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The Influence of Biotic and Abiotic Parameters on the
Distribution and Abundance of Two Sympatric
Species of Hirudinoidea

by

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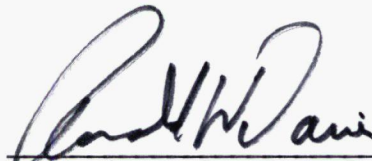DEPARTMENT OF BIOLOGY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "The Influence of Biotic and Abiotic Parameters on the Distribution and Abundance of Two Sympatric Species of Hirudinoidea" submitted by Frederick John Wrona in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

The ecology of two sympatric, freshwater, predatory leeches,
Nephelopsis obscura Verrill, 1872 and Erpobdella punctata (Leidy,
1870) (Erpobdellidae) was investigated. In Alberta, N. obscura is
generally numerically dominant in lentic habitats, while E. punctata
is most abundant in colder lotic ecosystems. Davies, Reynoldson and
Everett (1977) hypothesized that inter-specific competition for food
resources was responsible for the dominance of N. obscura in lentic
habitats of Alberta. The objective of this study was to determine
whether the differences in distribution and abundance of N. obscura
and E. punctata in lentic and lotic habitats could be explained by:
1) inter-specific competition, 2) differing physiological responses to
temperature and/or oxygen concentration as measured by respiration,
growth and reproduction, and 3) differences in substrate preference.

In Cairn Pond, the competition hypothesis was investigated
serologically when E. punctata was unusually dominant and during the
immediate transition period to dominance by N. obscura. At the
species level, no significant differences in prey utilization occurred
as a result of the change in dominance. However, intra-specifically
E. punctata displayed food resource partitioning on a temporal and
weight-class basis while N. obscura showed only differential
weight-class utilization. Inter-specific niche overlap decreased
significantly with the change to N. obscura dominance, supporting the
competition hypothesis.

Temperature, oxygen concentration, nutritional condition, re-
productive state and activity level were found to influence the oxygen

uptake of both species. N. obscura displayed higher aerobic metabolic
scope than E. punctata at warmer temperatures, with the converse true
at colder temperatures. When exposed to either short-term or
long-term hypoxia, both species were oxy-conformers. Under anoxia,
N. obscura survived longer than E. punctata, but survival time for
both species declined with increasing temperature.

The onset of cocoon production in both species was temperature
related, with the lowest proportion of individuals laying cocoons at
5 ^{o}C, and the highest proportion at 20 ^{o}C. Based on the mean number
of cocoons/individual, the mean number of eggs/individual, cocoon
hatching success, and the growth rates of young leeches, E. punctata
was more successful at colder temperatures than N. obscura. At warmer
temperature regimes, neither species showed a clear ecological ad-
vantage.

Since both species selected solid substrates, substrate pref-
erence could not explain the observed differences in distribution
between lentic and lotic habitats. However, the success of both
species is dependent on the presence of solid substrates for laying
cocoons and attachment.

It was concluded that the differential responses of N. obscura
and E. punctata to the abiotic parameters examined could explain the
dominance of E. punctata in lotic habitats. However, biotic factors
were believed to be primarily responsible for the dominance of
N. obscura in lentic habitats of southern Alberta.

ACKNOWLEDGEMENTS

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1.0 GENERAL INTRODUCTION

Hirudinoidea (leeches) are common members of many freshwater macroinvertebrate communities. They are phylogenetically closely related to the Oligochaeta having a thin, flexible cuticle, prostomium, peristomium, pygidium, hermaphroditic reproductive system, and metameric segmentation (Dales, 1967). However, Hirudinoidea are distinct from all other Annelida by having a fixed number of body segments (34), a circum-oral anterior sucker, a ventral posterior sucker, usually no chaetae, a greatly reduced coelom, and fused gonads and gonadoducts (Dales, 1967; Davies, 1971). In Canada there are four families of Hirudinoidea, the Piscicolidae, Glossiphoniidae, Hirudinidae and Erpobdellidae (Davies, 1972, 1973). Pisicolid and glossiphonid leeches lack jaws and denticles and are characterized by having a mouth which is a small pore in the anterior sucker through which a muscular pharyngeal proboscis is protruded. Members of both families feed by penetrating the soft tissues of a host or prey and sucking the body fluids. The Pisicolidae are all ecto-parasites of fish and are rarely free-living. They have a cylindrical body and reproduce by attaching chitinized cocoons, which encapsulate the fertilized eggs, to solid substrates like stones and plants. Members of the Glossiphoniidae are either free-living predators, feeding on invertebrates, or temporary ecto-parasites of fish, amphibians, snails or birds (Davies, 1972, 1973). The Glossiphoniidae are unique in displaying the brooding of their fertilized eggs and newly hatched young on their ventral body surface (Davies, 1971). The commonest glossiphonid leeches in the sloughs and ponds of Alberta are

Helobdella stagnalis (L., 1758) and Glossiphonia complanata (L., 1758) (Moore, 1964, 1966), which primarily feed on Gastropoda, Chironomidae, Oligochaeta and Amphipoda (Wrona, Davies, Linton and Wilkialis, 1981), Theromyzon rude (Rathke, 1862), a temporary blood-sucking ecto-parasite of waterfowl (Davies and Wilkialis, 1981), and Placobdella papillifera (Verrill, 1872), also a temporary ecto-parasite with unknown definitive hosts (Davies and Wilkialis, 1982).

Hirudinidae and Erpobdellidae leeches differ from the Pisicolidae and Glossiphoniidae by lacking a muscular eversible proboscis and having a mouth which is a large aperture often occupying the entire anterior sucker (Davies, 1971). The Hirudinidae have a buccal cavity containing jaws or denticles and are either temporary blood-sucking ecto-parasites of vertebrates or macrophagous predators of invertebrates. They reproduce by attaching their cocoons either to solid substrates underwater or some species leave the water and deposit their cocoons in moist micro-habitats (Sawyer, 1972).

The only family in which all members are predators is the Erpobdellidae, which lack jaws or denticles in the buccal cavity and are raptorial predators of invertebrates. The prey are either swallowed whole or their body fluids sucked out (Davies and Everett, 1975). Erpobdellidae reproduce by attaching their cocoons to solid substrates such as macrophytes and stones occurring underwater.

This study examines the ecologies of two sympatric species of Erpobdellidae, Nephelopsis obscura Verrill, 1872 and Erpobdella punctata (Leidy, 1870). In lentic habitats of western Canada,

N. obscura is generally numerically dominant, while in eastern Canada, E. punctata is the more abundant (Davies, 1973; Davies, Reynoldson and Everett, 1977). However, in lotic ecosystems of southern Alberta, E. punctata is usually the numerically dominant erpobdellid.

One of the principal objectives in the discipline of ecology has been to understand the environmental factors that determine the distribution and abundance of organisms (Townsend, 1980). Using the classification of Andrewartha and Birch (1954), the environment of aquatic Hirudinoidea can be divided into four major components: 1) weather (i.e. temperature, light regimes), 2) food, 3) interaction with other animals (intra- and inter-specific), predation and organisms causing disease, and 4) a place in which to live (i.e. physical and chemical characteristics of the habitat, such as pH, concentration of certain ions and gases, substrate composition, magnitude of water flow).

Sawyer (1974) forwarded specific abiotic and biotic environmental factors he considered having a major influence on the distribution and abundance of freshwater leeches in North America: availability of food organisms; nature of the substrate; water currents (lentic versus lotic); water chemistry; and the range of temperature and dissolved oxygen.

Over the last ten years, Davies and co-workers have conducted a detailed research program on the environmental factors responsible for the observed differences in the distribution and abundance patterns of N. obscura and E. punctata in lentic habitats in western Canada. Specifically, these studies have quantitatively examined species

differences in life histories and feeding ecologies (reviewed in Chapter 2), osmoregulation and respiration (Chapter 3), and reproduction (Chapter 4). However, no quantitative studies have been performed to determine the abiotic and/or biotic environmental factors responsible for the differences in their distribution and abundance between lentic and lotic habitats.

The overall aim of this study was to determine whether the observed differences in the pattern of distribution and abundance displayed by N. obscura and E. punctata could be explained on the basis of species-specific differences in the responses to the environmental factors Sawyer (1974) reported to influence leech distribution. Three general hypotheses were examined:

1) When sympatric and food limited, the co-existence of N. obscura and E. punctata in lentic habitats is accommodated through inter-specific food resource partitioning on a temporal and/or weight basis.

2) The differing distribution and abundances of N. obscura and E. punctata in lentic and lotic ecosystems in southern Alberta is in part determined by differing physiological responses to temperature and/or oxygen concentration as measured by respiration, growth and reproduction.

3) Species-specific differences in substrate preference accounts for the dominance of N. obscura in lentic and E. punctata in lotic habitats.

1.1 Site Descriptions

1.1.1 Cairn Pond

Cairn Pond is a typical small, ephemeral, slough in the knob and kettle topography of the prairie-foothills transition zone (Legget, 1961), located 4.5 km west of Calgary city limits and approximately 2 km north of highway 1A (114° 15' W, 51° 10' N). The pond is bisected into two separate basins, having no direct connection, by an east-west municipal district road. Located within a depression of a drainage basin of about 16 hectares with no visible surface drainage, the northern basin has a surface area of about 1 hectare and a maximum depth of 2.5 meters.

The west bank has a heavy cover of Poplus balsamifera L. and Salix sp. down to the supra-infra littoral zone while the north and south banks are grass covered, interspersed with small shrubs. The littoral zone consists primarily of mud, with small amounts of scattered rock along the east shoreline and gravel constituting about 25% of the south shoreline. During summer there is a dense growth of macrophytes, especially Potamogeton richardsonii (Benn.) Rydb., Myriophyllum exalbescens Fern. and Elodea canadensis Michx..

Monthly water chemistry samples were taken and Cairn Pond was found to be limnologically (and climatologically) similar to neighboring Newsome Pond, described in detail by Davies and Reynoldson (1976). The seasonal changes in water temperature of Cairn Pond follow the pattern shown in Figure 1.1, with open water from late April to early November.

The northern basin was used to examine the reproductive and feeding ecologies of sympatric populations of <u>N. obscura</u> and <u>E. punctata</u>.

1.1.2 Stephenson Pond

Stephenson Pond is located near Cairn Pond (114° 16' W, 51° 9' N) in similar topography with a surface area of about 2.2 hectares and maximum water depth of 2.5 meters. The pond has been enlarged by the construction of an earth-stone dike at the east end, and has its water level maintained partially by a small intermittent stream entering the western end. The catchment basin is heavily utilized by grazing livestock and consequently, there is high nutrient loading in the water body resulting in a blue-green algal bloom every summer. <u>P. balsamifera</u> and <u>Salix sp.</u> cover a hill down to the shoreline on the south end, with open grass and shrub constituting the remaining riparian vegetation. The littoral zone is mostly soft mud and sand with large rocks in the dike area and <u>P. richardsonii</u> and <u>M. exalbescens</u> being abundant in the summer. Stephenson Pond was climatologically and limnologically very similar to Cairn Pond and Newsome Pond (Davies and Reynoldson, 1976). <u>N. obscura</u> was extremely abundant with <u>E. punctata</u> only rarely encountered.

1.1.3 Bow River

All the <u>E. punctata</u> used in laboratory experiments were collected from the Bow River, about 1 km downstream from the outfall of the City of Calgary sewage treatment plant at Bonneybrook. Boulders, gravel

and sand were the predominant substrate types, but during July -

September, a dense growth of _Potamogeton filiformis_ Pers. occurred.

Current velocity ranges from 0.5 - 1.0 m·sec^{-1} and dissolved oxygen

levels were always near saturation.

The seasonal temperature pattern at the site is displayed in

Figure 1.1. During the winter months, the river never completely

froze due to the thermal influence from the sewage treatment plant.

Leeches could only be collected in months of low discharge (July -

April).
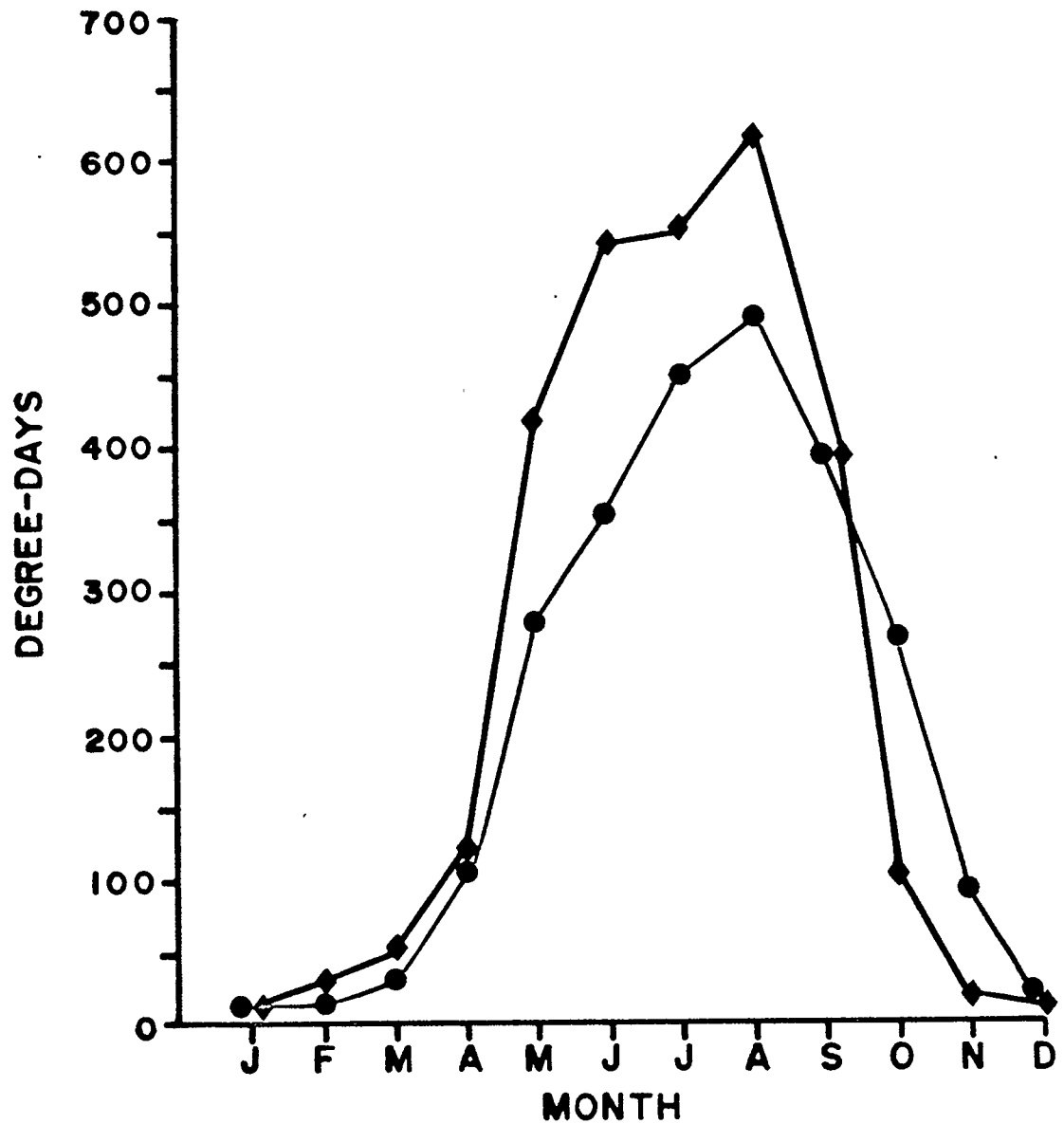
Figure 1.1:   Comparison of annual celsius degree-days of the
              lentic (◆) and lotic (●) sites where
              N. obscura and E. punctata occur (lentic data
              based on measurements at 1 m depth from Cairn
              Pond and four neighboring ponds; lotic data
              courtesy Alberta Environment, Calgary).

1.2 Statistical Methods

The following statistical methods were used for data analysis:

1.2.1 Analysis of Variance

Analysis of variance (ANOVA) was used whenever three of more experimental groups were tested for differences in population means with respect to treatment. The null hypothesis was always, H_o : $u_1 = u_2 = u_3 = ...u_k$, where, k = the number of experimental groups. Depending on the number (n) of treatment variables (factors) examined, an n-factor ANOVA was performed following the protocol of Zar (1974) using SPSS (Statistical Package for the Social Sciences) (Nie, Hull, Jenkins, Steinbrenner and Brent, 1975). When a single-factor ANOVA rejected the null hypothesis of equality of population means, a Student-Newman-Keuls (SNK) a posteriori multiple range test was used to determine between which population means the differences occurred (Zar, 1974).

A 3 X 3 Latin square experimental design (adopted from Cochran and Cox, 1957; Gale, 1971) which is a special type of ANOVA, was used in the analysis of substrate preference by N. obscura and E. punctata (Chapter 5). In a Latin square design, the treatments are grouped into replicates with every row and column of any square a complete replication (Cochran and Cox, 1957). This double replication min-imizes the errors in differences among rows (row effects) and columns (column effects) (Cochrane and Cox, 1957). The dispersal patterns of the leeches can be determined by examination of the row and column effects.

Since the substrate treatments are applied in sequence (both in row and column), there is a risk of residual or carry-over effects of one treatment onto the succeeding treatment. To overcome this problem, the Latin square design was balanced with respect to residual effects by having each treatment proceeded twice by each of the other treatments and the 3 X 3 Latin squares replicated four times within the apparatus. Subsequently, using the protocol forwarded by Cochran and Cox (1957), a computer program was written to calculate the direct effects of substrate treatment free from the influence of residual effects.

1.2.2 Regression and Covariance Analysis

Least-squares linear regression analysis (Zar, 1974) was used to examine the relationship between two variables (correlation) and provided an equation relating one variable to another. The independent and dependent variables of curvilinear data (i.e. power function) was log transformed to obtain a linear regression. The null hypothesis that the slope of a regression was not significantly different from zero was tested using a Students t-test and equality of slopes and intercepts amoung regressions was tested using analysis of covariance (Zar, 1974).

1.2.3 Cluster Analysis

Cluster analysis is a multivariate statistical technique designed
to delimit any underlying subpopulation (i.e. group) structure that
may exist in a large data set of multiple measurements. The algorithm
defines the subpopulations by determining group membership on the
basis of maximizing the differences between groups while minimizing
within group differences on the measured parameters.

Cluster analysis (Clustan 1C (Wishart, 1975)) was used to examine
intra- and inter-specific differences in temporal and weight class
prey utilization by N. obscura and E. punctata (Chapter 2). Ward's
method of hierarchical fusion, an agglomerative polythalic algorithm,
which calculates minimum variance spherical clusters was used
(Wishart, 1975). As the prey categories were generally
non-orthogonal, to maximize the separation of the derived clusters,
the data for each test used were transformed into a set of un-
correlated variables using Principal Component Analysis (PCA) (Cooley
and Lohnes, 1971; Wishart, 1975). The similarly matrix for cluster
analysis was calculated from the principal factor scores derived from
Principal Component Analysis, using Euclidean distance as the sim-
ilarity coefficient.

