) Temperature Adaptation and (iii) Respiration.

To determine the adaptive abilities of P. alaskensis larvae, comparisons will be made both with other chironomids in different environments and with other insect species in general. Finally, the original hypothesis, that the degree of regulatory ability may be ultimately responsible for the survival of this species in the intertidal zone, will be argued.

I. Ionic and Osmotic Regulation

The focus of this section is on the well-developed ability of P. alaskensis larvae, as shown in the laboratory and field, to tolerate a range of external environments varying in salinity (tapwater, seawater, concentrated seawater) and in composition (seawater, Ca^{++}-free seawater). The ability of larvae to withstand water loss is poor and this is also discussed.

The osmoregulatory abilities of organisms have been examined by researchers for many years. With the advent of increasingly sophisticated microtechniques, these studies can now be performed on smaller organisms and on fluids from individual organs. The amount of literature available from which comparisons among organisms can be made is enormous. Some terms have emerged which apply to common osmoregulatory abilities of organisms and these will be followed. Among the references used in this study, the concentration of fluids has been measured in a variety of units, differing from researcher to researcher. A conversion chart (Appendix D) was used to standardize units to facilitate comparisons among species. The unit chosen for all species was mM (for ions) and mM NaCl (for solutions). Therefore, m-eq./L, g % and mOsm, in most cases have been converted to mM NaCl .

a. Regulator or Conformer ?

According to the terminology of several major reviews (Bayly, 1972; Krogh, 1939; Potts and Parry, 1964; Shaw and Stobbart, 1963), P. alaskensis larvae are euryhaline and are regulators, that is, the larvae can survive in a large range of salinities and do so by maintaining the osmotic pressure of the internal body fluids at a near constant level throughout the environmental changes. According to the terminology of Bayly (1972), P. alaskensis larvae are both hyper- and hyposmotic regulators, the isotonic point being 475 mOsm. As Graph 2 indicates, the osmotic pressure of the internal medium (P_i) of P. alaskensis larvae is maintained within 107 mOsm over a 874 mOsm change in the osmotic pressure of the external medium (P_o). Where $\Delta P_i/\Delta P_o$ approaches zero, a high degree of regulation (R_o) is indicated (Shaw and Stobbart, 1963). Of several salt-water dipteran larvae examined by Sutcliffe (1960), the mean values of $\Delta P_i/\Delta P_o$ were found to lie between 0.03 and 0.10, with the exception of the only chironomid larvae examined (*Chironomus salinarius*) which had a higher mean value of 0.20. The mean value of R_o for *P. alaskensis* larvae is 0.12, only slightly above the range of the strong regulators examined. It is important to note that, of the dipterans examined by Sutcliffe (1960), none respires through the cuticle except *C. salinarius*. For example, *Aedes detritus*, *Cricotopus vitripennis* and *Ephydra riparia* have relatively impermeable cuticles and respire via air tubes or specialized plastrons (Hinton, 1967; Sutcliffe, 1960). Chironomid larvae are apneustic and require a more permeable cuticle in order to respire, a theory suggested by Hinton (1976) which will be discussed further under the section on respiration. More energy expenditure would be required to maintain a low value of R_o when cuticle permeability is high.

Insects restricted to only freshwater have a highly permeable cuticle and high R_o values indicating that the mechanisms of osmoregulation are not adapted to cope with high salinities. Freshwater larvae cannot tolerate high blood concentrations of ions, as can some brackish water conformers. The mechanisms of cell tolerance are not yet understood (Shaw and Stobbart, 1962).

In brackish water dipteran larvae, classified as conformers, P_i is not well regulated but the insect may survive with high blood concentrations up to a certain point (isosmotic point) beyond which P_i increases rapidly and the insect dies (Bayly, 1972; Foster and Treherne, 1976).

P. alaskensis larvae occupy a position close to that of true regulators but since they do not have as well developed abilities as some dipterans, they tend also to conform to changes in the salinity of the external environment

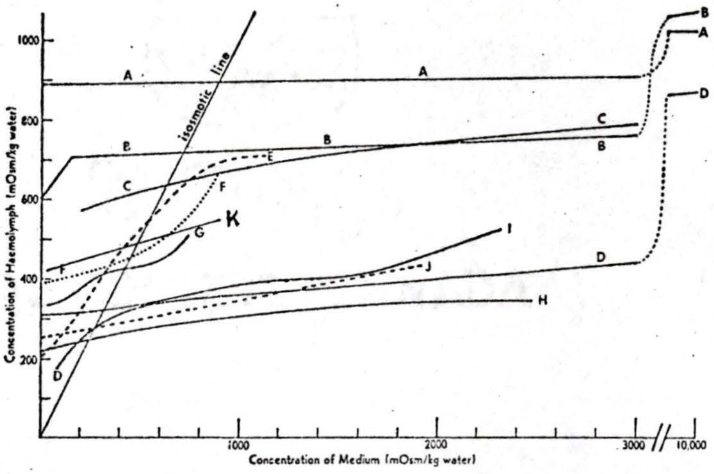
(Figure 7). They are strikingly similar in their abilities to Chironomus salinarius investigated by Neumann (1961). If more points along the osmoregulation line of P. alaskensis were measured, curves similar to those of C. salinarius might be evident. Bayly (1972) has placed C. salinarius midway between a salt marsh and marine insect in its abilities to cope with high concentrations of salts in the medium.

b. The role of cuticle permeability

As noted previously, chironomids respire cutaneously whereas most other salt water dipterans examined use air tubes and plastrons or carry air bubbles. The cuticle of salt water insects has normally been accepted as being impermeable (Beadle, 1939; Ramsay, 1950; Nemenz, 1960; Shaw and Stobbart, 1963). It was not until Sutcliffe (1961) investigated Limnephilus plebeius, that the concept of a highly permeable cuticle (equivalent to freshwater insects) in a salt water insect was discovered. Since then, Leader(1972) has further demonstrated the differences in cuticle permeability of direct air breathing versus cuticle respiring insects. Two species of trichopterans were examined. Limnephilus affinis can maintain its blood osmotic pressure at a high level up to 75% seawater and relies on uptake of dissolved oxygen through tracheal gills. Philanisus plebeius can maintain its blood osmotic pressure at a low level over a wide range of external concentrations, but is unable to complete its development in freshwater and relies on transcuticular diffusion to obtain oxygen. Unfortunately, not all cases have been as thoroughly examined as these have. Therefore, cuticle permeability and osmoregulatory ability cannot be compared equally among many species examined.