1.2.4 Goodness-of-Fit

The Kolmogorov-Smirnov two-sample (two-tailed) test was used following the procedures outlined in Siegel (1956) to determine whether two independent samples populations have the same distributions. The two-sample test investigates the agreement between two cummulative distributions and is sensitive to differences in central tendency, dispersion, and skewness. The Kolmogorov-Smirnov test was used to: 1) verify cluster membership, 2) test for between group (cluster) differences in proportional utilization of prey types (Chapter 2), and 3) to determine significant intra- and inter-specific differences in substrate preference (Chapter 5).

The Chi-square approximation statistic (d.f.= 2) was utilized for hypothesis testing when small, unequal sample sizes were encountered (Siegel, 1956).

1.2.5 Niche Metrics

A number of indices were used to quantitatively examine the food niches of N. obscura and E. punctata (Chapter 2). Intra- and inter-specific differences in food niche breadth were examined on a temporal and weight-class basis using the Shannon-Wiener diversity index and its related statistics (Poole, 1974). The Shannon-Wiener index summarizes in one value, the number of prey categories utilized (species richness) and the proportional contribution of each prey item to the overall diet. Maximum niche breadth occurs when all prey

categories consumed are taken in equal proportions. The measure of utilization Evenness is a metric that represents the deviation in prey use from the maximum potential niche breadth.

Schoener's (1970) index for niche overlap was used to examine inter-specific food resource utilization as Linton, Davies and Wrona (1981) using Monte Carlo computer simulations found this index to best quantify true overlap in the 10 - 90% overlap range.

All hypothesis testing was performed at the $p = 0.05$ level of probability.

2.0 EVIDENCE FOR COMPETITION

2.1 Introduction

In the lentic leech communities of Alberta and western Canada

N. obscura is usually the numerically dominant erpobdellid and

E. punctata the sub-dominant (Davies, 1973; Davies, Reynoldson and

Everett, 1977; Linton, Davies and Wrona, 1982). Davies, Reynoldson

and Everett (1977) studied the reproductive strategies of E. punctata

in two limnologically similar temporary ponds and found that in Jail

Pond, where E. punctata was sub-dominant, it had a simple annual

reproductive cycle, while in Cairn Pond, where E. punctata was un-

usually dominant, a more complex life cycle was found with repro-

duction occurring annually or semi-annually. Additionally, when

sub-dominant, E. punctata was found to reproduce at a smaller size,

after a shorter maturation period, earlier in the breeding season and

over a longer period than when dominant, where it reproduced at a

larger size, later in the breeding season and over a more restricted

period of time (Table 2.1). Therefore, when sub-dominant, E. punctata

displayed many of the characteristics of r-selection but when dom-

inant, it showed attributes more resembling the correlates of

K-selection (Pianka, 1970) (Table 2.2).

In contrast, irrespective of whether numerically dominant or

sub-dominant, N. obscura populations were found to produce two

generations of young annually, one in the spring and the other in late

TABLE 2.1

Ecological characteristics displayed by E. punctata in
two limnologically similar ponds where it was sub-
dominant (Jail Pond) and unusually dominant (Cairn Pond)
(from Davies, Reynoldson and Everett, 1977).

Characteristic	Jail Pond	Cairn Pond
Life Cycle	Annual	Annual or bi-annual
Reproductive Period	April-October	May-July
Mature Size	300-900 mg	600-1800 mg
Proportion Mature in potentially mature range	High	Low

TABLE 2.2

Morphological and life history characteristics a species would
theoretically possess under competitively lax (r-selected) and
competitively intense (K-selected) environments. (after Pianka,
1970).

r-Selection	K-Selection
Rapid development	Slower development
High maximum rate of increase	Greater competitive ability
Early reproduction	Delayed reproduction
Smaller body size	Larger body size
Single reproduction	Repeated reproduction
Intra-and inter-specific competition lax	Intra-and inter-specific competition keen

summer (Davies and Everett, 1977). The spring cohort produces young after either 12 or 15 months, while the fall cohort produces young after 12 or 19 months, with all individuals being semelparous.

It therefore appeared that the type of reproductive strategy used by E. punctata was correlated with the relative abundance of N. obscura, which together with the great dietary overlap in prey eaten by the two species, led Davies, Reynoldson and Everett (1977) to hypothesize that inter-specific competition for food resources was responsible for the observed differences.

Reynoldson and Bellamy (1970), Lock and Reynoldson (1976) and Williamson (1972) identified a number of criteria that could be used as appropriate evidence for the existence of inter-specific competition in the field. Competition in this context is defined as "the biological interaction between two or more organismic units that occurs when a) a necessary resource is in limited supply or b) resource quality varies and demand is quality dependent, resulting in reduced fitness and/or equilibrium population size of each organismic unit" (McNaughton and Wolf, 1973; Pianka, 1974). The criteria suggested are:

1) The comparative distribution and/or relative abundance of the two potentially competing species should be amenable to explanation based on competition.

2) The two species utilize a common resource which may provide the basis for competition.

3) Evidence from the performance of the species in the field that intra-specific competition is occurring.

4) The existence of character divergence between species suggesting that competition is or has been operating.

5) The existence of ecological (spatial or temporal) differences between the two species inferring that competition is or was operating.

6) That both the resource which is being competed for and population abundance should be manipulated separately in the field with predictable results based on the hypothesis that competition is occurring.

7) Events following the introduction or reduction of one of the potentially competing species should be consistent with the competition hypothesis.

Review of the available data on N. obscura and E. punctata in relation to the suggested criteria for inter-specific competition is given below:

Criterion 1:

The studies by Reynoldson and Davies (1976,1980) revealed a considerable overlap in the range of water conductances in which N. obscura and E. punctata can regulate their weights. However, as E. punctata appeared to osmoregulate more successfully at higher salinities than N. obscura, Reynoldson and Davies (1976, 1980) suggested that E. punctata could have a competitive advantage over N. obscura in waters of higher salinity. The more detailed study of Linton, Davies and Wrona (1982) found that both N. obscura and E. punctata were hypo-osmoregulators and concluded that the physio-

logical responses of the two species to ionic composition, total dissolved solids or temperature were not sufficiently different to cause the observed marked differences in relative abundances of sympatric populations. The sub-dominance of E. punctata in lentic habitats could thus not be explained on the basis of differential reaction to components of water chemistry, making the comparative distribution and abundance of N. obscura and E. punctata amenable to explanation based on competition.

Criterion 2:

In the laboratory, prey range experiments revealed that a considerable overlap existed between the diets of N. obscura and E. punctata with Chironomidae, Oligochaeta and Amphipoda being consumed in highest proportions (Davies and Everett, 1975).

Serological investigation of N. obscura and E. punctata in the field also revealed a considerable overlap in their diets (Davies, Wrona and Everett, 1978; Davies, Wrona, Linton and Wilkialis, 1981). For any given month, Chironomidae, Oligochaeta and Amphipoda formed the major components of the diet of both species. Copepoda/ Cladocera were consumed by N. obscura and E. punctata in low proportion in the summer and the only food resource not utilized by both species were Gastropoda, consumed occasionally by N. obscura (Davies, Wrona, Linton and Wilkialis, 1981).

Criterion 3:

Indirect evidence that intra-specific competition is occurring in the field was obtained from the studies of Everett (1974) and Davies,

Reynoldson and Everett (1977), who found for both N. obscura and

E. punctata that mortality rate of small individuals occurred in the

period immediately following recruitment. This is comparable to the

results given by Reynoldson and Bellamy (1970) and Lock and Reynoldson

(1976), who used a similar argument as partial evidence for the

occurrence of intra-specific competition in freshwater triclad spe-

cies. It should be noted that in lentic ecosystems of Alberta,

leeches do not have important predators and thus predation could not

be involved to explain this phenomenon (Everett, 1974).

Intra-specific partitioning of food resources as a function of

differential weight class utilization by N. obscura and E. punctata

(Davies, Wrona, Linton and Wilkialis, 1981) is also indirect evidence

for intra-specific competition operating. Since natural selection

operates to maximize fitness, predators such as N. obscura and

E. punctata would be expected to subdivide their life cycles into size

(age) classes, each differing in terms of resource utilization since

this would decrease intra-specific competition and thereby increase

population fitness (Giesel, 1974). Thus the performance of popu-

lations of N. obscura and E. punctata in the field suggests that

intra-specific competition for food resources is occurring.

Criterion 4:

The existence of morphological character divergence between

N. obscura and E. punctata has not been quantitatively examined in the

literature and this criterion can not be used to support the com-

petition hypothesis.

Criterion 5:

Intra- and inter-specific partitioning of food resources was found to occur on a temporal and weight (size) age basis between sympatric populations of *N. obscura* and *E. punctata* (Davies, Wrona, Linton and Wilkialis, 1981). Inter-specific food niche overlap occurred through the spring, summer and autumn, but Davies, Wrona, Linton and Wilkialis (1981) hypothesized that the probability for inter-specific competition was highest in the late summer and early fall when the niche breadths of both species were found to decline, indicating increased specialization in prey use. These results support the hypothesis that inter-specific competition for food is occurring between the two species.

Criterion 6:

No field experiments involving the manipulation of the food resources and population abundances yielding results that are pre-dictable from competition theory have been successfully completed on *N. obscura* and *E. punctata*.

Criterion 7:

It was hypothesized by Davies, Reynoldson and Everett (1977) that the differences in the type of reproductive strategy displayed by *E. punctata* was functionally related to the relative abundance of its potential competitor *N. obscura* and therefore resulted from inter-specific competition for food resources.

2.2 Objectives

The objectives of this study are to further examine the question of whether inter-specific competition potentially occurs between N. obscura and E. punctata and can therefore be used as an ecological explanation for the observed differences in their distributions and abundances. The study will quantitatively investigate the following criteria;

Criterion 4: Determine whether morphological character divergence exists between N. obscura and E. punctata.

Criterion 7: Determine whether the feeding strategies of N. obscura and E. punctata in Cairn Pond, when E. punctata was numerically dominant (1976) and during the immediate transition period to dominance by N. obscura (1978) are compatable to explanation by the competition hypothesis.

2.3 Materials and Methods

2.3.1 Collection Methods

N. obscura and E. punctata, when occurring in shallow water (early spring to late summer) were collected by hand from the undersurface of stones. After the fall migration to deeper water (Davies and Everett, 1977; Davies, Reynoldson and Everett, 1977), an Ekman grab ((0.23 m^3) was used through holes cut in the ice with a chain saw. Samples were taken monthly for each of the two years investigated. The first 50 specimens collected were identified, to give the proportional representation of each species and collections continued until 50 of the dominant and 25 of the sub-dominant species were obtained. After collection the specimens were blotted with tissue to remove excess water, immediately frozen on dry ice, and returned to the laboratory. The specimens were weighed to the nearest 0.1 mg on a Mettler H10 analytical balance and stored at -20 °C for later serological analysis.

2.3.2 Morphological Analyses

The morphological parameters measured on both species were wet weight, dry weight, length, and the diameters of the posterior sucker, anterior sucker and mouth. Wet weights were obtained from thawed specimens blotted dry to remove excess water and subsequently weighed on a Mettler H10 analytical balance to the nearest 0.1 mg. Length was measured to the nearest 1.0 mm using a gridded petri dish. Measurements of anterior and posterior sucker and mouth diameters were

obtained using a Wild M5 dissecting microscope fitted with a stage
micrometer with an accuracy of 0.05 mm. To obtain dry weights, the
leeches were re-frozen at -20 °C, placed in a Thermovac (Model
FD-Port) freeze-dryer for 24 hours, and re-weighed to the nearest
0.1 mg. Morphological measurements were made on both N. obscura and
E. punctata in the 10 - 1000 mg wet weight range.

2.3.3 Serological Methods

Gut content analyses of field specimens were performed using
serological techniques (Davies, 1969; Davies, Wrona and Linton, 1979).
Specific rabbit antisera were produced against Copepoda/Cladocera,
Chironomidae, Oligochaeta, Amphipoda and Gastropoda, covering the
range of prey utilized by both species (Davies and Everett, 1975).
The titer and the detection period were determined for each antiserum
(Table 2.3). Titer is the immunological strength or capacity of an
antiserum to detect proteins, which is based on the reciprocal value
of the highest two fold serial dilution of a known standard (10 mg
prey antigen in 1 ml physiological saline). Detection period is the
length of time a meal can be serologically detected in the gut of a
predator after ingestion.

All serological testing was performed using a modified
Oakley-Fulthorpe precipitin test (Davies, 1969) using leech gut
content solutions as the antigens (Davies, Wrona and Linton, 1979).
Serological testings were performed five times for each field
specimen, utilizing each of the five prey antisera. All tests were

incubated at room temperature for 72 hours before being read. A positive precipitin reaction from a field specimen indicated that the particular prey category had been consumed within the defined detection period for that prey item, while a negative reaction indicated that it had not.

Since N. obscura and E. punctata both feed on prey of different sizes and frequently group feed, the quantification of the serological techniques was determined on the basis of full gut equivalence rather than the number of prey consumed (Davies, Wrona, Linton and Wilkialis, 1981). Quantification was performed by feeding groups of starved N. obscura and E. punctata until satiated with one of each of the five potential prey categories. Groups of five leeches were subsequently killed at fixed time intervals after ingestion and tested serologically. In this manner the time period over which the prey category could be detected in the leech gut by the antiserum was determined and was defined as the detection period (Table 2.3). As the detection periods were determined at 20 °C, the estimate of predation on a given prey item will be correct for the majority of the field season (spring - summer). Feeding in colder months (fall - winter) will be overestimated because at low temperatures the actual detection period will probably be longer than that determined at 20 °C (Davies and Reynoldson, 1971). To allow for comparisons in prey utilization within and between the two leech species, it was necessary to adjust for differences in detection period between the prey categories by multiplying the serological data by a correction factor. The calculated correction factors standardized the differences in detection

times amoung prey to a common length of time, insuring that the corrected number of observed positive reactions were integers (Table 2.3).

TABLE 2.3

The final titer of the antisera, the detection period (hours) of the prey antigens
and the correction factors used to adjust for differences in the detection periods of the prey for
Nephelopsis obscura and Erpobdella punctata together with the uncorrected number of positive precipitin
reactions, the percentage of individuals with multiple (ie. more than one) positive precipitin
reactions and the percentage of individuals giving a positive precipitin reaction from Cairn Pond,
Alberta for 1976 and 1978.

	Nephelopsis obscura					Erpobdella punctata				
	Titre	Detection Period	Correction Factor	No. + 1976	1978	Titre	Detection Period	Correction Factor	No. + 1976	1978
Antiserum										
Cladocera/ Copepoda	128	24	6	4	6	128	36	4	15	7
Chironomidae	128	36	4	44	93	128	36	4	62	38
Oligochaeta	64	36	4	44	76	64	36	4	53	30
Amphipoda	64	48	3	24	51	64	48	3	63	46
Gastopoda	64	48	3	5	0	64	48	3	0	0
No. Tested				300	600				600	300
% +				38.3	37.5				31.5	35.3
% Multiple Positives				2.0	0.3				0.7	5.0

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2.4 Results

2.4.1 Morphological Comparison

A simple linear regression model through the origin was found to best represent the relationships between dry and wet weight for N. obscura (r^2 = 0.93, P<0.05) and E. punctata (r^2 = 0.94, P<0.05) (Figure 2.1). Analysis of covariance revealed no significant inter-specific differences in the relationship between dry and wet weight, thereby allowing the common regression equation;

$$DWT = 0.143 \cdot WWT$$

where,

$$DWT = \text{dry weight (mg)}$$
$$WWT = \text{wet weight (mg)}$$

to be used for both species for conversion of wet weight data. The linear measures of length, anterior and posterior sucker diameter, and mouth diameter were found to be related to wet weight by a power function (Figures 2.2 - 2.5). Covariance analysis showed no significant differences between N. obscura and E. punctata in the manner posterior sucker diameter was related to wet weight (Figure 2.2). However, significant inter-specific differences were found in the relationships between length, anterior sucker and mouth diameter in relation to wet weight (Figure 2.3 - 2.5). E. punctata was longer for

a given wet weight than N. obscura (Figure 2.3), while N. obscura had

a larger anterior sucker (Figure 2.4) and mouth diameter (Figure 2.5)

on a wet weight basis than E. punctata.
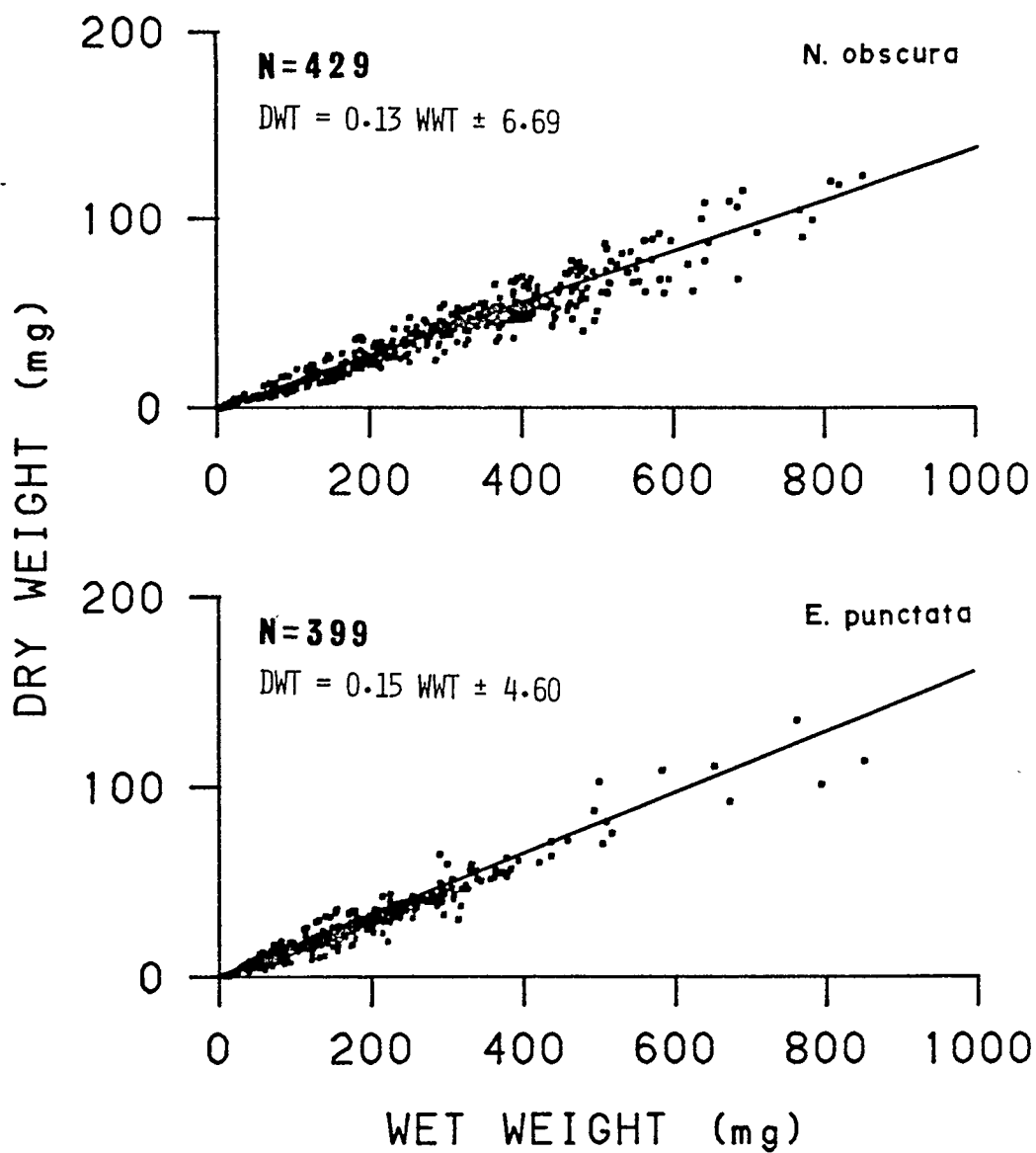
Figure 2.1: The relationship between dry weight and wet weight (± SE of regression) for N. obscura and E. punctata.