Sweeping statements have been made in reference to cuticle permeability which conflict and at the same time are unfounded. For example, Bayly (1972) states that "the permeability of the body wall of saline water dipterans is fairly similar to that of those living in freshwater", while, according to

Figure 7. Osmoregulation curves of saline water species.



Osmoregulation curves for:

A. *Ephydra cinerea* at 3° C	(Nemenz, 1960)
B. *Ephydra cinerea* at 21° C	"
C. *Haloniscus searlei* at 20° C	(Bayly & Ellis, 1969)
D. *Artemia salina* at 21° C	(Croghan, 1958)
E. *Tanypus nubifer* at 9° C	(Lauer, 1969)
F. *Enallagma clausum* at 9° C	"
G. *Chironomus salinarius* at 25° C	(Neumann, 1961)
H. *Ephydra riparia* at 20° C	(Sutcliffe, 1960)
I. *Cricotopus vitripennis* at 20° C	"
J. *Aedes detritus* at 20° C	(Beadle, 1939)
K. *Paraclunio alaskensis* at 15° C	

(adapted from Bayly, 1972)

Shaw and Stobbart (1963), "the relative impermeability of their cuticles" allows salt-water larvae to become hyperosmotic to dilute external solutions.

The apparent discrepancies appear to be due to the fact that osmoregulation, cuticle permeability and respiration are studied as separate entities in various organisms. But osmoregulation processes may depend on the permeability of the cuticle which in turn is determined by the mode of respiration of an insect. A high osmotic load is placed on an insect in a hypersaline environment if the cuticle is permeable. Hence, P. alaskensis larvae inhabit freshwater. Most of the insect species which have adapted to saline waters have impermeable cuticles.

In examining Figure 7 , one can visualize that, on the left side of the isosmotic line, the medium is hyposmotic and would tend to cause larvae to swell Examination of the shape of the regulation curves of Figure 7 shows that those larvae with air tubes (A,B,C,D,H,J) do not alter the concentration of the hemolymph as the salinity of the medium increases. On the other hand, those insects which respire via the cuticle (E,F,G) undergo large adjustments in the regulation of the hemolymph as the salinity of the medium increases. It would seem practical, with a permeable cuticle, to vary the level of solutes in the body as the external medium varies in salinity, thereby decreasing the passive flow of water and the energy required to maintain homeostasis.

On the right side of the isosmotic line, those relying on cuticular respiration come under great stress in desiccating environments. Those insects not involving the cuticle in respiration seem to be able to regulate indefinitely as the salinity increases and little or no stress is evident.

c. Volume regulation

According to Lange and Mostad (1967), two methods exist for the evaluation of volume regulation in animals; direct volumetric measurements which are likely to yield the most reliable results but which have limited applicability and measurements of the change in the animal's body weight during the period of osmotic adjustments. The second method may also suffer from various uncertainties such as weight variations caused by excretion. But, overall, valuable information can be drawn from the latter method, as exemplified by the curves in Graph 4 .

In all cases, if a steady-state in body weight was reached, this occurred within the first four hours of the experiment. Certainly this shows that the period of acclimatization may not require days, as is often thought in experimental procedures. Bayly (1972) was surprised that the fish, Cyprinodon rubrofluviatilis required only 8 hours to establish equilibrium upon being transferred directly from freshwater to seawater. This may be the case, as more species are examined. The implications at the cellular level of rapid osmotic and ionic adjustments are intriguing.

When larvae were placed directly from seawater into freshwater and weighed several times within the first hour, a curve very similar to that of a model curve produced by Lange and Mostad (1967) is apparent. The model curve is the result of the theoretical application of mathematical modelling.

Calcium-free seawater is known to interfere with osmoregulatory mechanisms (Krogh, 1939) and this was evident in P. alaskensis larvae. A lack of calcium in the medium causes a rapid increase in weight after two hours followed by a slow decline in weight. Eventually, although weight loss was not severe (7.72%, n = 8), all larvae appeared limp and lifeless after 48 hours and were dead after 92 hours. This indicates that, after initially interrupting the process of osmoregulation, the lack of calcium then began to interfere with metabolism, eventually leading to death.

The cuticle of chironomid larvae may be highly permeable, as indicated by the fact that larvae lose water rapidly in concentrated seawater and air (Graph 4). In most cases, an increase in the external salinity is accompanied by an increase in the $Q\ O_2$ (oxygen uptake), demonstrating that an increase in energy is required to maintain homeostasis (Foster and Treherne, 1976). An increase in salinity beyond the concentration of seawater stresses P. alaskensis larvae and they do not survive, indicating that, energetically, the load is too great to enable larvae to maintain homeostasis and so regulation breaks down. If the cuticle were less permeable, less energy would be required to counteract the desiccating effects of a highly saline environment. It would be of great value to determine the diffusional permeability of the cuticle of P. alaskensis larvae.

Bradley and Phillips (1975, 1977a,b,c), Maddrell and Phillips (1975) and Phillips and Meredith (1969a,b) have elucidated in detail the mechanisms of osmoregulation in Aedes campestris and Aedes taeniorhynchus. These processes may be characteristic of hyposmotic regulators in general. The drinking rate increases as the salinity of the medium increases. This has also been observed in Aedes togoi (Asakura, 1978) in conjunction with an increase in the activity of alkaline phosphatase localized in the caecal epithelium which may then be involved in the transport of ions across the caecal wall. It is well known in diadromous fish that the pituitary and endocrine secretions play an important role in osmoregulation (Lagler et al., 1977) and it may well be expected that the brain and neurosecretions of insects are involved in a similar role. The neuro-endocrine system of insects is known so far to initiate and control such processes as moulting, reproduction and diapause (Wigglesworth, 1972). Excess salts imbibed are excreted in the form of a hypertonic urine, the process being completed mainly in the rectum. The Malpighian tubules form an isosmotic secretion which appears to be further adjusted by active transport of Na^+, K^+, Mg^{++}, and Cl^- ion into the rectal area. The concentration of fluid in the Malpighian tubules can be adjusted changing the rate of secretion along the tubules in response to varying external environmental salinities. Water follows passively into both the Malpighian tubules and the rectum. Ultrastructural changes observed in the rectum and Malpighian tubules associated with changes in the external medium have been well documented (Marshall, 1974; Meredith and Phillips, 1973). The above system is flexible and active transport of ions as well as the rate of secretion of fluids can be adjusted to meet the needs of the animal, but at the same time a steady-state system over a wide range of environmental salinities can be maintained. *P. alaskensis* larvae may prove to show some furthe adaptations in the mechanisms of osmoregulation known to occur in dipteran larvae thus far. Due to the fact that the cuticle is probably as permeable as its freshwater counterparts and no anal papillae or respiratory tubes are present, a further load may be placed on the osmoregulatory mechanisms. If one interprets from the rate of water lost by larvae in concentrated seawater that the cuticle is highly permeable, and from the calculated drinking rate that the turnover of ions and water in the body is high, then it can be predicted that in a medium hyperosmotic to the blood, a urine hyperosmotic to the blood will be secreted and vice versa. A study of the fluid concentrations secreted by the gut, the Malpighian tubules and the rectum under varying salinities would further the understanding of the mechanisms osmoregulation in *P. alaskensis* larvae.

d. Hemolymph composition

Table 10 indicates the percentage that the ions, Na^{+}, Mg^{++} and Cl^{-}, contribute to the total osmotic pressure of the hemolymph of *P. alaskensis* larvae. A similar ratio is found in the salt-water mosquito, *Aedes taeniorhynchus* investigated by Bradley and Phillips (1975). The remaining percentage of the osmotic pressure would be contributed by such components as K^{+}, Ca^{++}, inorganic phosphates, carbohydrates and amino acids (Wigglesworth, 1972). Sutcliffe (1963) has compared the chemical composition of hemolymph in insects and some

Table 10. Summary of hemolymph analyses of <u>P. alaskensis</u> larvae reared in freshwater and salt water with a comparison to <u>Aedes taeniorhynchus</u> (Bradley & Phillips, 1975)

	P. alaskensis larvae in freshwater	*P. alaskensis* larvae in seawater	*A. taeniorhynchus* larvae in seawater
Osmolarity (mOsm/L)	417 (100)	524 (100)	350 (100)
Components (mM) Cl^-	60 (14.39)	126 (24.05)	98 (28.00)
Mg^{++}	8 (1.92)	14 (2.67)	5 (1.43)
Na^+	191 (45.80)	246 (46.95)	149 (42.57)
Σ Components (mM)	259 (62.11)	386 (73.67)	252 (72.00)
% components remaining	(37.89)	(26.3)	(28.00)

( ) = % of total osmolarity

other arthropods in relation to their phylogeny. Overall, the total concentration of solutes in the hemolymph of insects is not dissimilar to that found in a wide variety of other terrestrial and freshwater animals. The freezing point depression can range from 0.4 to 1.3°C $\simeq$ 120-376 mM/L NaCl (Shaw and Stobbart, 1963).

In all marine, brackish and freshwater Crustacea-Malacostraca the blood composition is of the type where sodium chloride accounts for nearly all of the blood osmolar concentration, and potassium, calcium and magnesium each account for only about 1-3 per cent of the total. In the Exopterygota insects, sodium and chloride both represent major proportions of the total osmolar concentration. On the other hand, in the Endopterygota (which includes P. alaskensis) chloride accounts for only a minor proportion, usually less that 10 per cent of the total, although in a few instances chloride is more important, e.g. 19 per cent of the total in larvae of Aedes aegypti. The analyses of the hemolymph of both P. alaskensis and A. taeniorhynchus larvae show considerably higher levels of chloride (24% and 28%) when in seawater. This pattern is exemplified by Sutcliffe (1963) in his Type III category in which sodium is high (21-48%) and chloride is low relative to this. Calcium, magnesium, potassium and inorganic phosphate are low but amino acids are high, up to 25%. Species included in this group are Osmyba fulvicephalus and Sialis lutaria, both neuropterans, and Tipula montium, a dipteran. Included, in general, are the orders Trichoptera, Diptera, Neuroptera, Mecoptera and most Coleoptera.

To summarize, osmotic and ionic regulation in P. alaskensis larvae is well developed ($\Delta P_i/\Delta P_o = 0.31$) and the haemolymph ionic concentrations are regulated both hyper- and hyposmotically. Their ability to regulate is not as extensive as some salt water insects that have been examined and it appears that their abilities are probably limited by the fact that they respire via the cuticle. Experiments indicate that the cuticle is highly permeable and therefore more energy is required to maintain homeostasis. In high salinities, regulation breaks down. Larvae lose weight and die in 150% seawater, calcium-free seawater and in air. Water regulation is steady in both freshwater and seawater. The composition of the hemolymph is characterized by high sodium levels with lower chloride, very low magnesium and probably low amino acids. A similar apportionment is evident in other dipterans, particularly species inhabiting saline waters.

Temperature Adaptation

According to the definitions given in a review of frost tolerance by Asahina (1969), Paraclunio alaskensis larvae can be considered to be freezing-susceptible, that is, they cannot survive the formation of ice in the cells of the body (Results, section IV). Two methods by which an insect can avoid cold injury are supercooling and frost tolerance. The former is the avoidance of freezing, the latter the tolerance to ice formation in the tissue fluids. In a freezing-susceptible insect, cooling to temperatures below the supercooling point, i.e. the highest temperature at which spontaneous freezing occurs in the insect's body, invariably means death, although a frost tolerant insect would survive this event (Asahina,1969). Freezing in an insect's body can be induced either by (1) spontaneous freezing in some part of the body, or (2) inoculation through the cuticle from an external ice crystal.

At the Gonzales Weather Station, the lowest recorded temperature from 1975-1978 was -7.3°C. Therefore, freezing may occur in the winter, although never for prolonged periods. In addition, the ameliorating effect of the sea on the intertidal zone would decrease substantially the probability of freezing. Morley and Ring (1972b) recorded inshore water temperatures for 1969-70 ranging from 7 - 13°C. Frost and snow have been observed in the upper intertidal zone, but at no time have the algae been observed to be in a frozen state. Even so, the effect of cold on the survival of organisms in the intertidal zone is a consideration. Generally it can be stated that, if freezing air temperatures occurred, P. alaskensis larvae would be prepared physiologically to avoid any freezing down to $-11.48 \pm .636^{\circ}C$. A temperature lower than this would result in cold injury due to the damaging effects of ice formation on cells.