$$LOG_{10}L = 0.915 + 0.312 \ LOG_{10}WWT \pm 0.059$$

$$LOG_{10}L = 0.839 + 0.361 \ LOG_{10}WWT \pm 0.048$$
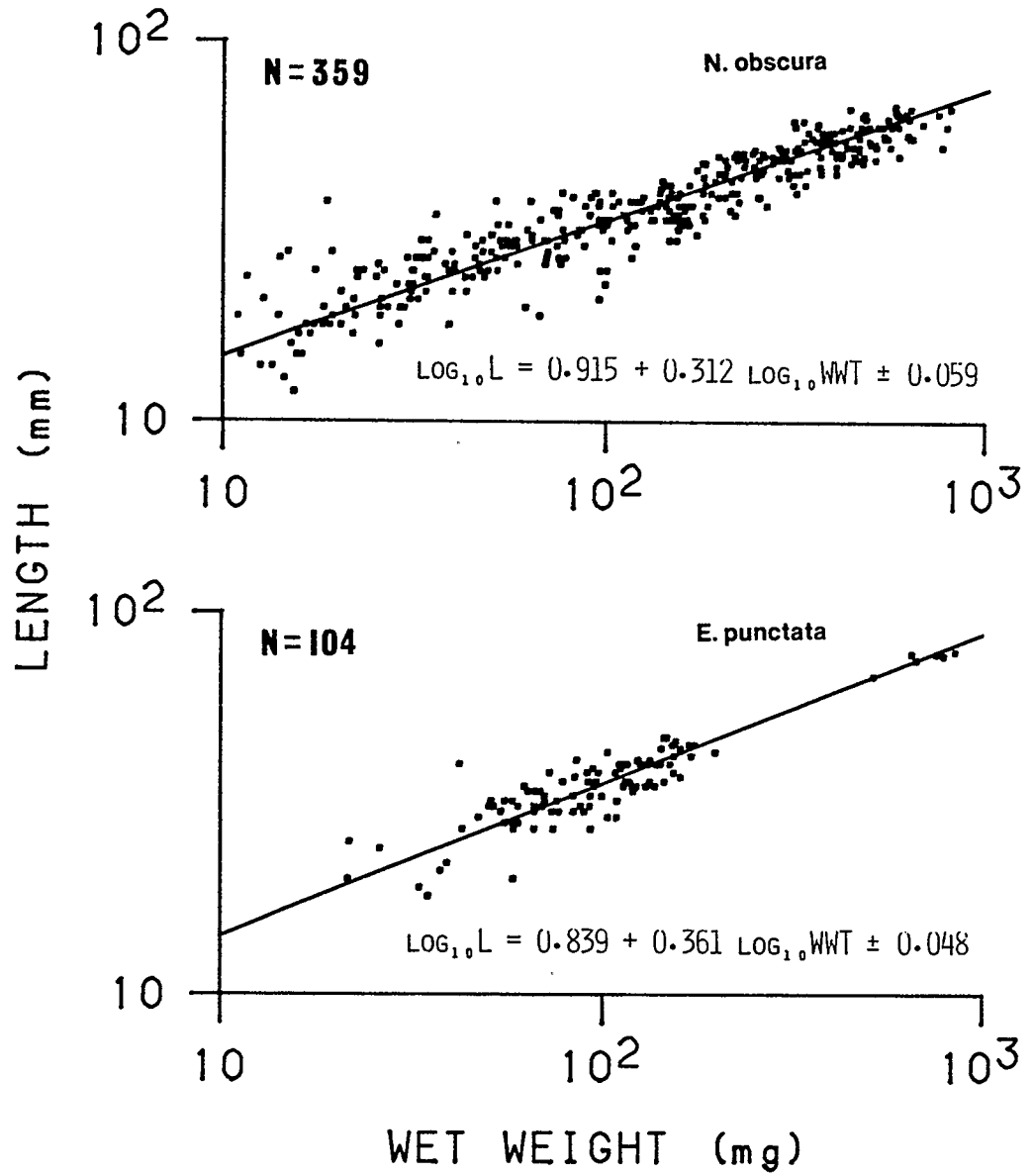
Figure 2.2:   The relationship between length and wet weight
(± SE of regression) for N. obscura and
E. punctata.

$$\text{LOG}_{10}\text{AS} = 0.013 + 0.147 \; \text{LOG}_{10}\text{WWT} \pm 0.030$$

$$\text{LOG}_{10}\text{AS} = -0.022 + 0.186 \; \text{LOG}_{10}\text{WWT} \pm 0.046$$
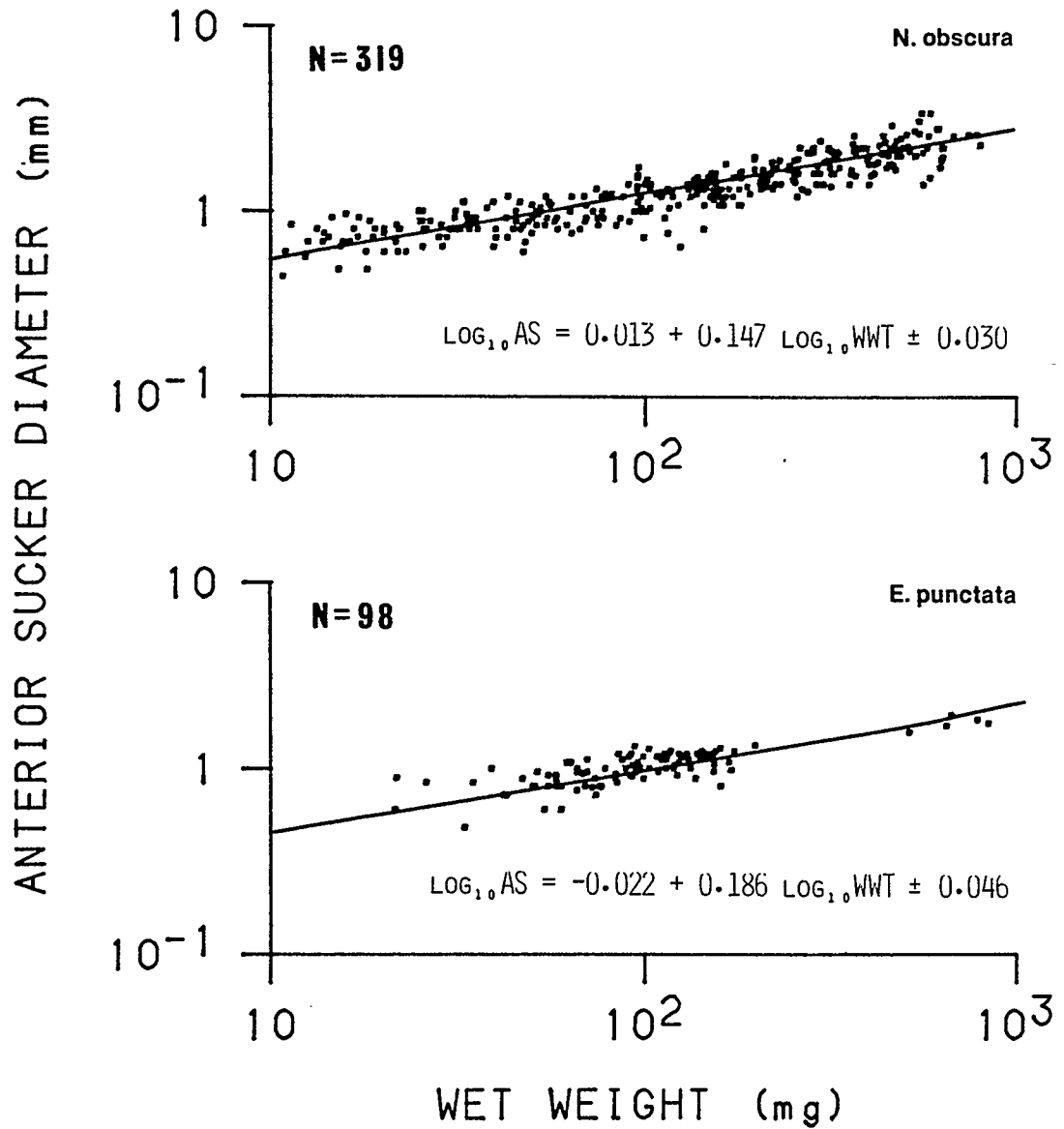
Figure 2.3: The relationship between anterior sucker
diameter and wet weight (± SE of regression) for
N. obscura and E. punctata.
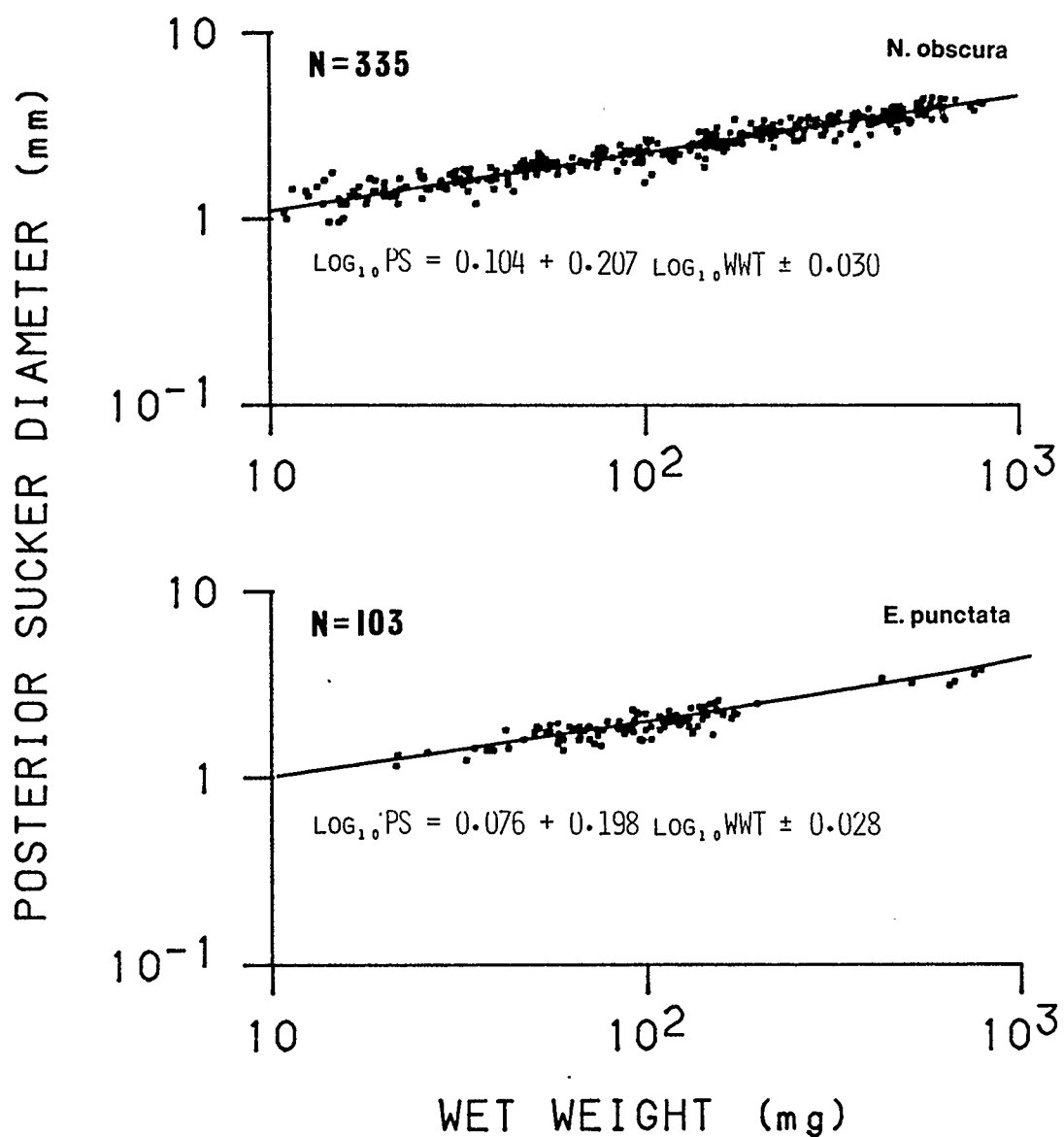
Figure 2.4:   The relationship between posterior sucker
diameter and wet weight (± SE of regression) for
N. obscura and E. punctata.

N. obscura

N=310

$LOG_{10}MD = -0.078 + 0.157 LOG_{10}WWT \pm 0.045$

E. punctata

N=95

$LOG_{10}MD = -0.006 + 0.104 LOG_{10}WWT \pm 0.029$
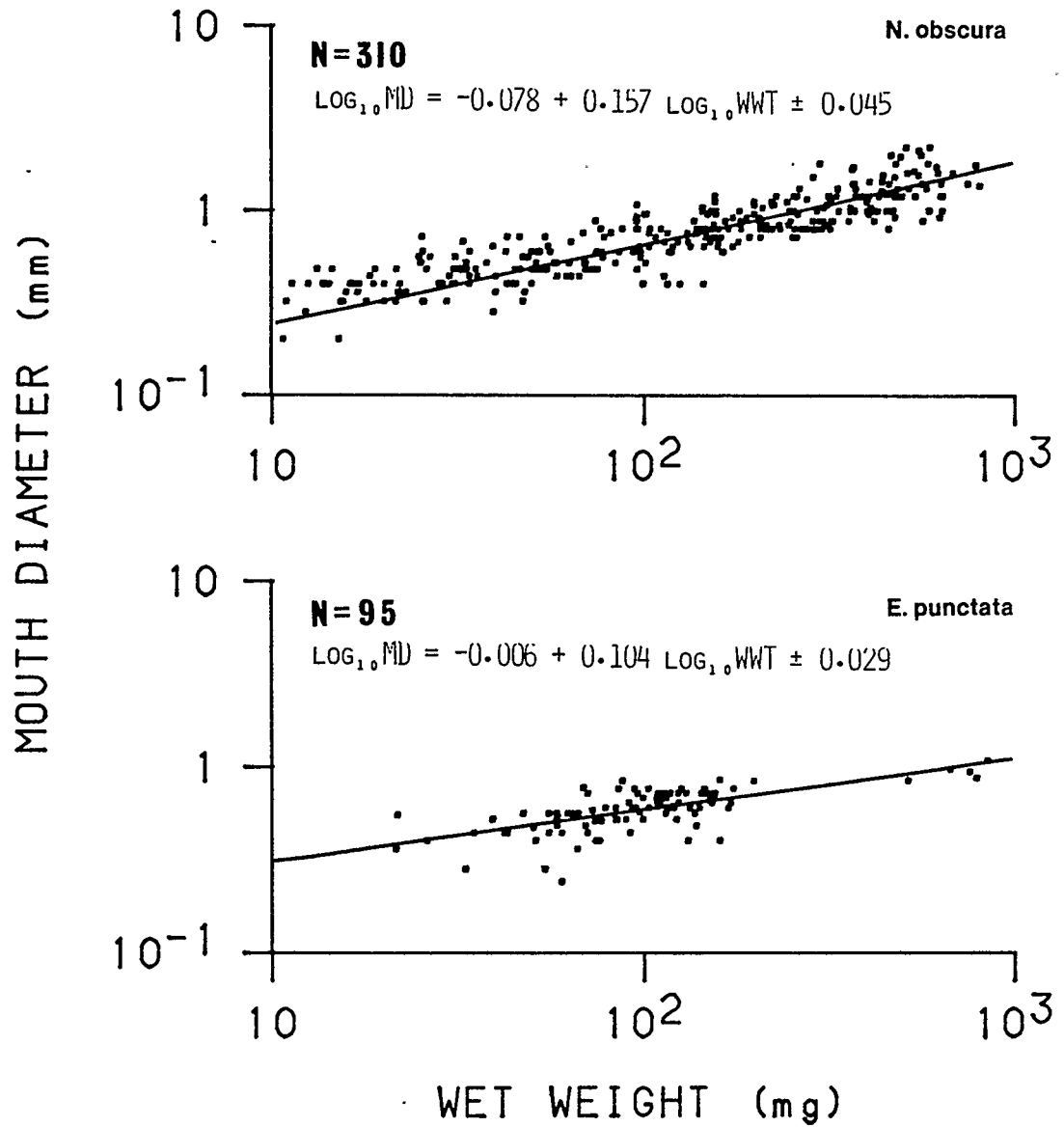
Figure 2.5:   The relationship between mouth diameter and wet weight (± SE of regression) for N. obscura and E. punctata.

2.4.2 Serological Field Study

In both 1976, when numerically dominant and in 1978 when
sub-dominant, E. punctata showed decreases in proportional abundance
from 82% to 70% in 1976, and 24% to 8% in 1978 (Figure 2.6).
Conversely, in both years N. obscura showed corresponding increases in
proportionality.

Defined as the percentage of individuals tested that gave a
positive precipitin reaction, the feeding activity of N. obscura and
E. punctata (Figure 2.7) showed no significant differences between
species or years, with maximum feeding activity occurring in the
spring and summer months for both species. Additionally, the feeding
activity of E. punctata was generally lower in the fall and winter
than observed for N. obscura.

At the species level, no significant differences in prey uti-
lization were found for either leech species between years (Figure
2.8) despite the very low consumption of Gastropoda by N. obscura in
1976. However, N. obscura and E. punctata did differ significantly
from each other in 1978, but not in 1976.