As summarized by Asahina (1969), the majority of ice forms in the blood and extracellular fluids. The initial spontaneous freezing of these fluids is shown in Graph 5 (point A). Most insects will survive, upon thawing, if cooled only to this point. As the freezing process continues, however, more damage occurs and death will result. The long plateau characteristic of the freezing curve of P. alaskensis larvae (point B, Graph 5) indicates that extracellular freezing between the cells persists but that the rate of ice formation rapidly decreases. The rate of ice formation slows down because the increased amount of ice in the haemocoel after the initial spontaneous freezing has caused the remaining fluid to become more concentrated thus slowing down the freezing rate of the remaining fluid. The initial spontaneous freezing event releases heat of fusion causing the increase in temperature. If, after this event, most of the available water is in a frozen state, the temperature will return rapidly to its original point. If extracellular freezing continues, then the long plateau seen in Figure 5 will be apparent. In some insects, one or more rebounds can be observed which may be caused by a small fraction of body tissue which has remained in a supercooled state (Ring, 1980).

Hibernating insect stages are usually more tolerant to freezing than actively feeding larvae and nymphs. This is probably attributable to nucleation by food and contaminants in the digestive tract at higher temperatures, thereby acting as nucleating agents (Asahina, 1969). Also, the probability of such nucleation increases as the volume of water increases. In an insect's body, the blood in the bulk space of the body cavity may be the fraction most susceptible to freezing (Asahina, 1969). The larval stage of P. alaskensis is the only actively feeding stage of the life cycle. Larvae contain an average of 71.7% water and are not covered by any protective casing to prevent seeding from externally frozen water. They are, then, very susceptible to freezing.

Variations in freezing-susceptibility between summer and winter forms of the same species have been well documented and are correlated with the amount of glycerol produced by the insects and the state of dehydration of the tissues which decreases their susceptibility to freezing (Danks, 1978). No such variations were observed in the avoidance or tolerance of P. alaskensis larvae to freezing. It is often difficult to compare the frost tolerance abilities of insects since experimental methods can vary - i.e. precooling temperatures and cooling rates - but, in general, chironomids are well known for their abilities to overwinter as larvae in the Arctic and Antarctic regions. The Diptera make up 60-90% of the Arctic fauna and of that 40-60% are chironomids (Danks, 1978).

Enteromorpha linza can tolerate some freezing (Kanwisher, 1957). The effects of prolonged cold temperatures (1^o to 2^oC) on intertidal organisms have not been determined. Larvae stored at 2^oC for an hour appear limp and lifeless, as if in a thermally quiescent state, and require several hours to recover after being warmed to 15^oC.

In summary, P. alaskensis larvae are freezing-susceptible although they supercool to a temperature low enough to be rarely encountered in the intertidal zone.

Respiration

It must be concluded from microscopic and experimental work, that *P. alaskensis* larvae are apneustic and respire through the cuticle. No other respiratory mechanism is present (Fig. 3a,b,c). According to Hinton (1976) nearly all intertidal insects have adapted cutaneous respiration in the larval form. Other adaptations do exist and these are 1) the plastron or permanent physical gill found in pupae and some larvae (Ring,1978) and 2) the compressible or shrinking physical gill occasionally found in some adults. Absent from the intertidal zone are 1) the ability of an insect to surface for air 2) air siphons, spiracles and tubes and 3) the ability to tap the intercellular spaces of plants.

Respiratory membranes must have holes large enough to allow oxygen molecules to enter, but any holes large enough for these molecules will, according to Hinton (1976), allow water to escape because the water molecules are smaller. As Hinton (1976) points out, respiratory mechanisms are moist not because this assists in the diffusion of oxygen but because they cannot help being moist if they have holes large enough to pass oxygen molecules. A moist, permeable skin restricts animals to damp places. Confirming this theory, Buck (1962) states that

> "since water vapor is a gas....water must be lost during respiration and an arrangement for making O_2 uptake more rapid or efficient will usually increase transpiration correspondingly. The operation of these principles has long been recognized in a) the vanishingly small cutaneous respiration of some terrestrial species, particularly in inactive forms denied water intake for long periods; (b) the rapid desiccation of hyperventilating insects; (c) cuticular coatings that reduce water loss without impeding gas exchange; and d) at least by implication the very existence of valvular spiracles."

P. alaskensis larvae appear to be restricted to moist crevices, in amongst algae and barnacles and in runoff areas. Desiccation during low tide is avoided by the selection of these sites. At high tide, the tubes of P. alaskensis were observed to be silvery in appearance and filled with air.

In the laboratory, the respiration rate of larvae was calculated to be 0.032 μg 0_2/mg dry wt. per hour in seawater at 15°C. It is very difficult to make any comparison of this rate with that found in other insects because the rate of respiration is highly dependent on a number of variables such as surface/volume ratios and weight (Mill , 1974). The main point of establishing a "base-line" respiratory rate was to determine whether or not there is a measurable respiration rate of larvae when submerged at high tide in seawater. Since there is a small but distinctly measurable rate, it can be assumed that larvae do actively respire when submerged.

Houlihan (1969) has shown an interesting comparison of respiration rates in Donacia simplex larvae living in the mud of salt marshes. The following respiration rates (in μl 0_2/mg/hr) were obtained:

	aerial respiration	cutaneous respiration
moist air	0.04	
aerated water	0.12	0.015
deoxygenated water	0.15	0.020

In addition to cutaneous respiration, larvae may use ventilation movements when submerged to assist with respiration. These movements have been described in many organisms (Buck, 1962; Hinton, 1976; Mill , 1974). On several occasions larvae were observed to rhythmically ventilate their tubes with their body. This would act to circulate fresh water past the cuticle. Theoretically, the bending of the body during the ventilation motions which causes the sclerites to separate would then temporarily expose the membraneous cuticle between each of the sclerites. The cuticle between the sclerites is much thicker (Fig. 8) than the sclerites themselves but also appears to be less sclerotized as indicated by the difference in optical properties when viewed under the microscope. It is possible that the result of this bending, is that the exposed areas between the sclerites can "breathe". Figure 1c shows that the tracheoles occur in distinct vertical bands in these areas. Ebeling (1976) comments on "the folds of soft and flexible membranes that allow for stretching and distention, as well as for articulation" by stating that due to "the fact that they are not sclerotized and probably because they are usually thinner, the membraneous portions of the cuticle are usually thinner". Mill (1976) has also commented on a similar type of arrangement, using the term "respiratory organs" for that observed in Ceratopogonidae larvae. Upon tensile mechanical hysteresis, solid cuticles exhibit cyclic-hardening whereas intersegmental membranes undergo stress-softening (Hepburn, 1976).

The above evidence indicates that the cuticle can vary its properties considerably within an organism. This leads to the question of understanding the material itself and its limitations. Many reviews are available covering the subject of cuticle structure and permeability (Ebeling, 1976; Hinton, 1976; Neville, 1967; Prusch, 1976; Wigglesworth, 1972). Experimentation has recently developed to test artificial membranes and this has lead to a better understanding of the nature of 1) the very thin outer lipid film and 2) the thick inner hydrophilic membrane which is predominantly chitin, protein and mucopolysaccharide (Ebeling, 1976). Neville (1976) has shown the orientation of the fibrillar structure in cuticle and how these patterns can vary. EM work has demonstrated that the membrane (which is hydrophilic) is pierced with a myriad of pores. To date, the permeability of the cuticle has been shown to vary as the external vapor pressure, temperature, and the nature of the outer wax covering varies. As more evidence in this area is gathered, the study of osmoregulation will benefit from the increased understanding of the processes found in the cuticle in such areas as the body wall, the gut and the rectum.

In summary, P. alaskensis larvae have been shown to respire cutaneously at a rate of 0.032 μl 0_2/mg/hr in seawater at 15°C. Since the cuticle is permeable to oxygen, and therefore probably also to water, P. alaskensis larvae are limited to moist areas to prevent desiccation. A permeable cuticle, as discussed earlier, may also limit the osmoregulatory capabilities of larvae inhabiting the intertidal zone.

Conclusion

P. alaskensis larvae have successfully established a niche in the intertidal zone. Very few other insect species have been so capable. The reasons behind this success are due in part to the abilities of larvae to regulate well in both seawater and freshwater. Another major contribution to their success is their ability to anchor themselves onto the substrate using both the hooks on the pseudopods and the tubes which they secrete from salivary glands. Few of their freshwater counterparts possess the same degree of methods of anchoring. The life cycle of *P. alaskensis* fluctuates with the seasonal cycling of the algae and several other environmental factors such as sunshine, temperature, rainfall and wind. But, by maintaining stock populations of larvae in protected areas, the population is regulated somewhat throughout the year. When habitat availability increases, the population can rapidly colonize the new areas. In the intertidal zone, where conditions are always changing, a flexible, rapidly dispersing type of population is essential.

Physiologically, *P. alaskensis* larvae can regulate the osmotic and ionic concentrations of the hemolymph in the salinities expected to occur in the midlittoral zone (they are also well adapted to prevent freezing down to temperatures well below those expected in the intertidal zone). The cuticle appears to be permeable due to the fact that larvae depend on transcuticular diffusion to obtain oxygen. Undulatory movements by larvae exemplified possible ventilation processes. Larvae are limited to areas in the intertidal that are continuously moist. *P. alaskensis* larvae cannot regulate water loss in a desiccating environment such as in air or concentrated seawater. It is because of the drastic effects of desiccation that larvae inhabit only the green algae zone instead of the upper littoral zone, in tidepools or among the littorinids or barnacles.

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Appendices

A. Sample of data collection sheet from Log Book

B. Culture methods

C. Na^+, Mg^{++}, and Cl^- : Calculations

D. Chart for computing osmolarity, freezing point depression and molarity of salt solutions.

E. Water regulation: data for Experiments A & B

F Respiration rate: data and calculations

G. Isotope counts

Appendix A. Data Collect Sheet from Log Book

Location:

Coll. #: ____________

Date : ____________

Time: ____________ Tide: ____________ Weather: ____________

Previously: ____________

Sketch of area:

Organisms	Collection Area
A. Density #/in^2	
Egg masses	
Larvae	
Pupae	
Adults	
B. Algal Cover	
Type	
Height	
Density	
Moisture	
C. Organisms	
Tipulids	
Mites	
Swallows	
Other	
D. Photos	
E. Relevant Data	(drain areas, larval activity, tubes, etc.)

Appendix B. Culture Methods

No substrate:

1. Small glass bowls, not-aerated: Bacterial growth was high. Larvae floated and struggled on the surface. Survival rate was poor over extended time periods.

2. Small glass bowls, aerated: The seawater evaporated rapidly and therefore salinity fluctuations were difficult to monitor. Growth of bacteria was less than in non-aerated dishes but the flotation of larvae continued to be a problem.

3. "Drip" Apparatus: A sheet of glass, standing vertically in a screened cage, was equipped with a hose running across the top edge. The hose was punctured with numerous small holes to create small but steady streams of water which ran down the glass. This system was to imitate the seawall. The problems were that the water was able to evaporate and crystallize on the glass so that no organisms (i.e. diatoms and algae) grew on the glass to provide a substrate for larvae

Substrate:

4. Glass tubes: Small pieces of capillary tubing were placed in an aerated aquarium tank with larvae already introduced into the tube. The larvae did not remain in the tubes at all times and consequently would float and become caught in the surface tension.

5. Cotton batting: Glass bowls containing cotton batting were aerated and carefully monitored to control salinity. This method proved to be the most successful.