In both years, on a monthly basis, Amphipoda were more heavily
utilized by E. punctata and Oligochaeta more heavily utilized by
N. obscura (Figures 2.9, 2.10) although with the change in dominance,
utilization of Amphipoda by E. punctata increased in 1978. Chi-
ronomidae were utilized most heavily by E. punctata in late winter -
early spring months and by N. obscura throughout the remainder

1976
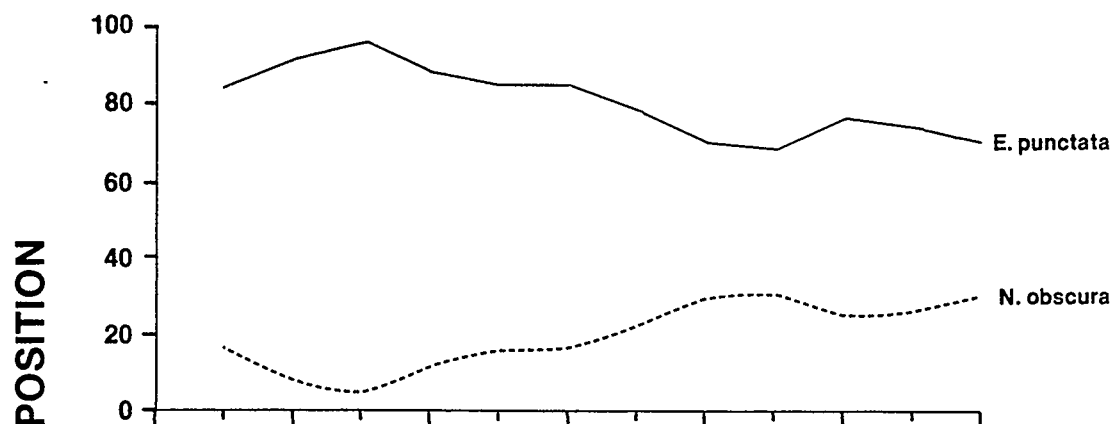
1978
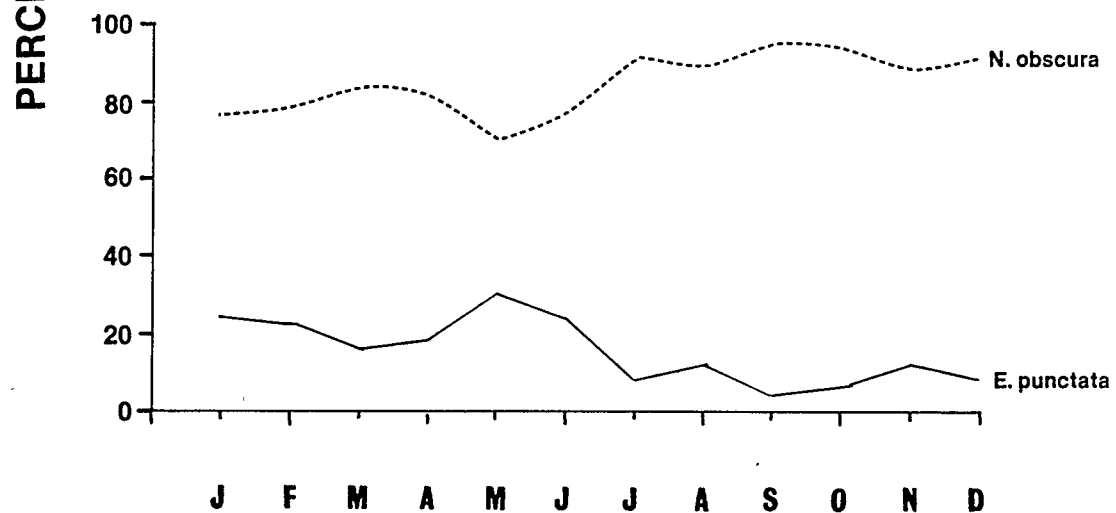
Figure 2.6:   Percent composition of N. obscura and E. punctata in Cairn Pond.
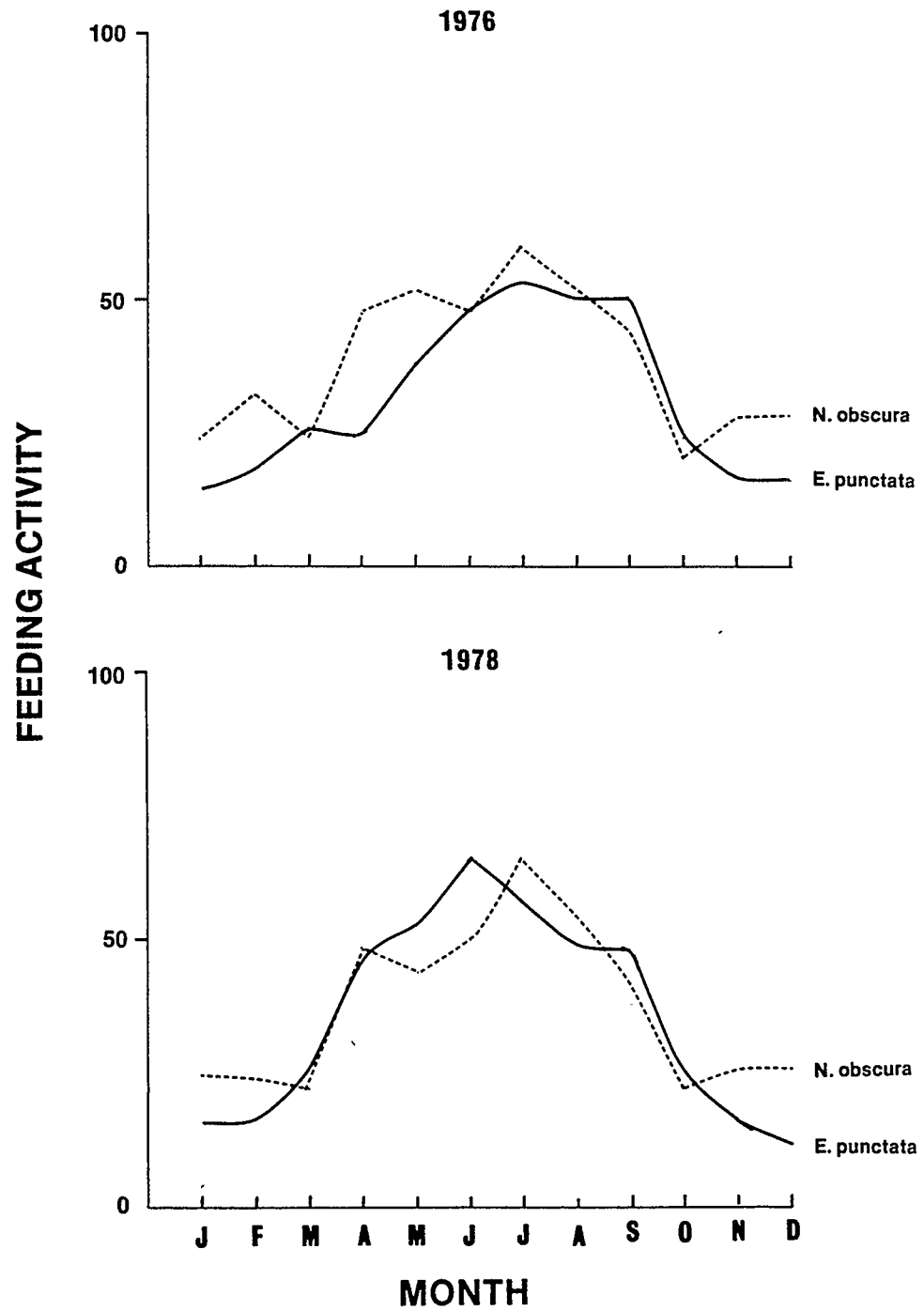
Figure 2.7:   Temporal feeding activity of N. obscura and
              E. punctata.

1976

1978
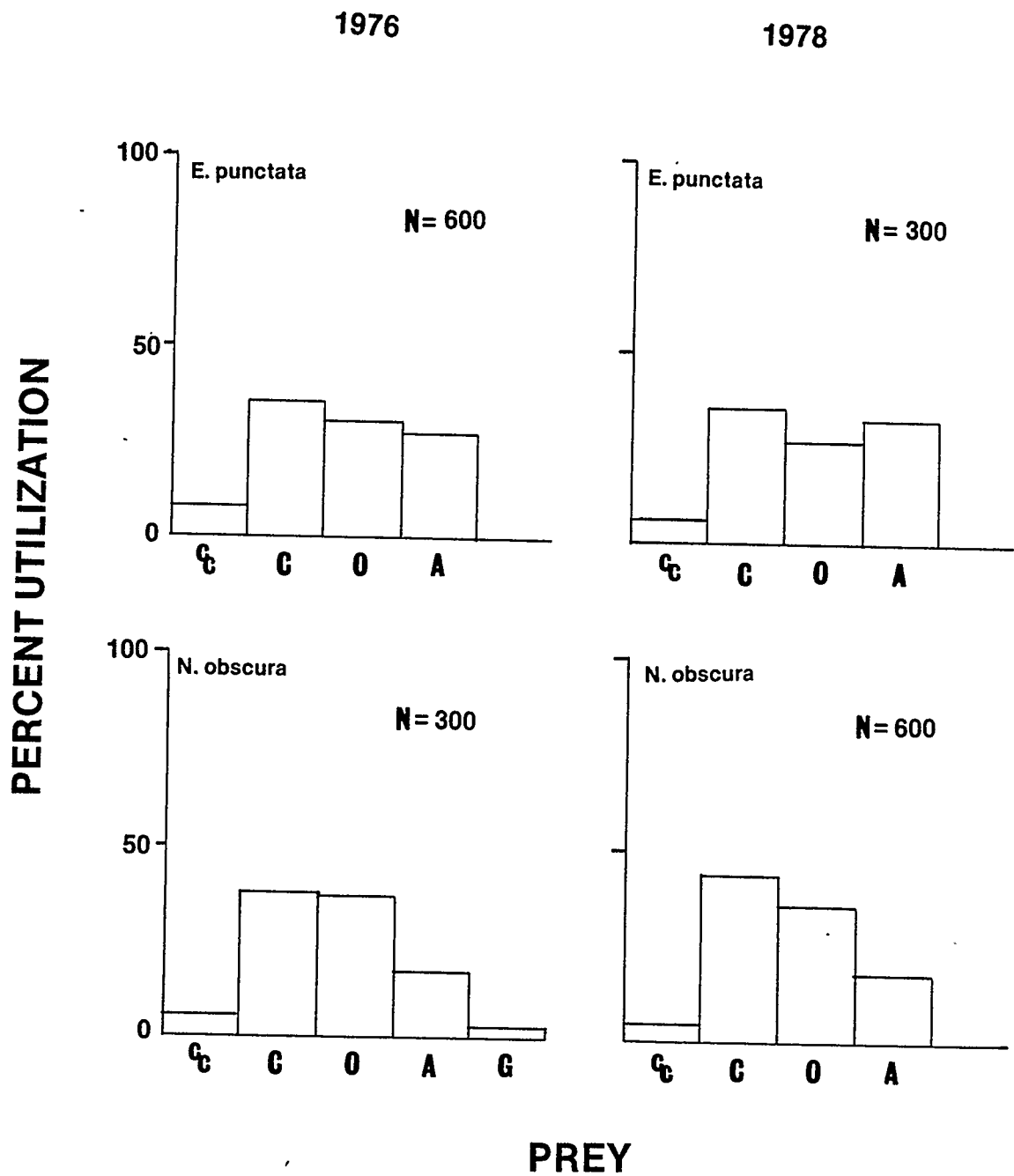
Figure 2.8: Percent frequency prey utilization histograms for <u>N. obscura</u> and <u>E. punctata</u>.

1976

$\bar{x} = 0.77$

OVERLAP

Copepoda/Cladocera

Chironomidae

DIET

Oligochaeta

OF

Amphipoda

PROPORTION

Gastropoda

J F M A M J J A S O N D

MONTH
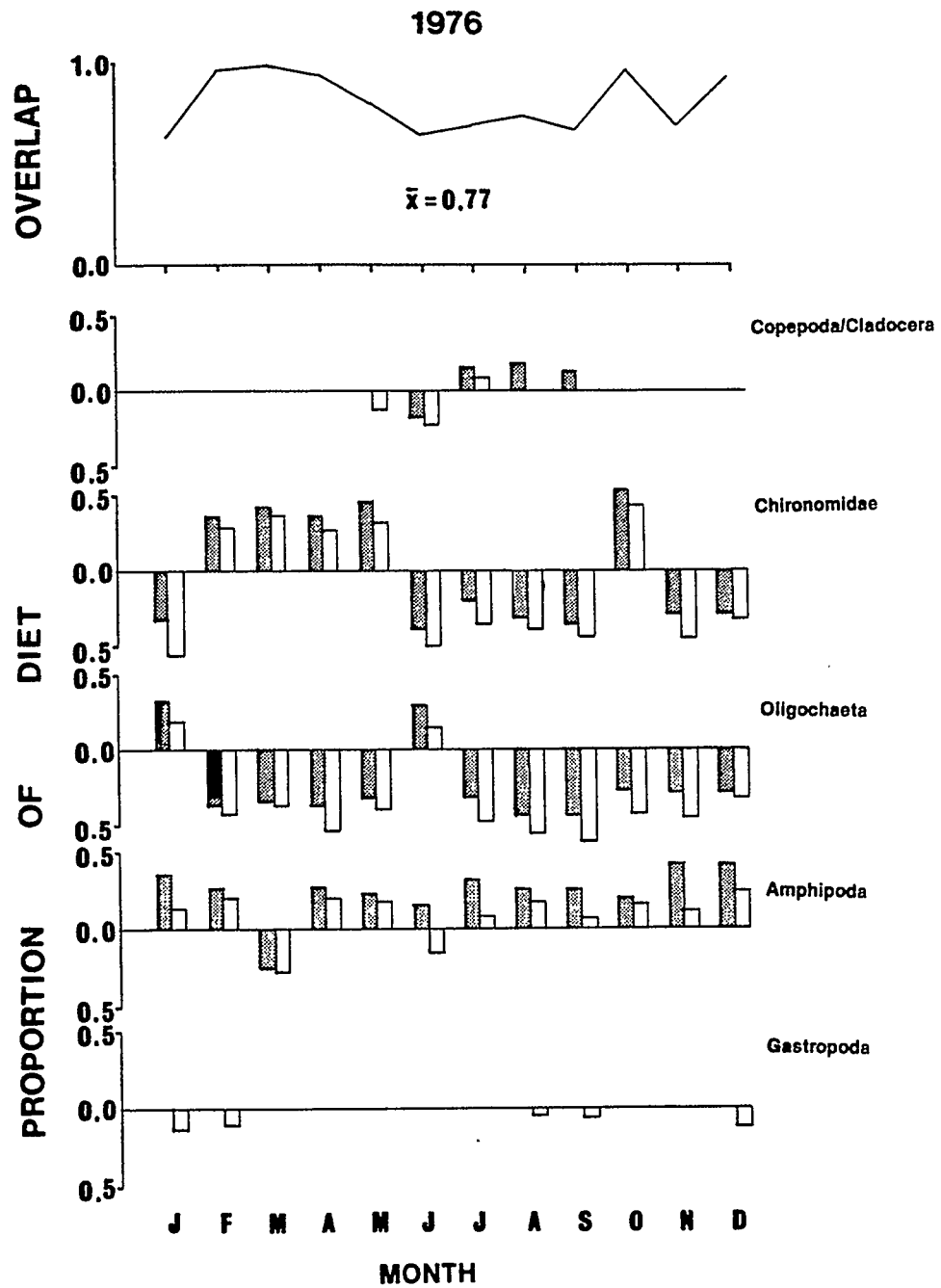
Figure 2.9:   Niche overlap between N. obscura and E. punctata in 1976 and the proportional utilization of each prey category. E. punctata (hatched) plotted above the line when the major consumer and N. obscura (clear) plotted below the line when the major consumer.

1978

$\bar{x} = 0.68$
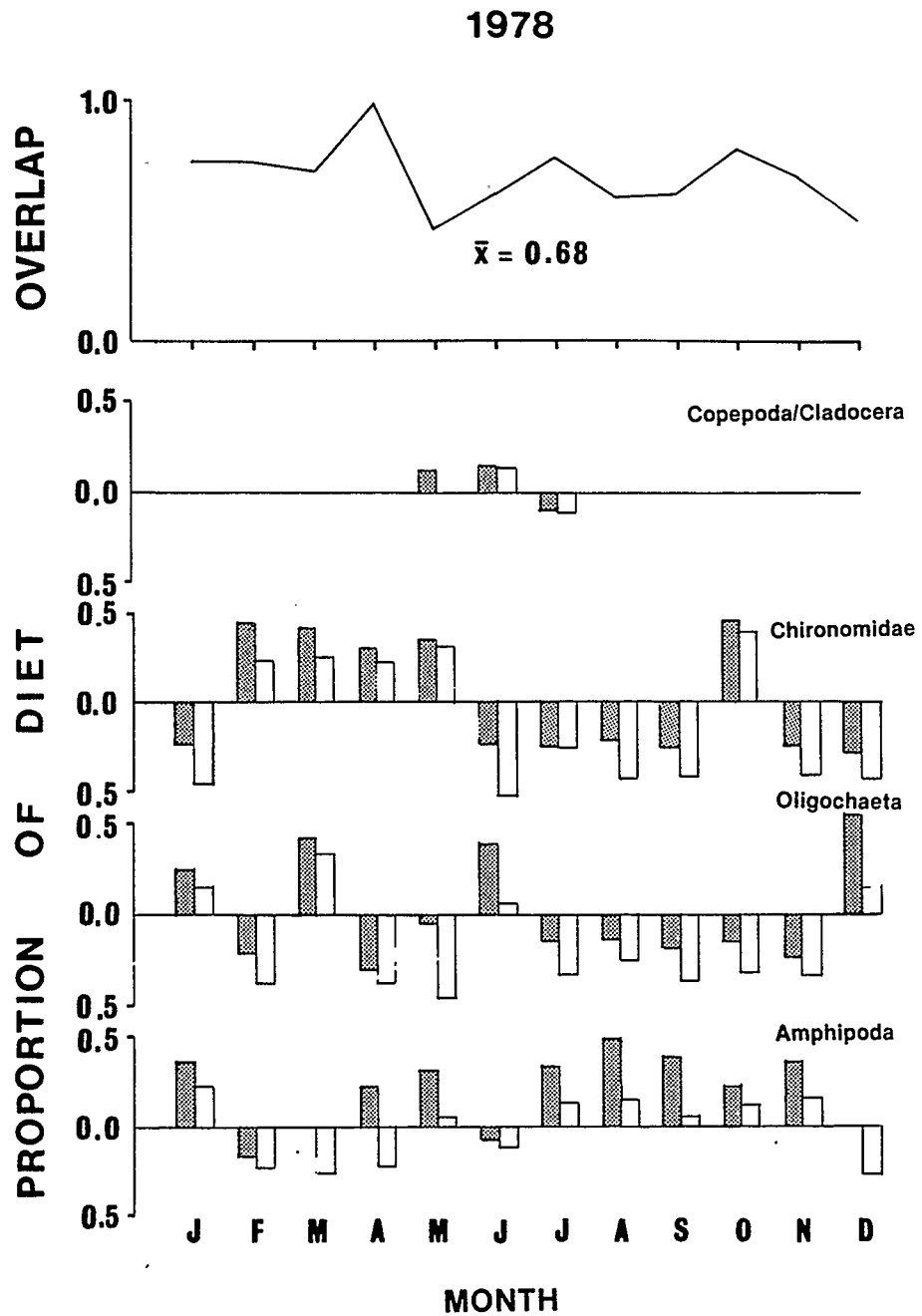
Figure 2.10: Niche overlap between N. obscura and E. punctata in 1978 and the proportional utilization of each prey category. E. punctata (hatched) plotted above the line when the major consumer and N. obscura (clear) plotted below the line when the major consumer.

of the year, regardless of dominance (Figures 2.9, 2.10). N. obscura however increased its proportional use of Chironomidae when it became dominant in 1978. Both species only utilized Copepoda/Cladocera to a small extent during parts of the spring and summer (Figure 2.9, 2.10).