Appendix C. Na^+, Mg^{++} and Cl^- Calculations

i) Calculations of mM Na^+, and Mg^{++} in hemolymph of *P. alaskensis* larvae

$$\text{Molarity} = \#\text{moles/L}$$

$$\text{Mole} = \frac{\text{gm mol. wt.}}{\text{L}}$$

$$1 \text{ mole} = \frac{X \text{ gm}}{\text{L}} = \frac{X\ \mu\text{g}}{\text{mL}}$$

$$1 \text{ m mole } Na^+ = \frac{23\ \mu\text{g}}{\text{mL}} \qquad 1 \text{ m mole } Mg^{++} = \frac{24.3\ \mu\text{g}}{\text{mL}}$$

$$\text{mM of Na in hemolymph} = \frac{\#\ \mu\text{g/mL of hemolymph}}{23\ \mu\text{g/mL } Na^+} \qquad \text{mM of } Mg^{++} \text{ in hemolymph} = \frac{\#\ \mu\text{g/mL hemolymph}}{24.3\ \mu\text{g/mL } Mg^{++}}$$

ii) Calculations of m-eq./L of Cl^- in hemolymph of *P. alaskensis*

Standard: A full capillary ($\bar{X}$ = 32.05 mm) of 50 mM Cl^- is titrated by 25 'units' of $AgNO_3$

Sample: 11 'units' of $AgNO_3$ are required to titrate the capillary with 5 mm of hemolymph

$$\frac{5 \text{ mm}}{32.05 \text{ mm}} = .156 \quad \text{and} \quad 11 \text{ units } \times \frac{0.05 \times 10^{-6}}{25 \text{ units}} \text{ moles } (Cl^-)$$

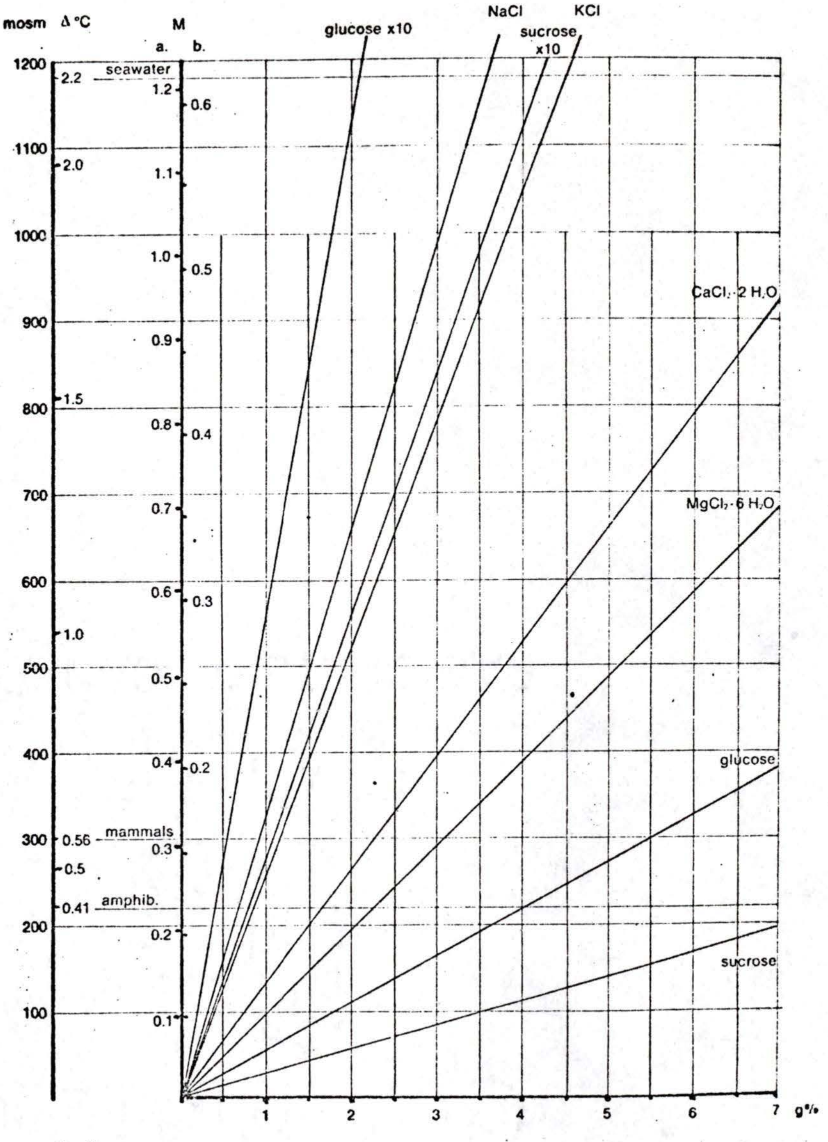
$$= \frac{2.2 \times 10^{-8} \text{ moles } Cl^-}{0.156 \times 10^{-6} \text{ L}}$$

$$= 1.41 \times 10^{-1} \frac{\text{moles } Cl^-}{\text{L}}$$

$$\therefore [Cl^-] = \frac{1.41 \times 10^{-1} \text{ moles}}{\text{L}} \times \frac{1000 \text{ m moles}}{1 \text{ mole}} = 141 \text{ mM } Cl^-$$

$$= 141 \text{ mEq./L } Cl^-$$

Chart for computing osmolarity, freezing point depression, and molarity of salt solutions



Ordinate:

1. osmolarity in mosm/1000 g H_2O
2. freezing point depression Δ in °C
3. molarity of *a.* non-electrolytes
   b. monovalent salts

Abscissa:

grams/100 ml

Curves for:

glucose — glucose × 10 — sucrose — sucrose × 10 — NaCl — KCl
(the concentrations are correct for molarity and tonicity)

$CaCl_2$ — $MgCl_2$
(the concentrations are correct only for molarity since Δ are slightly different, but negligible)

(Millonig, 1972)

Appendix E. Water Regulation Experiment A.
(weights expressed in mg)

1. FW

Time (hrs.)

Larva	0	:05	:15	:40	1	2	17	24	48
1	7.5	7.2	7.3	7.2	7.2	6.9	6.7	7.0	6.1
2	4.5	4.7	4.9	5.0	4.3	4.5	4.5	4.7	4.2
3	6.0	6.0	6.1	6.4	5.9	5.8	5.6	5.6	5.5
4	4.8	4.9	5.1	4.8	4.3	4.5	4.2	4.5	3.8
5	8.8	9.0	9.0	8.9	8.6	8.7	8.2	8.5	8.5
6	7.2	7.3	7.4	7.1	6.8	7.0	6.6	7.0	6.6
$\overline{X}$	6.46	6.51	6.63	6.56	6.18	6.23	5.96	6.21	5.78
±S.E.	0.68	0.66	0.63	0.62	0.69	0.66	0.61	0.63	0.69

mean % Δ wt. = 10.47%
rate = −0.014 mg/hr.

2. SW

Time (hrs.)

Larva	0	1	2	4	23	48
1	9.5	9.4	9.3	9.1	8.9	8.7
2	8.1	8.2	7.6	6.9	6.4	7.3
3	10.4	10.7	10.4	10.5	10.2	10.6
4	8.6	8.9	8.8	8.7	8.2	9.0
5	9.7	9.4	9.4	8.9	7.7	8.6
6	9.2	9.4	9.5	8.8	8.1	9.3
7	10.0	10.2	10.1	9.2	9.3	10.8
8	6.7	7.0	6.6	6.6	6.4	7.3
$\overline{X}$	9.02	9.15	8.96	8.58	8.15	8.95
±S.E.	0.42	0.47	0.45	0.44	0.47	0.46

mean % Δ wt. = 0.77%
rate = −0.001 mg/hr.

3. Ca^{++}-free

Time (hrs.)

Larva	0	1	2	4	24	48
1	8.3	7.9	8.2	7.9	7.8	7.3
2	8.5	8.6	9.0	8.3	8.6	8.6
3	9.0	8.9	9.2	8.0	8.1	8.0
4	6.9	7.1	7.6	7.5	6.9	6.5
5	7.3	7.4	7.5	7.5	7.5	6.5
6	6.4	6.5	6.6	5.2	6.1	5.9
$\overline{X}$	7.73	7.73	8.01	7.40	7.50	7.13
±S.E.	0.41	0.37	0.40	0.45	0.36	0.41

mean % Δ wt. = 7.72%
rate = −0.12 mg/hr.

Appendix E. cont'd.

4. Conc. SW

Time (hrs.)

Larva	0	1	4	6	10	22	27	48
1	6.6	6.5	6.2	6.0	5.8	5.7	5.6	5.6
2	8.7	8.7	8.1	8.0	7.7	7.6	7.5	7.6
3	4.6	4.6	4.4	4.3	4.1	4.1	4.2	4.0
4	5.2	5.2	5.0	4.9	4.8	4.8	4.8	4.0
5	4.5	4.6	4.4	4.2	4.1	4.1	4.2	3.9
6	4.6	4.7	4.4	4.4	4.4	4.3	4.3	4.2
7	5.8	5.8	5.3	5.1	5.1	4.9	- out of dish -	
8	3.8	3.9	3.6	3.4	3.3	3.3	3.3	3.2
$\overline{X}$	5.47	5.50	5.17	5.03	4.91	4.85	4.84	4.64
±S.E.	0.55	0.53	0.49	0.50	0.47	0.46	0.51	0.56

mean % Δ wt. = 15.17%
rate = -0.17 mg/hr.

5. Air

Time (hrs.)

Larva	0	4	22
1	7.3	5.5	2.6
2	2.1	1.6	1.0
3	6.1	5.2	3.4
4	6.2	4.8	2.6^d
5	7.4	5.7	2.0^d
6	6.1	5.1	3.8
$\overline{X}$	5.91	4.65	2.56
±S.E.	0.79	0.62	0.40

mean % Δ wt. = 56.68
rate = -0.15 mg/hr.

d = dead

Appendix E. cont'd.

Experiment B. (weights expressed in mg)

1. SW

Larva	0	1	2	4	23	48	mean % Δ wt.
			Time (hrs.)				
1	4.32	4.20	4.07	4.08	4.17	4.19	3
2	5.28	5.12	5.06	4.99	5.01	4.93	10
3	5.14	5.00	4.87	4.81	5.01	5.03	8
4	5.25	5.07	4.98	4.92	4.96	4.85	10
$\bar{X}$ =	4.99	4.84	4.74	4.70	4.78	4.75	
±S.E. =	22.7	21.7	27.8	20.9	20.6	19.0	

mean % Δ wt. = 4.8%

2. FW

Larva	0	1	2	3	5	17	24	42	mean % Δ wt.
				Time (hrs.)					
1	5.42	5.21	5.08	5.13	5.19		5.04	4.95	8
2	2.64	2.49	2.47	2.49	2.47		2.41	2.48	6
3	6.84	6.64	6.52	6.54	6.53		6.50	6.43	5
4	5.78	5.44	5.42	5.52	5.59		5.45	5.33	7
$\bar{X}$ =	5.17	4.94	4.87	4.92	4.94		4.85	4.79	
±S.E. =	89.5	87.6	85.7	86.2	87.1		86.9	83.3	

mean % Δ wt. = 7.3%

3. Conc. SW

Larva	0	1	2	3	4	24	48	mean % Δ wt.
				Time (hrs.)				
1	4.28	4.11	4.00	4.00		3.95	3.89	18
2	5.79	5.63	5.50	5.44		4.88	4.62	18
3	5.98	5.76	5.68	5.61		5.17	4.92	17
$\bar{X}$ =	5.35	5.16	5.06	5.01		4.66	4.47	
±S.E. =	53.7	52.9	53.2	51.0		36.7	30.5	

mean % Δ wt. = 16.4%

4. Air

Larva	0	1	2	4	22	23	46	mean % Δ wt.
				Time (hrs.)				
1	6.42	5.98	4.82	4.33		3.63	3.15	50
2	4.60	4.00	3.68	3.42		2.37	1.79^d	61
3	6.05	5.43	4.72	4.24		3.40	2.80^d	54
4	6.18	5.78	5.48	5.22		4.32	3.77	39
$\bar{X}$ =	5.81	5.17	4.67	4.30		3.43	2.87	
±S.E. =	41.1	39.8	37.2	36.8		40.3	41.4	

d = dead

mean % Δ wt. = 50.6%

Appendix F. Respiration Rate Data

Date/Sample	n	Time 0	:15	:50	1:15	1:30	1:45	2:45	3:45	17:00	18:00	21:00	22:00	Respiration rate µl O_2/mg/hr. after 1st injection
Oct. 23 E	10	129		116.5	113.4	103[a]								.020
Oct. 23 F	20	129		112.6	85.8	69.2[a]								.019
Oct. 29 A	6	110	103.2		95.6				67.4[a]					.089
Oct. 29 D	10	110	106.6		82.5			49.7				14.7[b]†		.026
Oct. 30 B	6	125								92	84.3[c]			.006
Oct. 30 C[c] dead (control)	6[b]	126		127		129.5	127[b]							no rate

$\overline{X} = .032 \pm .0146$

a - all larvae alive

b - all larvae dead

c - 50% alive

† Sample D - 1 larvae somewhat alive after 2 hrs. in fresh solution.

Appendix F cont'd. Calculations used to convert pO_2 to μl O_2/mg dry wt./hr.