Cluster analysis delineated two groups of sampling months in 1976 and three groups in 1978 (Figure 2.11) which displayed unique trends in prey utilization by E. punctata. These temporal differences were a function of Copepoda/Cladocera utilization and decreased proportional utilization of Chironomidae in the summer (May - August) of 1976 compared to the rest of the year. In 1978, June was characterized by high proportional utilization of Oligochaeta followed by Chironomidae, Amphipoda and Copepoda/Cladocera compared to the rest of the summer, which showed high Amphipoda and Chironomidae utilization, and lower Oligochaeta, and Copepoda/ Cladocera utilization. In the winter, when Copepoda/Cladocera were not utilized, Chironomidae and Oligochaeta formed the major components of the diet. In neither year did N. obscura show temporal differences in feeding (Figure 2.12). Chironomidae, Oligochaeta and Amphipoda constituted the major com- ponents of the diet of N. obscura when sub-dominant or dominant (Figure 2.12).

Within each of the defined temporal groups, cluster analysis segregated the weight classes of E. punctata into weight ranges displaying unique trends in prey utilization. In both temporal groups delineated in 1976, the majority of weight range groups
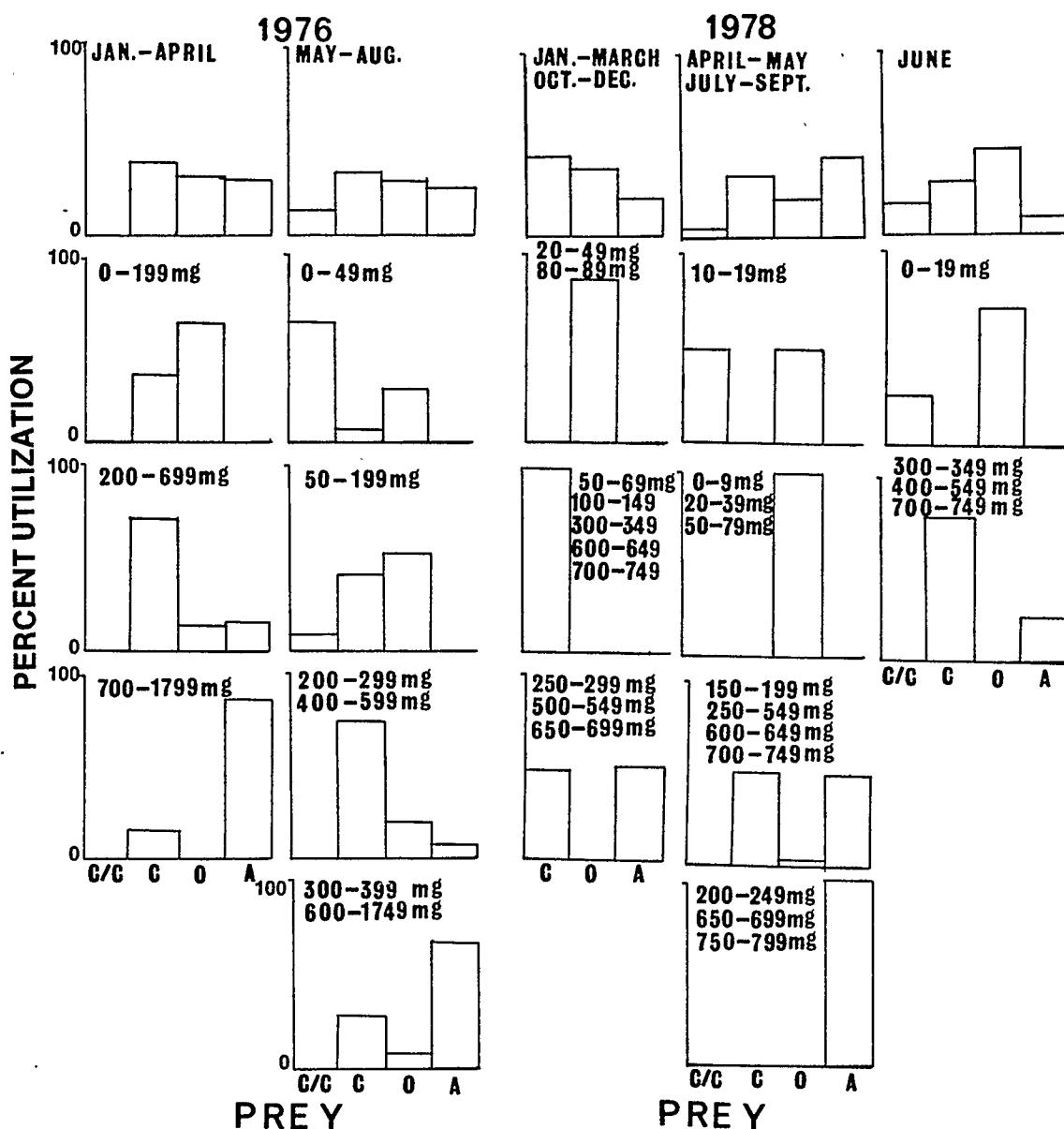
Figure 2.11: The percentage utilization of prey (C/C = Copepoda/Cladocera, C = Chironomidae, O = Oligochaeta, A = Amphipoda, G = Gastropoda) shown by E. punctata for these groups of months and those weight ranges delineated by cluster and Kolmogorov-Smirnov analyses as displaying unique trends in prey utilization.

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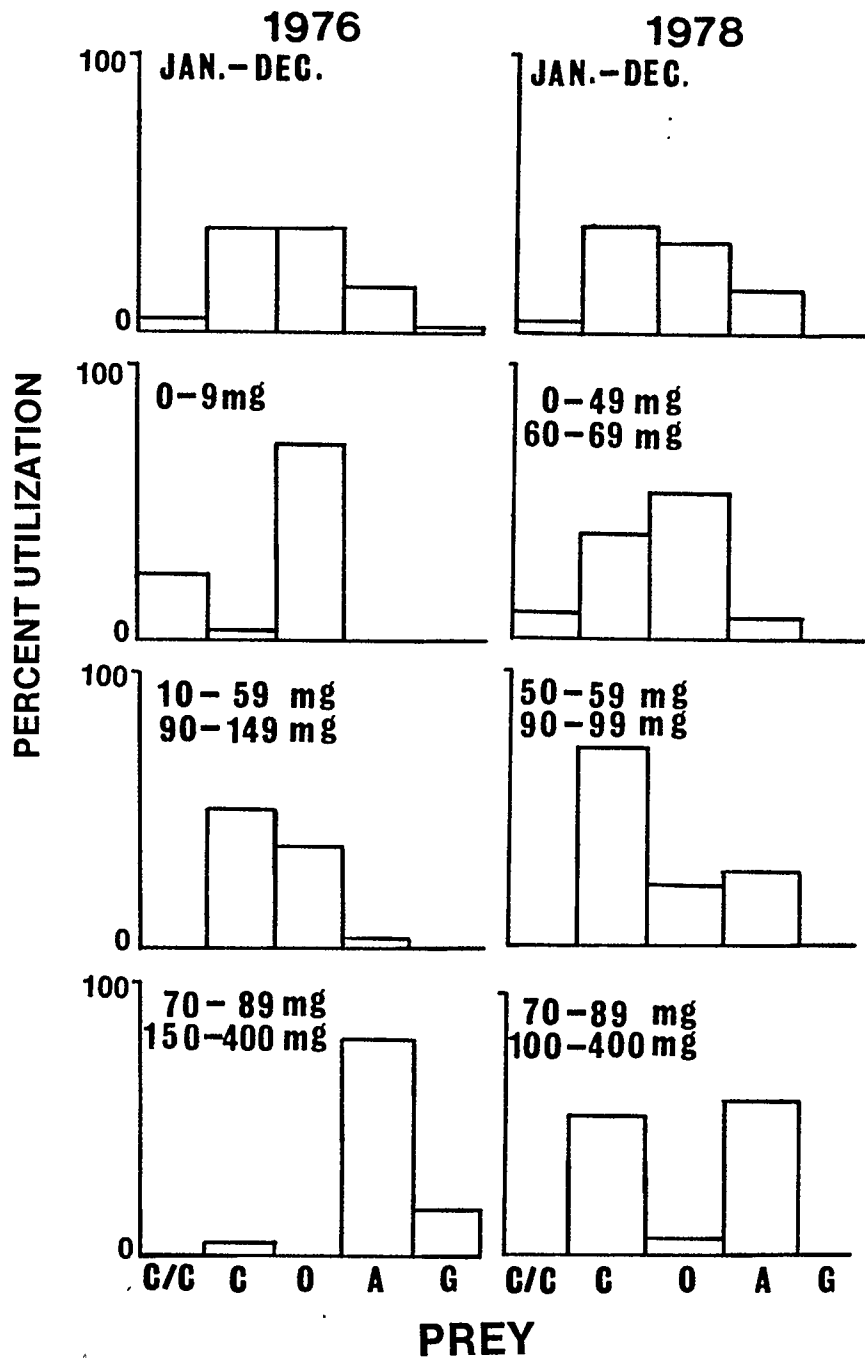
Figure 2.12: The percentage utilization of prey (C/C = Copepoda/Cladocera, C = Chironomidae, O = Oligochaeta, A = Amphipoda, G = Gastropoda) shown by N. obscura for these groups of months and those weight ranges delineated by cluster and Kolmogorov-Smirnov analyses as displaying unique trends in prey utilization.

consumed three prey types, with smaller leeches feeding heavily on Oligochaeta and Copepoda/Cladocera and larger leeches feeding more on Chironomidae and Amphipoda (Figure 2.11). In 1978 when sub-dominant, there was a reduction in prey types taken, with each weight group of E. punctata showing greater specialization (Figure 2.11). The weight groups within each temporal period were less cohesive with very different weight classes clustered within a weight group. Additionally the 800-1700 mg weight range present in 1976 was absent in 1978 (Figure 2.11). However, similar to 1976 the smaller leeches tended to utilize more Oligochaeta and Copepoda/Cladocera and the larger individuals more Chironomidae and Amphipoda.

N. obscura showed three weight groups with unique feeding trends, with a minimum of three prey types utilized by each (Figure 2.12). The weight groups of N. obscura were more cohesive in weight class membership and could be summarized as being representative of the feeding trends of small, medium and large individuals (Figure 2.12). Small N. obscura tended to utilize more Oligochaeta than other weight groups and were also the only weight range that consumed Copepoda/Cladocera. Chironomidae were taken in highest proportion by the medium sized individuals while Amphipoda were consumed the most by large individuals (Figure 2.12).

Analysis of the temporal niche breadth and utilization evenness values for E. punctata (Table 2.4) shows lower mean niche breadth when sub-dominant than when dominant, although the seasonal pattern of change in niche breadth was similar in both years. Niche evenness was

higher and less variable in E. punctata when it was dominant. N. obscura had a higher mean niche breadth in 1976 but the evenness values were similar in both years.

Schoener's niche overlap index showed a mean overlap of 0.77 when E. punctata was dominant in 1976 (Figure 2.11) and a lower value of 0.68 when N. obscura was dominant in 1978. In both years niche overlap was higher in the spring and early fall.

Table 2.4

Niche breadth (B) and Evenness (E) for Erpobdella punctata and
Nephelopsis obscura for each sampling month.

Month	E. punctata				N. obscura			
	1976		1978		1976		1978	
	B	E	B	E	B	E	B	E
January	1.58	1.00	1.56	0.98	1.71	0.85	1.44	0.91
February	1.57	0.99	1.46	0.92	1.85	0.92	1.54	0.97
March	1.55	0.98	1.00	1.00	1.57	0.99	1.57	0.99
April	1.57	0.99	1.57	0.99	1.46	0.92	1.54	0.97
May	1.53	0.96	1.72	0.86	1.86	0.93	1.27	0.80
June	1.90	0.95	1.77	0.89	1.80	0.90	1.52	0.76
July	1.94	0.97	1.86	0.93	1.66	0.83	1.87	0.93
August	1.97	0.99	1.40	0.88	1.74	0.87	1.46	0.92
September	1.92	0.96	1.53	0.96	1.56	0.78	1.30	0.82
October	1.46	0.92	1.44	0.91	1.47	0.93	1.44	0.91
November	1.56	0.98	1.56	0.98	1.39	0.88	1.40	0.89
December	1.56	0.98	0.92	0.92	1.91	0.96	1.46	0.92
Mean	1.68	0.97	1.48	0.93	1.67	0.89	1.48	0.90

2.5 Discussion

When the data from this study and the information previously collected are examined in relation to the established criteria (Reynoldson and Bellamy, 1970; Williamson, 1972; Lock and Reynoldson, 1976), the hypothesis that inter-specific competition is occurring between N. obscura and E. punctata is further reinforced.

Reynoldson and Davies (1976, 1980) showed a considerable overlap in the range of water conductances in which N. obscura and E. punctata could osmotically regulate their weights. However, when sympatric in lentic habitats, there usually is a strong inverse relationship between the numerical abundances of N. obscura and E. punctata (Davies, Everett and Reynoldson, 1977) with the dominance of E. punctata over N. obscura in lentic habitats in Alberta a very ephemeral event.

In 1971 Cairn Pond was a temporary autumnal pond (Wiggins, Mackay and Smith, 1980) and no leeches were recorded. In February 1974, E. punctata was dominant (Davies, Reynoldson and Everett, 1977) and remained so until some time in 1977 (i.e. a period of 3-6 years). The dominance of E. punctata in Cairn Pond until 1977 could have been due to E. punctata arriving in the pond before N. obscura either as a function of chance dispersal or of its better dispersive abilities. Alternatively the temporary dominance of E. punctata could have been due to its ability to withstand dessication and low water levels better than N. obscura (Kenk, 1949; Wiggins, Mackay and Smith, 1980). During 1974-75, E. punctata declined from 92% to 83% (Davies, Reynoldson and Everett, 1977) and from 82% to 70% in 1976 (Figure

2.6). The switch to dominance by <u>N. obscura</u> in 1977 was rapid with <u>E. punctata</u> reduced to 24% by the beginning of 1978 (Figure 2.6). Additionally, <u>E. punctata</u> displayed a different reproductive strategy when it was numerically dominant than when it was sub-dominant (Davies, Reynoldson and Everett, 1977).

Higher feeding activities occurred in the spring and summer months while lower activities were observed in the fall and winter for both species (Figure 2.7). This was probably a function of lower metabolic activity of the leeches rather than reduced prey availability as the winter Ekman grabs contained large numbers of potential prey. However, <u>E. punctata</u> had lower feeding activity in the fall and winter than <u>N. obscura</u> in both years.

The prey ranges of <u>N. obscura</u> and <u>E. punctata</u> in this study were very similar to those recorded by Davies, Wrona, Linton and Wilkialis (1981) in Bruce Lake. Chironomidae, Amphipoda and Oligochaeta formed the major components of their diet, with Copepoda/Cladocera being consumed in low proportions by both species in the spring and summer, and Gastropoda only taken to a limited extent by <u>N. obscura</u> (Figure 2.7). Amphipoda were always proportionally more heavily utilized by <u>E. punctata</u> through the majority of the year, while Oligochaeta were generally more heavily utilized by <u>N. obscura</u>. Only during the spring did <u>E. punctata</u> utilize Chironomidae more heavily than <u>N. obscura</u>. (Figures 2.9, 2.10).

As <u>E. punctata</u> and <u>N. obscura</u> both forage in the same habitats, the differences in prey utilization are probably indicative of differences in preference (selection) rather than differences in prey

availability or encounterability. Ekman grabs taken in 1976 and 1978 revealed no significant changes in the overall abundance of any of the prey types. The preference (selection) of the prey by N. obscura and E. punctata is therefore due to differences in prey palatability, food quality, or to differences in the ability of N. obscura and E. punctata to handle (capture) the prey.

Clearly, differences in prey utilization occurred between N. obscura and E. punctata . However, a more robust test of the competition hypothesis is to look for changes in feeding strategies in relation to changes in relative densities of the leech species. At the species level, N. obscura and E. punctata showed no significant differences in prey utilization in 1976 when E. punctata was dominant (Figure 2.8). However, intra-specifically both temporal and weight range differences in the diet occurred between N. obscura and E. punctata. When dominant, E. punctata showed two groups of sampling months with significant differences in prey utilization (Figure 2.11). In contrast, N. obscura displayed no temporal separation in the types of prey consumed in either year (Figure 2.12). This differs from the results obtained by Davies, Wrona, Linton and Wilkialis (1981) from Bruce Lake, where both N. obscura and E. punctata showed temporal differences in prey utilization. Being limnologically and climatically similar, the differences between the results obtained from Bruce Lake and Cairn Pond are likely due to the permanence and persistence of the dominance of N. obscura in Bruce Lake.

Intra-specific resource partitioning as a function of differential weight class utilization of prey was shown by both species.

Such resource partitioning minimizing intra-specific competition would increase population fitness (Giesel, 1974). N. obscura showed cohesive weight range groups of small, medium and large individuals, each displaying unique feeding trends. E. punctata similarly reduced the potential for intra-specific competition when dominant in 1976.

At the species level, prey utilization by N. obscura and E. punctata in 1978 when N. obscura became dominant was similar to 1976, with E. punctata utilizing Amphipoda in higher proportions than N. obscura, which primarily consumed Chironomidae and Oligochaeta (Figure 2.10). However, when sub-dominant, E. punctata displayed three groups of sampling months with unique trends in prey utilization (Figure 2.11), rather than two when it was dominant, with N. obscura still displaying no distinct seasonal pattern (Figure 2.12). N. obscura maintained intra-specific resource partitioning with three cohesive weight range groups similar to those found in 1976, while E. punctata groups were not as cohesive, with small, medium and large individuals frequently utilizing prey in a similar fashion. The change in feeding ecology of E. punctata with changes in numerical dominance provides further evidence for inter-specific competition.

Examination of niche breadth and overlap values can provide additional information on competition. Without data on prey availability niche overlap alone cannot be used as an indicator of actual or potential competition. With high prey availability, high overlap indicates either high potential or low actual competition, while with low prey availability, high overlap indicates high actual competition. Niche overlap in combination with niche breadth can be

inferentially used to indicate potential or actual competition. If niche overlap is high and niche breadth remains unchanged or decreases, the potential for actual competition is greater.

Pianka (1972) proposed that maximal tolerable niche overlap should be lower in communities where inter-specific competition is intense. The Cairn Pond data conforms to this hypothesis in that a decrease in mean niche overlap (Figures 2.9, 2.10) and niche breadth (Table 2.4) was found for both species when N. obscura became dominant.

In both 1976 and 1978, niche overlap was highest In the spring and late fall although the probability of inter-specific competition for food resources existed at most times of the year. In the spring of 1976 increased overlap coincided with decreased niche breadth suggesting that prey availability increased and that competition was low. In the fall niche breadth of both species in both years tended to decrease which with high niche overlap indicates increased potential competition. The results are comparable to the findings in Bruce Lake, where the potential for competition was highest in the summer and early fall (Davies, Wrona, Linton and Wilkialis, 1981).