Eg. - Sample E (at 15°C and 35.5 °/oo)

Step #

1. Initial pO_2 - Final pO_2 : 129 - 116.5 = 12.5 mm HgO_2 consumed by 10 larvae over 1.2 hrs.
2. *Correct for salinity differences - not applicable
3. Barometric pressure of the room: 761.2 mm Hg
4. *Vapour pressure of water at 15°C: 12.8 mm Hg
5. Subtract #4 from #3: 761.2 - 12.8 = 748.4 mm Hg
6. *Multiply by % O_2 in air: 748.4 mm Hg x .209 = 156.4 mm Hg of Bath
7. % saturation of sample: Divide #1 by #6 = 1.67%
8. *The sample may hold 7.87 mg O_2/L. (at 15°C & 35.5 °/oo)
9. Adjust to % saturated (wet) bloat gas analyzer

   .0167 x 7.87 = 0.1314 mg O_2/L.
10. In a 2 m sample: ∴0.1314 x 0.002 = 0.00026 mg O_2 in 10 mℓ. sample
11. 1 mg O_2 = .7 mℓ O_2 : 0.00026 mg O_2 x $\frac{.7\ m\ell\ O_2}{1\ mg\ O_2}$ = 0.00018 mℓ O_2
12. Convert to μℓ : 0.00018 x 1000 = 0.1839 μℓ O_2
13. Convert to 1 hr.: 0.1839/1.2 hr. = 0.1533 μℓ O_2/hr.
14. Average dry wt. for 10 larvae = 7.5 mg ∴0.1533/7.5 = 0.020 μℓ O_2/mg dry wt./hr.

* Refer to Table I and III in J. Fish. Res. B. Can., Vol. 32 (12), 1975.

Appendix G. Isotope Counts : Data

This appendix outlines the raw data for Graph 7 . Corrected body counts were arrived at by i) correcting for quenching, and ii) subtracting the $\overline{X}$ values obtained for the controls from the $\overline{X}$ values for the experimental systems.

Time	Larva #	mg dry wt.	body counts (quenched dpm)	body counts (corrected dpm/mg dry wt.)	
2 hrs.	1	2.84	369	- 47	$\overline{X}$ = 119.8 S.E. = 49.926
	2	3.83	826	84	
	3	1.74	927	243	
	4	4.31	986	112	
	5	4.03	509	1	
	6	4.10	1156	159	
	7	1.63	1129	383	
	8	3.81	584	21	
	9	2.71	377 $\overline{X}$ = 503		
	10	1.59	629		
4 hrs.	1	2.00	463	116	$\overline{X}$ = 231.57 S.E. = 45.966
	2	3.00	885	218	
	3	3.00	2595	-	
	4	1.50	480	167	
	5	1.70	390	94	
	6	3.60	1745	421	
	7	1.40	576	247	
	8	2.50	1124	358	
	9	2.20	205 $\overline{X}$ = 230		
	10	3.30	254		

Time	Larva #	mg dry wt.	body counts (quenched dpm)	body counts (corrected dpm/mg dry wt.)	
8 hrs.	1	1.10	1329	384	$\overline{X}$ = 410.57
	2	1.70	1312	239	S.E. = 55.219
	3	1.10	1461	505	
	4	0.60	-	-	
	5	2.40	2466	650	
	6	2.20	1818	415	
	7	3.30	2376	445	
	8	3.70	1780	236	
	9	2.60	906 = $\overline{X}$	-	
	10	2.40	-		
24 hrs.	1	2.10	-	-	$\overline{X}$ = 3190.5
	2	2.30	23288	9601	S.E. = 1368.168
	3	2.20	9225	3645	
	4	0.10	-	-	
	5	0.30	4754	724	
	6	3.60	3268	573	
	7	2.00	2152	1891	
	8	0.40	2290	2709	
	9	4.90	874 } $\overline{X}$ = 1207		
	10	0.50	1539		

Appendix G Drinking Rate Calculations from Isotope Counts

Eg. 4 hrs. : 119 dpm/mg dry weight

$$\frac{\text{dpm in organism}}{\text{dpm in exp. system}} \times \text{amount of glucose available}$$

4.6×10^{-8}M solution used in the experimental system has a specific activity of 50.03 mCi/mmole glucose, therefore

$$4.6 \times 10^{-5} \text{ mmole glucose} \times 50.03 \text{ mCi/mmole glucose}$$

$$= 0.0023 \text{ mCi} - {}^{14}C$$

1 Ci= 2.22×10^{6} dpm, therefore,

$$2.3 \text{ Ci} \times 2.22 \times 10^{6} \text{ dpm/ Ci} = 5.106 \times 10^{6} \text{ dpm}$$

$$\frac{119 \text{ dpm/mg dry weight}}{5.106 \times 10^{6} \text{ dpm}} \times \frac{4.6 \times 10^{-5} \text{ mmoles glucose}}{\text{m}\ell}$$

$$= 1.07 \times 10^{-9} \text{ mmoles glucose/mg dry weight}$$

Since 1 mole glucose = 180.2 g/mole = 180.2 mg/mmole , then

$$\frac{1.07 \times 10^{-9} \text{ mmole glucose}}{\text{mg dry weight}} \times \frac{180.2 \text{ mg}}{\text{mmole}}$$

$$= 1.93 \times 10^{-7} \text{ mg glucose/mg dry weight}$$

SUMMARY:

4 hrs =	119 dpm =	1.93×10^{-7} mg glucose/mg dry weight
6	231	3.75×10^{-7}
8	410	6.65×10^{-7}
24	3190	5.17×10^{-6}

APPENDIX G cont'd.

If the maximum accumulation of dpm was 3190 units per mg dry weight and if this occured over 24 hrs., then by division the rate of accumulation (drinking rate) is 2.15×10^{-7} mg glucose/mg dry weight/hr. This is equivalent to 1.2×10^{-9} mmoles glucose/mg dry weight/hr

Since the concentration of glucose is 4.6×10^{-5}/ml , then larvae are drinking 1.2×10^{-9} mmoles of glucose per hour or 2.6×10^{-5} ml of fluid per hour. This can be converted to a final figure of

26 nℓ/mg dry weight/hr

Finally, since the dry weight of one larva is approximately 1 mg the drinking rate can be estimated to be

26 nℓ larva^{-1}hr^{-1}

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