Based on the relative abundance data, N. obscura appears to have an ecological advantage over E. punctata. If this ecological advantage is based on inter-specific competition in food limited situations, E. punctata would be expected to decrease its prey utilization overlap with N. obscura and specialize more on dietary components not heavily utilized by N. obscura. The field data presented here supports the concept of inter-specific competition

between N. obscura and E. punctata with E. punctata becoming more of a specialist in its diet and decreasing its overlap with N. obscura when sub-dominant and competitive pressures potentially high.

As the selection pressures stemming from competitive interactions would, over time, be expected to minimize inter-specific overlap in resource use and accommodate co-existence (Arthur, 1981), one possible evolutionary consequence of inter-specific competition is the divergence of certain morphological characters. This would allow each species to utilize non- or low overlapping ranges of the limited resources (Brown and Wilson, 1956; Grant, 1972; Arthur, 1981). The occurrence of morphological character displacement between two species may therefore be used as support for the hypothesis that inter-specific competition is, or was operating. Arthur (1981) forwarded a number of criteria which must be met before it can be concluded that character displacement has resulted from competitive selection pressures:

1) The variability in the mean value of the character at an allopatry/sympatry distributional border should not be predictable from phenotypic variation within either of these areas.

2) Sampling should be conducted along several geographic gradients from allopatry to sympatry, preferably differing in environmental conditions.

3) Heritability of the character should be estimated.

4) There should be evidence that inter-specific competition is

occurring or has occurred and that the characters investigated have

some potential bearing on the competitive process.

Only criterion 4 was quantitatively examined in this study by

comparing length, diameters of the posterior sucker, anterior sucker,

and mouth of N. obscura and E. punctata in relation to food resource

utilization.

Inter-specific morphological differences were found in length

(Figure 2.3), anterior sucker diameter (Figure 2.4), and mouth di-

ameter (Figure 2.5). As there were no observed species differences in

prey range (Figure 2.8) or niche breadth (Table 2.4), the morpho-

logical differences in anterior sucker and mouth diameters do not

appear to result in food resource partitioning on the basis of the

type of prey consumed. However, since N. obscura has a larger

anterior sucker and mouth diameter for a given size (age) than

E. punctata, it has the potential to exploit a wider size range of a

given prey type than E. punctata. The adaptive advantage, if any, of

E. punctata having a longer length for a given weight than N. obscura

is not apparent.

Thus, apart from the differences in the anterior sucker and mouth

diameters of N. obscura compared to E. punctata, no evidence for

morphological character displacement is found. Additionally, before

it can be argued that the observed differences in the anterior sucker

and mouth diameters are the result of competitive selection pressures, all the criteria forwarded by Arthur (1981) must be quantitatively examined.

Clearly, the majority of the criteria established to indicate inter-specific competition have been supported by the data collected on N. obscura and E. punctata. Both species have been found to be sympatric, share common resources, display different reproductive and/or feeding strategies when dominant or sub-dominant, partition food resources on a temporal and weight (size) basis and reduce inter-specific overlap in potentially competitively intense situations as theory predicts. However, the ecological consequences of the manipulation of food resources and/or species-populations (Criterion 6) needs to be examined to unequivocally prove that inter-specific competition occurs.

3.0 RESPIRATION

3.1 Introduction

The processing efficiency and fate of ingested food energy within an organism is generally considered to have a major influence on the ecological success of the species. Assimilated energy is partitioned between two major pathways within an organism; 1) synthesis and 2) respiratory metabolism (Figure 3.1). Synthesis involves the building of new somatic and gametic tissue, repair and maintenance of existing tissue, and storage of energy and/or nutrients for future utilization. From an evolutionary view point, reproductive output is probably the most important product of the synthesis pathway.

Respiratory metabolism is the biochemical pathway involved with the conversion of the chemical potential energy in food to usable high energy adenosine triphosphate (ATP) molecules, with the major by-product being dissipated heat. The efficiency of the conversion is highly dependent upon whether metabolism occurs under aerobic or anaerobic conditions. In the presence of oxygen, ATP is generated via aerobic glycolysis which incorporates the Krebs tricarboxylic acid cycle (TCA) and operates at a conversion efficiency of approximately 38% (Calow, 1981). In contrast, anaerobic glycolysis, which results in the formation of lactic acid, has a conversion efficiency of only 2 - 8% (Florey, 1966; Calow, 1981). The stored potential energy in the ATP molecules generated from respiratory metabolism is subsequently used to power the physiological processes involving chemical and physical work and synthesis. Thus, any biotic and/or abiotic en-

vironmental factor which influences respiratory metabolism can have a profound effect on an individual's fitness, by modifying survivorship and genetic contribution to subsequent generations through reproductive output. The ecological outcome of the organism-environment interaction will manifest itself through population growth or decline, which consequently determine the species distribution and abundance patterns (Andrewartha and Birch, 1954). Therefore, the examination of respiration rates under different environmental conditions provides a measurement of the physiological plasticity of a species, which has a major influence on its overall ecological success.
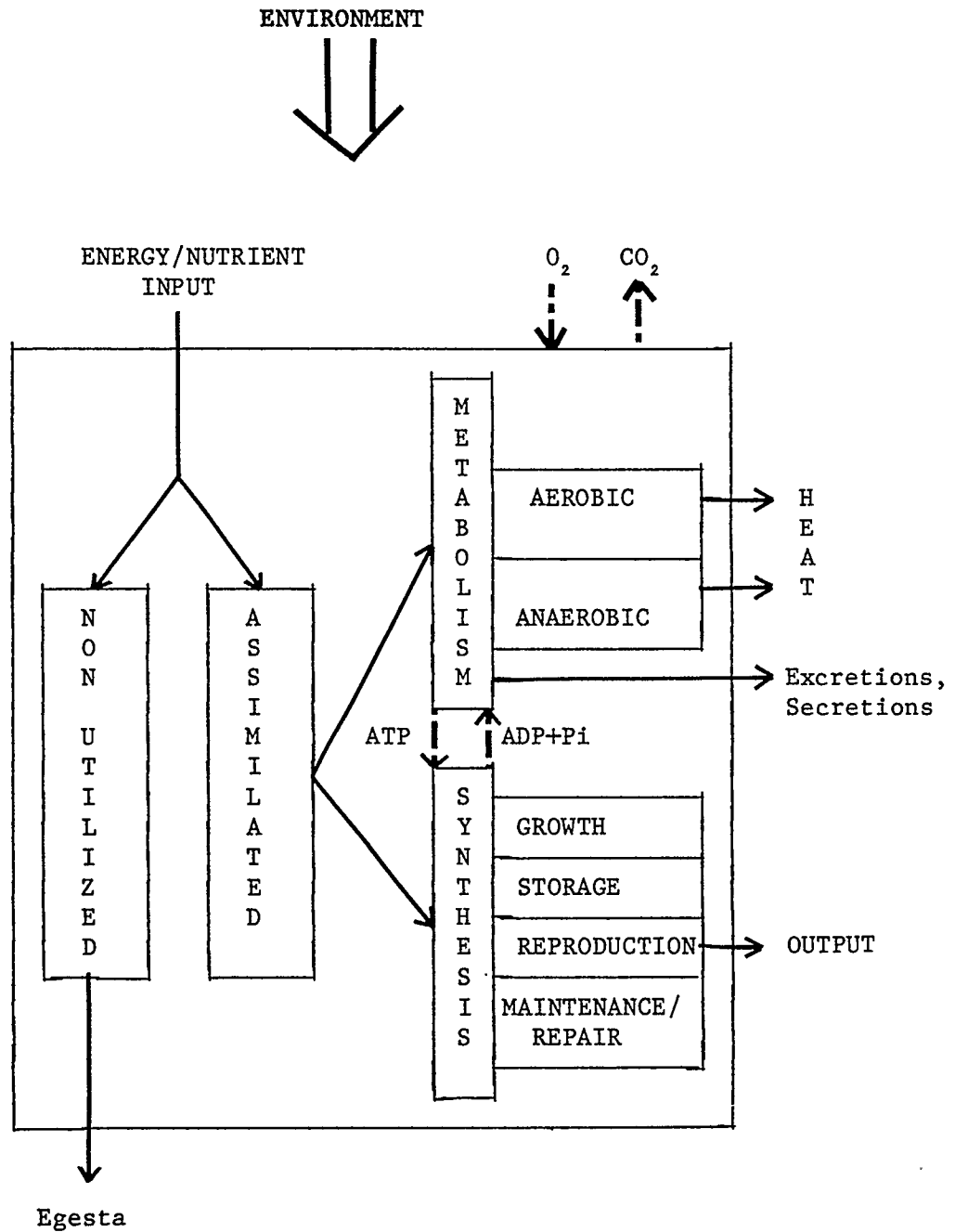
Figure: 3.1: Resources (energy/nutrient) partitioning
in a model leech.
Large Box = whole animal; small boxes =
sub-systems.

3.2 Oxygen Uptake

Energy expenditure (metabolism) of an organism is most commonly indirectly determined by measuring respiration, the process involving oxygen uptake and subsequent release of carbon dioxide (Florey, 1966; Phillipson, 1981). A potential weakness in the use of oxygen consumption as a quantitative estimate of metabolism is the assumption that only the pathways of aerobic glycolysis are being utilized by the organism. If however, anaerobic respiration is contributing a significant proportion to the energy expenditure, the metabolic costs will be grossly underestimated (Calow, 1981). Linton (1980) forwards three additional assumptions made when using oxygen consumption as an estimate of an organism's energetic requirements, these being: 1) energy production is linearly related to oxygen consumption, 2) a constant proportion of metabolism energy is being utilized by the organism while being monitored and 3) all oxygen required for aerobic metabolism is obtained from the external environment as molecular oxygen.

Of all the assumptions forwarded for the use of oxygen consumption as a quantitative estimate of metabolism, the one in greatest doubt is that N. obscura and E. punctata are obligate aerobes. Zebe, Salge, Wiemann and Wilps (1981) found Hirudo medicinalis (L.) to survive anoxia for several days at 20 ^{o}C, and based on an imbalance in the calculated energy budget between oxygen uptake and catabolic loss of tissue under starvation for Erpobdella octoculata (L.), Calow (personal communication) suggested the discrepancy was due to an-

aerobiosis. The proportion anaerobic respiration contributes to the overall metabolism of N. obscura and E. punctata is unknown, but will likely be similar as they are closely related phylogenetically and ecologically (Linton, 1980).

An array of environmental and physiological factors have been found to influence oxygen uptake rates of aquatic Annelida (Weber, 1978; Dejours, 1975) (Table 3.1). Physiological factors are modifiers of an individual's metabolism such as body size, hunger level, reproductive state, et cetera. Environmental factors comprise all biotic and abiotic influences on an organism's metabolism. The importance of the singular and combined effects of these factors in modifying leech respiration is evident in the following literature review.

TABLE 3.1

Factors influencing oxygen uptake rates
of aquatic annelids (after Dejours, 1975; Weber, 1978)

PHYSIOLOGICAL FACTORS   species differences
body size
stage of development
nutritional state
activity levels
acclimatization
diurnal and/or seasonal rhythms

ENVIRONMENTAL FACTORS   temperature
oxygen availability
salinity

3.3 Hirudinoidea Respiration - Literature Review

Few researchers have studied the effects of environmental factors
and physiological states on the respiration rates of ecto-parasitic
leeches belonging to the family Hirudinidae. Using sealed glass
containers as respirometers and the Winkler method (Anomynous, 1971)
for oxygen determinations, Lindeman (1932, 1935) examined the re-
lationship between oxygen tension and oxygen uptake in H. medicinalis.
H. medicinalis was found to be an oxy-regulator (Prosser and Brown,
1962), having oxygen consumption independent of oxygen tension between
20 - 100% saturation at 25 °C. When exposed to five temperatures
ranging from 10 - 34 °C, H. medicinalis displayed increased oxygen
uptake rates with increasing temperature, but remained an
oxy-regulator at all temperature treatments (Lindeman, 1935).

Madanmohanrao (1960) examined the influence of salinity on oxygen
consumption of the Indian cattle leech Poecilobdella granulosa
(Blanchard). P. granulosa had maximum oxygen uptake in 25% seawater
and decreasing respiration rates in response to progressively
increasing hypertonicity of the medium. Madanmohanrao concluded that
the lethal effects resulting from rapid water loss from the animals
were responsible for the observed decrease in respiration rates.

Ramamurthi (1968) repeated these experiments in more detail and
found that P. granulosa had its minimal oxygen consumption in isotonic
medium (25% sea water), and for a given osmotic gradient, had a higher
percentage increase in oxygen uptake in hypertonic than hypotonic
media. For lack of a better explanation, Ramamurthi (1968) believed
the discrepancy in results was due to the low sample size used by

Madanmohanrao (1960).

Nagabhushanam and Kulkarni (1977) examined alterations in the oxygen uptake of Poecilobdella viridis (Blanchard) in response to changes in oxygen tension, pH and salinity. P. viridis acted as an oxy-conformer (Prosser and Brown, 1962), in that its oxygen consumption was dependent upon oxygen concentration. Additionally, its respiration rate was lower in highly alkaline or acidic waters and higher in increased salinity. In a subsequent study, Nagabhushanam and Kulkarni (1978) found P. viridis to decrease its respiratory metabolism in response to dessication, but increase oxygen uptake with increasing temperature. They concluded that the respiratory responses of P. viridis physiologically allowed it to cope with the seasonal and diurnal fluctuations in water levels and temperatures in its natural habitat.

Zebe, Salge, Wiemann and Wilps (1981) found that in H. medicinalis, environmental anaerobiosis occurred as a result of reduced oxygen concentration in the external environment, while functional anaerobiosis developed within the tissues of the animal during excessive muscular activity (i.e. swimming, looping). H. medicinalis used two different biochemical modes of energy metabolism in response to the two types of anaerobiosis.

Mann (1956) conducted pioneering work on the eco-physiology of predatory Hirudinoidea when examining the influences of body size, starvation, oxygen tension, acclimation history, seasonal and behavioral patterns on the oxygen consumption of five common British species. Groups of leeches were placed in glass-stoppered bottles

serving as respirometers which were maintained at 20 °C, and oxygen consumption determined polarographically using a mercury-drop electrode. Two glossiphonid species, Glossiphonia complanata (L.) and Helobdella stagnalis (L.) were found to have differing respiratory strategies. G. complanata , abundant in hard water streams and lakes, behaved as an oxy-conformer when collected in the spring and summer months, and showed depressed oxygen uptake at higher oxygen concentrations when collected in the winter. H. stagnalis , commonly occuring in stagnant, eutrophic British lakes, displayed oxy-regulation. When fully acclimated, Erpobdella testacea (L.) (Erpobdellidae) was found to be an oxy-regulator. When acclimation was not allowed, oxygen consumption became dependent on oxygen tension. It was proposed that ventilation, dorsal-ventral movements of the body, provided irrigation over the body surface and was responsible for the maintenance of the high uptake rates at low oxygen tensions, thereby allowing for its occurrence in oxygen poor reed swamps. In contrast, sympatric E. octoculata is an oxy-conformer.

Pisicola geometra (L.) (Family Pisicolidae) which has a distribution limited to well-oxygenated habitats, displayed the highest oxygen uptake of all the species examined in air-saturated water and oxy-conformity under hypoxia.

Mann (1956) found that progressive starvation over a seven day period did not significantly alter the respiration rate of any of the species tested. Additionally, oxygen uptake rates were determined to be proportional to surface area rather than wet weight of the leeches.

Mann (1958) discussed the problems associated with measuring oxygen uptake of Hirudinoidea in his static respirometers which were; 1) oxygen uptake of the animals was measured under progressively increasing hypoxia, consequently limiting the duration of the experiment and 2) determination of quiescent respiration was difficult since the respirometers had to be shaken to ensure thorough mixing of the water. To overcome these problems, Mann (1958) developed a flow-through polarographic respirometer, in which groups of leeches could be placed in a constant flow of water of controlled oxygen concentration and temperature. Using this apparatus he quantitatively investigated the resting metabolism of E. testacea in relation to seasonality, differential acclimation to decreased oxygen tension, starvation, feeding and the relative contribution of blood haemoglobin at 20 °C. Summer collected E. testacea were found to acclimate to low oxygen tensions by maintaining constant oxygen uptake rates down to 33% air-saturation. Winter collected animals did not show this acclimation response. Feeding E. testacea with Tubifex sp. increased the resting respiration rate three fold as compared to starved individuals, although the respiration rates returned to starvation levels within four days. In air-saturated water, haemoglobin contributed 45% to overall oxygen transport to the tissues, while at 25% air-saturation, the haemoglobin contribution fell to 25%.

As ventilation was observed under low oxygen tensions, Mann (1956) concluded that this behavior accounted for the oxy-regulation displayed by E. testacea However, when utilizing the flow through respirometer, no ventilatory movements were observed although

E. testacea still showed oxy-regulation under low oxygen tensions. It was suggested that the acclimation response was a function of some internal mechanism, such as an increase in the rate of blood circulation (Mann, 1958). It is likely that both behavioral (ventilation) and physiological mechanisms contribute to the acclimation response of E. testacea to hypoxic conditions.

Mann (1961a, b) concluded that the differences in the field distributions of E. testacea and E. octoculata was attributable to their different respiratory strategies under hypoxic conditions. E. testacea , which was found to act as an oxy-regulator, can maintain a relatively high metabolic rate under low oxygen conditions, while E. octoculata behaved as an oxy-conformer, which implies that under low oxygen conditions the rate of feeding and/or reproduction may be depressed due to low aerobic metabolic output. Thus, on a physiological basis, E. octoculata would potentially be ecologically less successful than E. testacea under hypoxia.

The physiological explanation for the distribution differences between the two species forwarded by Mann (1961a, b) can be critisized because: 1) the physiological comparison between species was based on the respiration data collected using two different methodologies. E. octoculata was investigated using a static respirometer (Mann, 1956), the technique of which Mann (1958) himself critisized. The results for E. testacea were obtained using both the static and flow-through systems (Mann 1956, 1958); 2) only the resting rate of oxygen consumption was compared between the two species. Data is required on both the standard rate (quiescent respiration) and during

maximal activity (the active rate) (nomenclature after Fry 1947, 1957) before it can be concluded that the maintenance of a high, constant metabolic rate under increasing hypoxia gives E. testacea an eco- logical advantage over E. octoculata. The difference between the active and standard rates or aerobic metabolic scope (Fry 1947, 1957; Bennett, 1978) provides an index of the capacity to which activity (i.e. foraging, breeding) can be aerobically supported. Unless this information is obtained, oxy-regulation under hypoxia may in fact be indicative of a high metabolic cost associated with coping with declining oxygen conditions, consequently being disadvantageous to the species involved; 3) all the experiments performed by Mann (1956, 1958) were at only one temperature (20 $^{\circ}$C). As temperature has been recognized as one of the major environmental determinants of the rate of oxygen uptake and activity levels of poikilotherms (Bayne, Thompson and Widdows, 1976), it is difficult to be convinced by the explanation forwarded by Mann (1961a, b).

The little research carried out on the respiratory strategies of N. obscura and E. punctata in response to different environmental conditions and physiological states, has all been undertaken using the Gilson respirometer.

Reynoldson (1974) examined the effects of osmotic stress on the oxygen consumption of N. obscura, and recorded an increase in its respiration rate in response to increasing hypotonicity of the medium when not allowed to acclimate. However, if acclimation was allowed, a steady-state in oxygen uptake was achieved in hypo-osmotic media over the range of 230 - 3300 μmohs·cm^{-1}.

Osborne, Davies and Rasmussen (1981) studied the effects of total residual chlorine on the respiratory responses of N. obscura and E. punctata. In both species, temperature was found to significantly influence the respiration rate of the control animals. N. obscura and E. punctata showed 46% and 54% increases in uptake respectively in response to a temperature increase from 10 to 15 °C. Both species responded similarly to residual chlorine concentrations at 10 and 15 °C, having lowest oxygen uptakes at low and high chlorine concentrations and highest oxygen uptake at intermediate chlorine levels.

Linton, Davies and Wrona (1982) studied the respiratory responses of N. obscura and E. punctata to changes in media ionic composition (hard vs soft water), total dissolved solids (25 - 2000 $mg \cdot 1^{-1}$) and temperature (4 and 15 °C). No significant intra- or inter-specific differences were found in the respiratory responses of either species with respect to the tested parameters. Linton, Davies and Wrona (1982) thus concluded that the respiratory strategies shown by N. obscura and E. punctata could not account for the observed differences in their relative population abundances when sympatric.

Because a Gilson respirometer was used, all these studies suffered from the following potential problems: 1) since the respiration chambers are sealed, oxygen uptake is measured under progressively increasing hypoxia; 2) toxic metabolic waste products accumulate in the respirometers, thereby potentially stressing the leeches; 3) the required mechanical agitation of the respirometers between measurements may induce additional stress; 4) as bright flood-lamps are used to read the manometers for determination of

uptake rates and since leeches are generally photo-negative (Mann, 1961c), this could also be an additional stress; 5) it is not possible to determine whether the oxygen uptake rates are representative of standard, active or routine metabolism, the latter corresponding with spontaneous movements of the organism concerned (Newell, 1973).

3.4 Objectives

The objectives of the respiration experiments were to determine
whether differing respiratory strategies were displayed between
N. obscura and E. punctata in response to different environmental
factors (temperature, oxygen content of the water) while under
different physiological states (fed, starved, reproductive,
non-reproductive, resting, active, ventilating). The major hypothesis
is that the respiratory strategy adopted by N. obscura gives it an
ecological advantage over E. punctata in lentic habitats, with the
contrary being true for lotic situations, thereby providing a
physiological basis for the observed field distribution differences
between the two species.

3.5 Materials and Methods

3.5.1 Experimental Designs

3.5.1.1 100% Air-saturation Experiments

A series of experiments were conducted to examine the effect of temperature on the respiration rates of N. obscura and E. punctata as modified by different physiological states under 100% air-saturated conditions. The physiological states examined were reproductive condition, nutritional level and activity (Table 3.2).

Reproductively mature (clitellate) individuals of both species were obtained in May, 1981 and non-reproductive adults in November, 1981. Upon collection, the leeches were acclimated to test temperatures and starved in de-chlorinated, 100% air-saturated Bow River water for a minimum of 28 days. When examining the influence of feeding on the respiration rates, individuals were fed to satiation over a 24 hour period on Enchytraeus sp. 48 hours prior to the experiment. In all experiments, similar wet weight ranges (150 - 400 mg) of both species were used to minimize intra- and inter-specific weight (size) dependent differences in oxygen uptake. All respiration experiments were performed using 100% air-saturated water. The combinations of experimental factors (Table 3.2) were examined using ten individuals of each species with five replicate respiration readings taken per individual. All measurements were made at 15 °C on reproductive individuals and 5, 10, 15, 20 °C on non-

reproductive. The activity of an individual was recorded immediately prior to the measurement of oxygen uptake.

TABLE 3.2

Combinations of experimental factors examined in the
respiration analyses of N. obscura and E. punctata
under air-saturated conditions. X = experimental
combination performed.

TEMP. °C	STARVED		FED
	Reproductive	Non-Reproductive	Non-Reproductive
5		X	X
10		X	X
15	X	X	X
20		X	X

3.5.1.2 Hypoxia Experiments

The effects of environmental hypoxia on the respiration rates of
N. obscura and E. punctata were examined at different temperatures in
relation to both, short and long term exposure.

Short term responses were examined by measuring the oxygen uptake
of the experimental animals under progressively declining oxygen
tensions at each of 100%, 50%, 25% and 10% air-saturation on day 1 of
the experiment. Experiments were started in 100% air-saturated water,
and the animals were kept for 2 hours at each air-saturation level
before being exposed to the lower concentrations. Subsequently, the
leeches were maintained at 10% air-saturation for a period of five
days, with respiration readings being taken daily. Five
(150 - 400 mg) individuals of each species were examined at each
temperature (5, 10, 15, 20 ^oC), with five replicate respiration
readings taken at each experimental level of air-saturation on a given
day. The activity pattern of an individual was recorded immediately
prior to the respiration measurement. All leeches utilized in the
experiments were acclimated and starved for a minimum of 28 days in
de-chlorinated, 100% air-saturated Bow River water.

3.5.1.3 Anoxia Experiments

The survivorship of N. obscura and E. punctata under anaerobic
conditions was investigated at four temperatures, 5, 10, 15, 20 ^oC.
At each temperature treatment, groups of ten starved individuals of
each species previously acclimated to the test temperatures in

air-saturated, de-chlorinated, Bow River water for a minimum of 28

days, were placed separately in sealed 250 ml Erlenmeyer flasks

containing de-oxygenated water. Anoxic water was obtained by bubbling

nitrogen gas through the water for a period of one hour, and

subsequently checking the oxygen content with an oxygen electrode

(Section 3.5.3). Survivorship was determined daily.

3.5.2 Respiration Apparatus

All experiments were performed in a newly designed flow-through respirometer system (Figure 3.2). De-chlorinated Bow river water of known oxygen concentration and temperature was gravity fed into the respiration chambers from an insulated reservoir (20 l Nalgene carbuoy). The flow rate of the water through each respiration chamber ($0.1 - 1.0$ ml·min^{-1}) was individually controlled by using tygon tubing input lines fitted with valves (Travenol Flo-trol clamps).

The water temperature in the reservoir was regulated using an Instant Ocean Culture System (Model WM 500) which simultaneously circulated and cooled the water. Oxygen concentration of the water was controlled by bubbling the reservoir with compressed air or nitrogen using a Radiometer TOX-40 gas transmitter with a CME 40 on/off controller which automatically regulated the volume of gas required to maintain a preset level of oxygen saturation.

Modified 2 ml glass syringes constituted the respiration chambers, all of which were housed in a light-proof styrofoam insulating water jacket which kept the chambers dark and at the same temperature as the experimental input water. For each experimental run, twelve chambers were used, ten as experimental and two as controls.

Each respirometer was fitted with two electrodes (0.05 mm^2 copper magnet wire), one at each end. Activity of the leech in each chamber was monitored by connecting the two electrodes to an impedance

converter (Biocom. Inc. Model 2991) which generates a low oscillating current between the electrodes, with the water inside the respirometer acting as the electron carrier completing the circuit.

Any movement of the animal inside the chamber produces a change in the impedance of the circuit and this was monitored by coupling the converter to an amplifier (Grass P15 A.C. Pre-amplifier) and subsequently to an oscilloscope (Tektronix Model 5013N) and/or strip chart recorder (Cole Parmer Model 8373-20). Animal activity was observed as either beam movement on the oscilloscope screen or permanently recorded by the pen movement of the strip chart recorder.
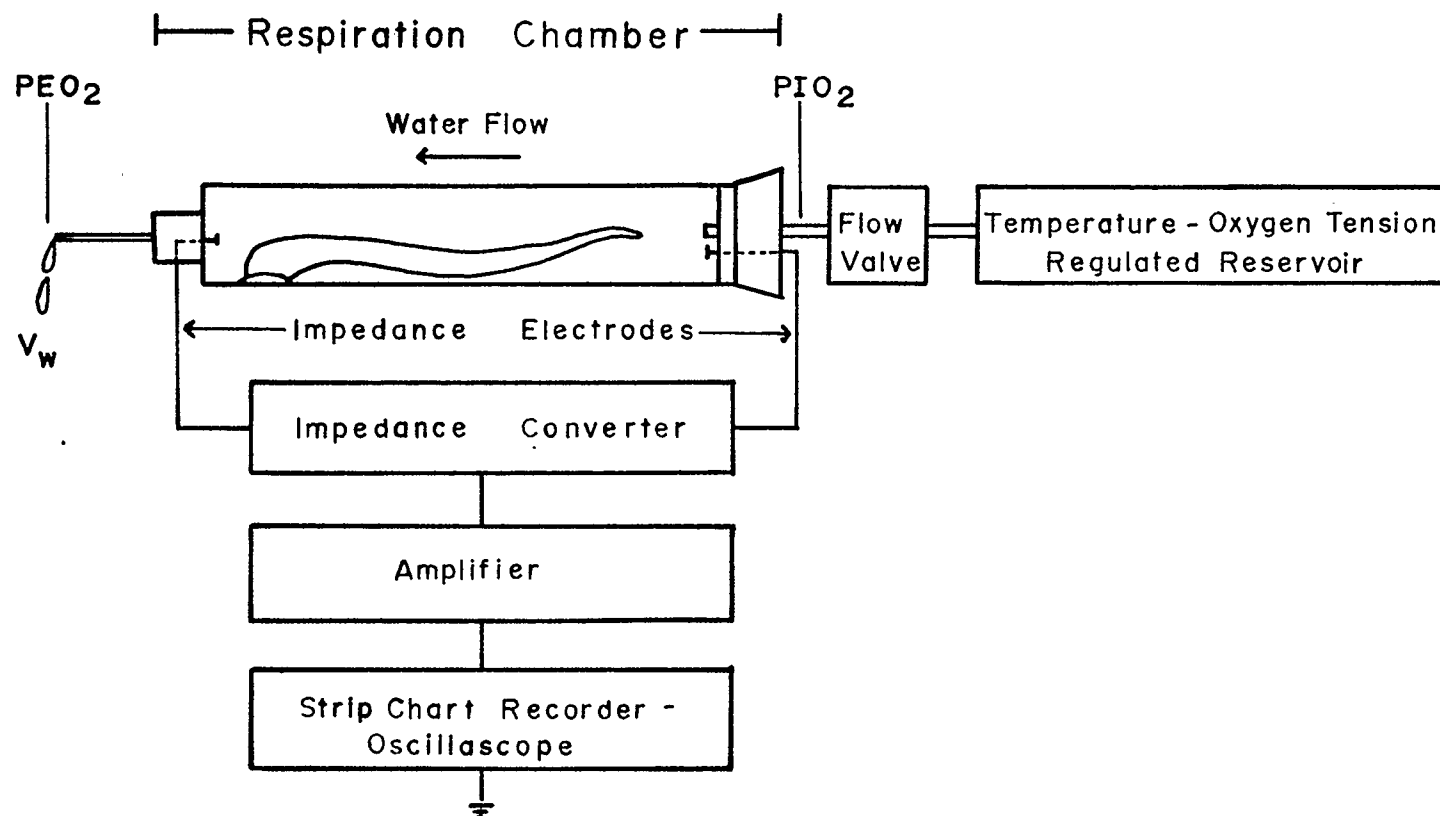
Figure 3.2: Schematic representation of respiration apparatus. See text (Section 3.5.3 for explanation of symbols).

3.5.3 Measurement of Oxygen Uptake

Oxygen uptake of the leeches was calculated using the relationship forwarded by Dejours (1975);

$$MO_2 = (PIO_2 - PEO_2) \cdot BWO_2 \cdot Vw$$

where,

MO_2 = oxygen consumption ($\mu Mol \cdot hr^{-1}$)

PIO_2 = partial pressure of oxygen in incoming water (torr)

PEO_2 = partial pressure of oxygen in exiting water (torr)

BWO_2 = solubility coefficient of oxygen in water at a defined temperature ($\mu Mol \cdot l^{-1} \cdot torr^{-1}$) (from Dejours, 1975)

Vw = flow rate of water through respirometer ($l \cdot hr^{-1}$)

The oxygen tensions of the water (PIO_2, PEO_2) were obtained by taking 100 ul samples using a 250 μl micro-syringe and injecting the sample into an Radiometer oxygen electrode (Type E5046) which was housed in a thermostatic jacket (Model D616). The thermostatic jacket kept the electrode at the same temperature as the experimental water. The partial pressure of oxygen was directly measured in torr (1 torr = unit of pressure equal to 1/760 of an atmosphere) using a Radiometer PHM 73 digital gas monitor (accuracy $\pm$ 4 torr), which was connected to the oxygen electrode. A stable reading was usually achieved within two minutes. The flow rate of experimental water through the

respirometers (Vw) was determined on each individual chamber at the time the oxygen readings were taken. A 5 ml volumetric pipette, with its tip sealed (accuracy $\pm$ 0.05 ml), was used to collect the exiting water from the respirometer for a 1 minute interval. Live wet weights of the experimental animals were obtained by weighing to the nearest 0.1 mg on a Sartorius analytical balance (Model 1602 MP) after being blotted with tissue paper to remove excess water both before and after each experiment. Wet weight used for each days calculations was obtained by interpolation of these two weights.

Oxygen consumption of the leeches was converted to $\mu l \cdot mg$ dry $wt^{-1} \cdot hr^{-1}$ at standard temperature and pressure (STP) using the relationship;

$$QO_2 = (MO_2 \cdot dry \ wt^{-1}) \cdot 22.414 \ (l \cdot Mol^{-1})$$

where,

QO_2 = oxygen consumption ($\mu l \cdot mg$ dry $wt^{-1} \cdot hr^{-1}$

at STP)

MO_2 = oxygen consumption ($\mu Mol \cdot hr^{-1}$)

dry wt = dry weight of individual (mg)

The wet to dry weight conversion was obtained for N. obscura and E. punctata using the regression:

$$DWT = 0.143 \cdot WWT \quad (\text{ Section } 2.4.1)$$

where,

DWT = dry weight (mg)

WWT = wet weight (mg)

All statistical analyses were performed using the QO_2 values.

3.6 Results

3.6.1 Activity Patterns

Three activity states were defined for <u>N.</u> <u>obscura</u> and <u>E.</u> <u>punctata</u> using the impedance monitoring system: 1) ventilation – rhythmic dorsal–ventral undulations of the body while maintaining the posterior sucker attached (Figure 3.3a), 2) random movement – associated with crawling, short bursts of swimming and random probing of the leeches' anterior (Figure 3.3b), and 3) resting – little or no activity (Figure 3.3c).

a) VENTILATION

b) RANDOM MOVEMENT
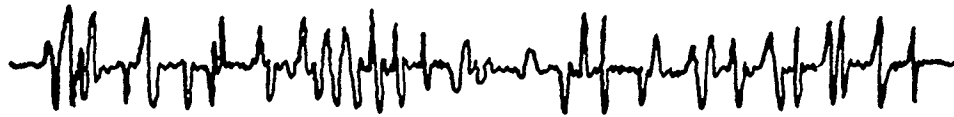
c) RESTING

TIME (min)

Figure 3.3: Impedance recordings of locomotory movements displayed by N. obscura and E. punctata during respiration analyses.

3.6.2 100% Air-saturation Experiments

Over the entire range of experimental temperatures, no significant differences were found in oxygen consumption between individuals ventilating and moving randomly. Therefore all subsequent results concerning active respiration are based on pooled data.

Under 100% air-saturated conditions, significant differences were found in oxygen uptake between N. obscura and E. punctata with respect to temperature, hunger and activity levels (ANOVA 4 way interaction F = 3.38, d.f. = 2,771) (Figures 3.4 and 3.5). Increasing temperature was found to have less of an influence on the oxygen uptake rate of starved, resting N. obscura than starved, resting E. punctata over the range of 5 - 15 °C (Figure 3.4). A significant increase in respiration rate was observed between 15 and 20 °C for N. obscura. Resting E. punctata had higher oxygen uptakes than N. obscura at 10 and 15 °C, with no inter-specific differences being apparent at 5 and 20 °C. When active, the respiration rates of both species were significantly higher than their respective resting values for a given temperature. Additionally, the oxygen consumption of active, starved E. punctata was significantly higher than N. obscura at 5 °C, significantly lower at 20 °C, and no inter-specific differences occurring at 10 and 15 °C (Figure 3.4).

The influence of temperature increase on the resting metabolism of fed N. obscura and E. punctata (Figure 3.5) was greater than for starved individuals (Figure 3.4) exposed to the same experimental conditions. Satiated, resting N. obscura displayed significantly higher oxygen uptake than E. punctata at 10, 15 and 20 °C, with no

inter-specific differences occuring at 5 °C (Figure 3.5). Both species showed no intra-specific differences in respiration at 15 and 20 °C. - The respiration rate of active N. obscura increased with temperature until 15 °C, where it reached an asymptote. In contrast, active E. punctata displayed temperature independent rates of oxygen uptake between 10 - 20 °C. In addition, the respiration rate of active N. obscura was significantly lower than E. punctata at colder temperatures (5 and 10 °C), but significantly higher at warmer temperatures (15 and 20 °C) (Figure 3.5).

The aerobic metabolic scope of starved E. punctata showed no correlation with temperature (Figure 3.6). However, a positive relationship was found between aerobic metabolic scope and increasing temperature for starved N. obscura. E. punctata displayed higher aerobic metabolic scope than N. obscura only at 5 °C. (Figure 3.6).

A negative trend was found between aerobic metabolic scope and increasing temperature with fed E. punctata (Figure 3.7). N. obscura in contrast showed a positive correlation with increasing temperature. The aerobic metabolic scope of fed E. punctata was lower than N. obscura at 15 and 20 °C, but higher at 10 °C.
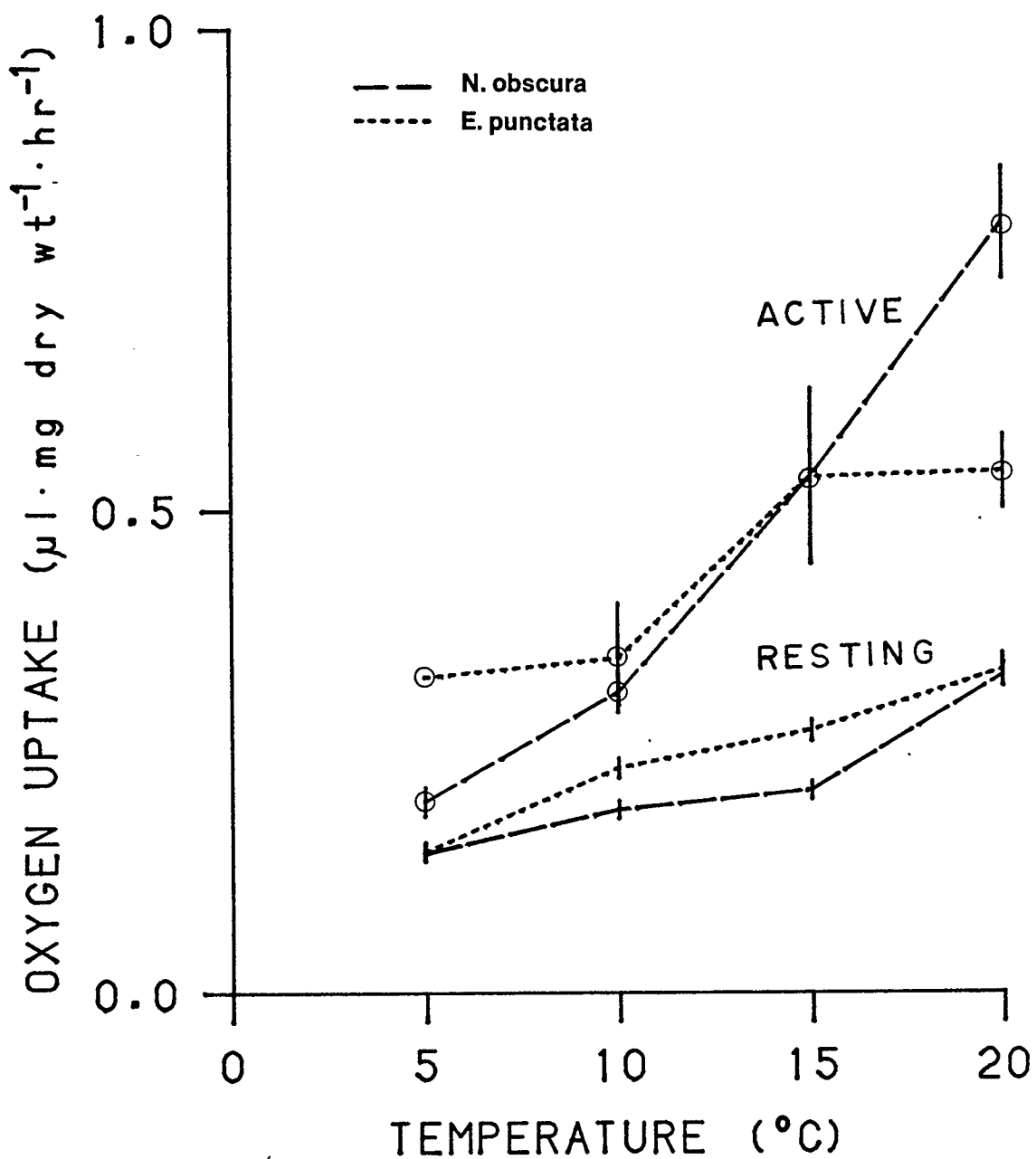
Figure 3.4: Mean oxygen uptake rates displayed by active (O) and resting, starved, non-reproductive, N. obscura and E. punctata acclimated at different temperatures. Vertical bars represent standard errors.
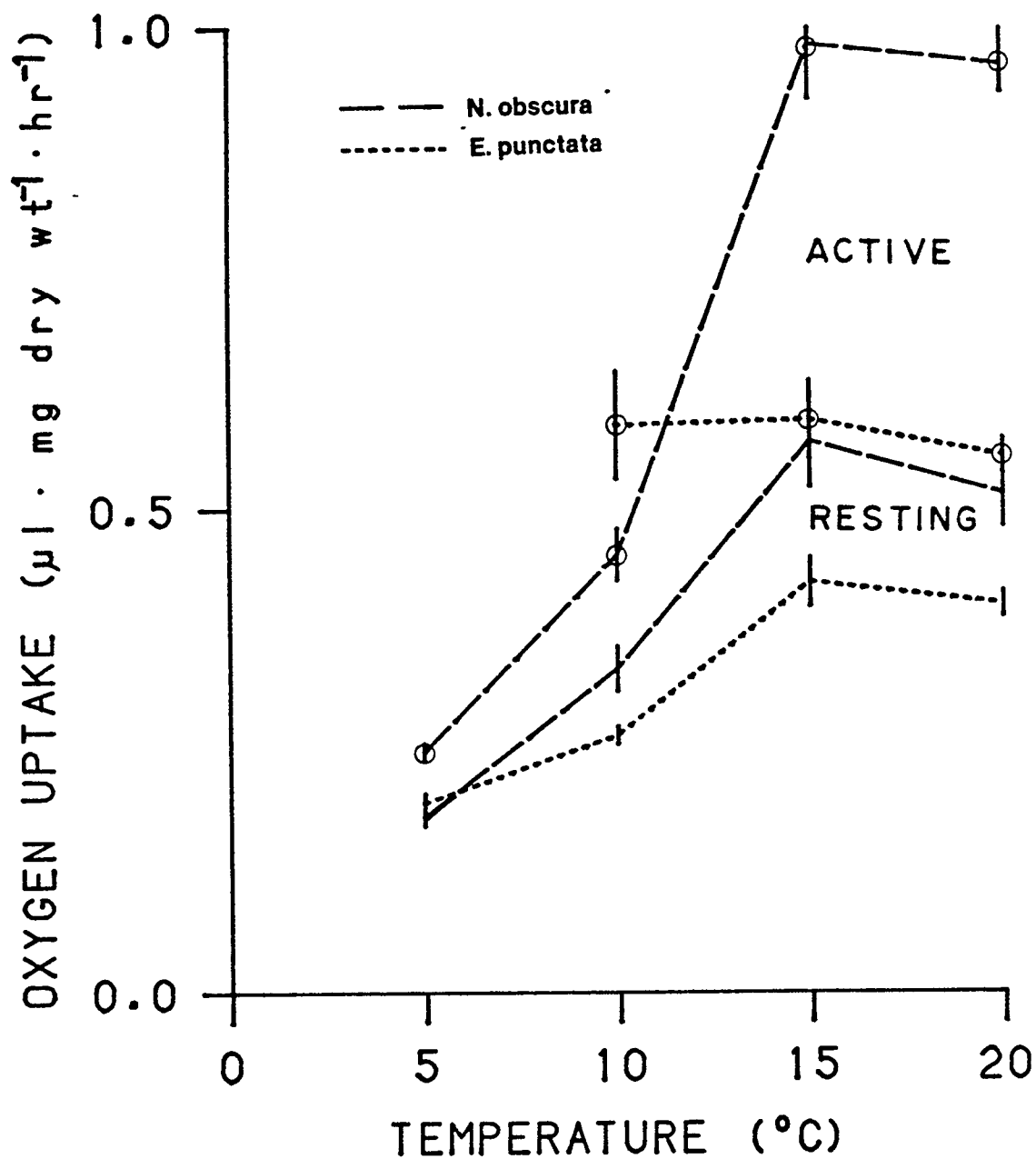
Figure 3.5: Mean oxygen uptake rates displayed by active (O) and resting, fed, non-reproductive, N. obscura and E. punctata acclimated at different temperatures. Vertical bars represent standard errors.
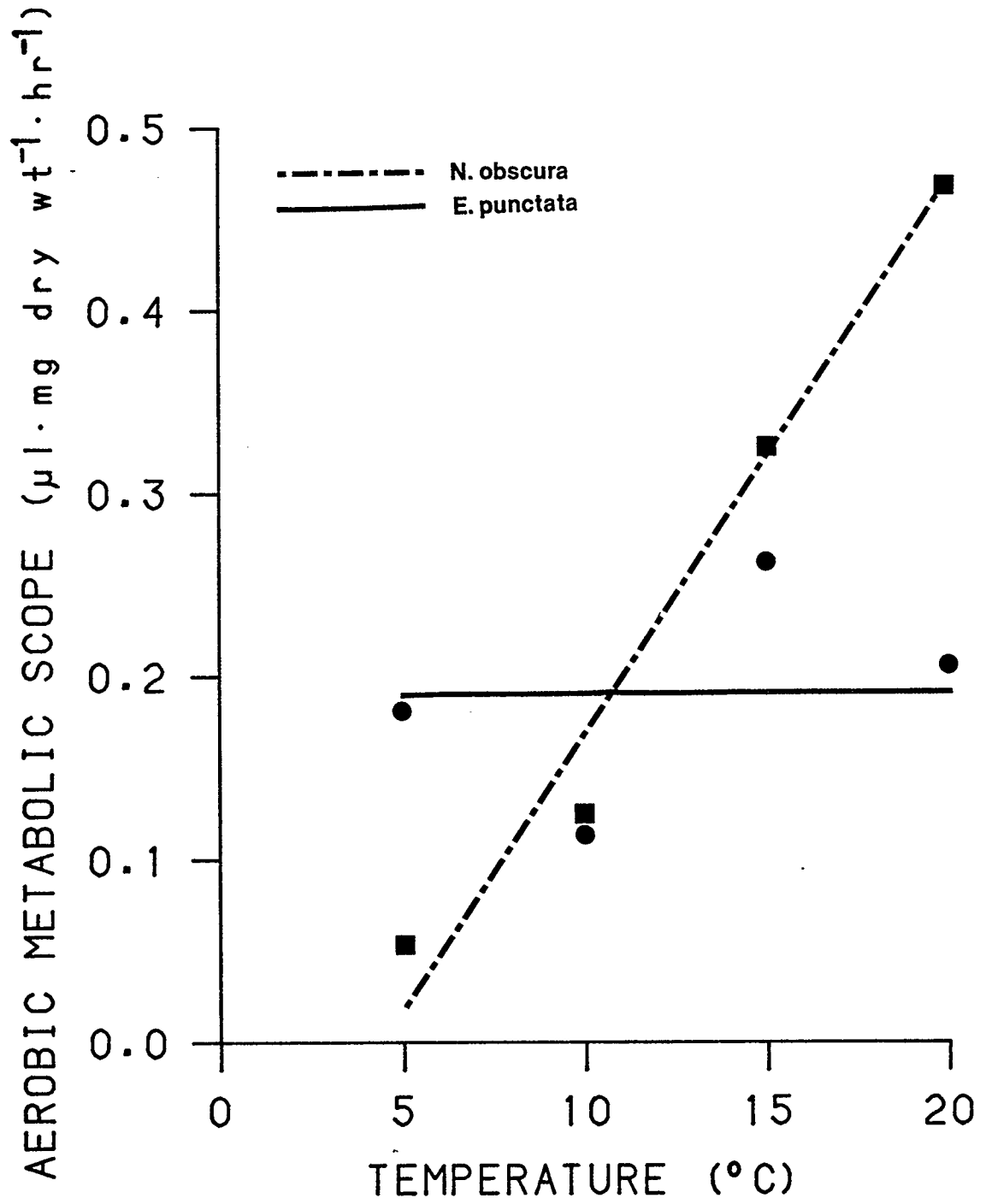
Figure 3.6: Aerobic metabolic scope of starved, non-reproductive N. obscura and E. punctata at different temperatures.
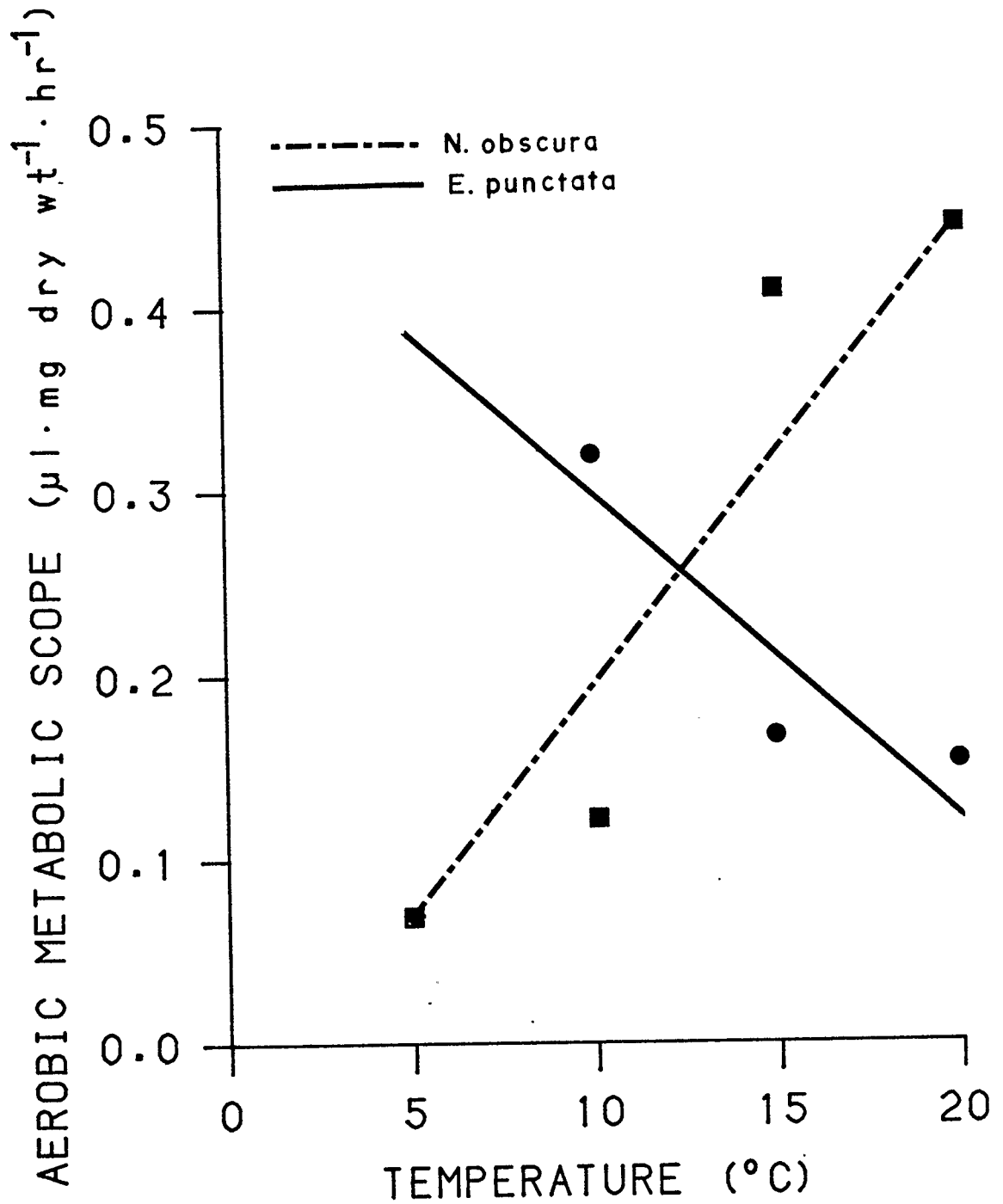
Figure 3.7: Aerobic metabolic scope of fed, non-reproductive
N. obscura and E. punctata at different temperatures.

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3.6.3 Short-term Hypoxia

Over the range of temperatures examined, resting, starved
N. obseura and E. punctata showed no significant inter-specific
differences in the rates of oxygen uptake in response to short-term
hypoxia (Figures 3.8 and 3.9). Both species displayed oxy-conformity,
with their rates of oxygen consumption being functionally dependent on
the oxygen concentration of the medium. Although the rates were
higher than that obtained for resting individuals, randomly moving and
ventilating animals of both species showed similar trends (Tables 3.3
and 3.4) by displaying oxy-conformity and no significant
inter-specific differences in their rates of oxygen uptake in response
to declining oxygen conditions.
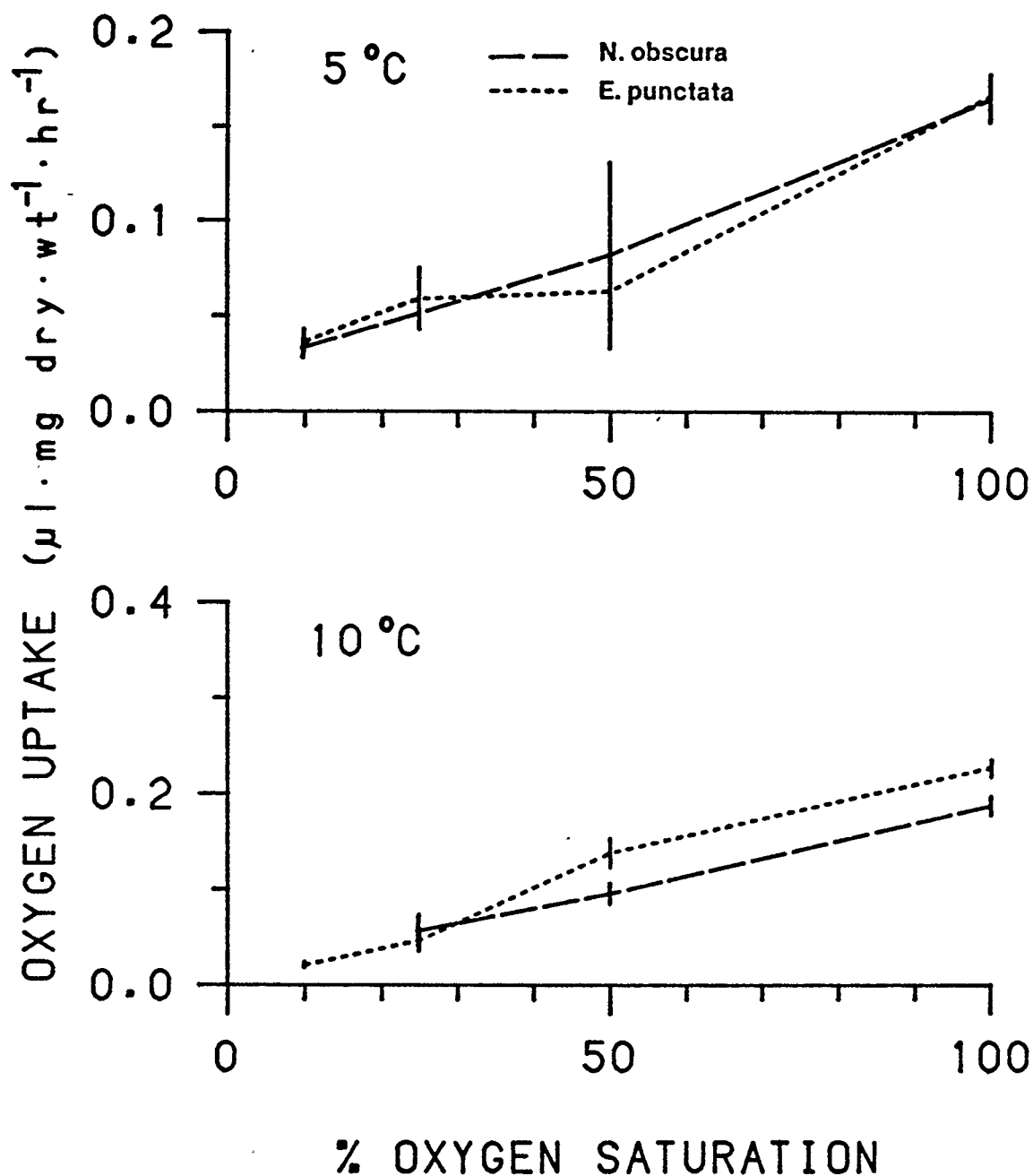
Figure 3.8: Mean oxygen uptake rates of resting, starved, non-reproductive N. obscura and E. punctata acclimated at 5 and 10°C when exposed to progressively increasing hypoxia over an 8 hour period (short term hypoxia). Vertical bars represent standard errors.
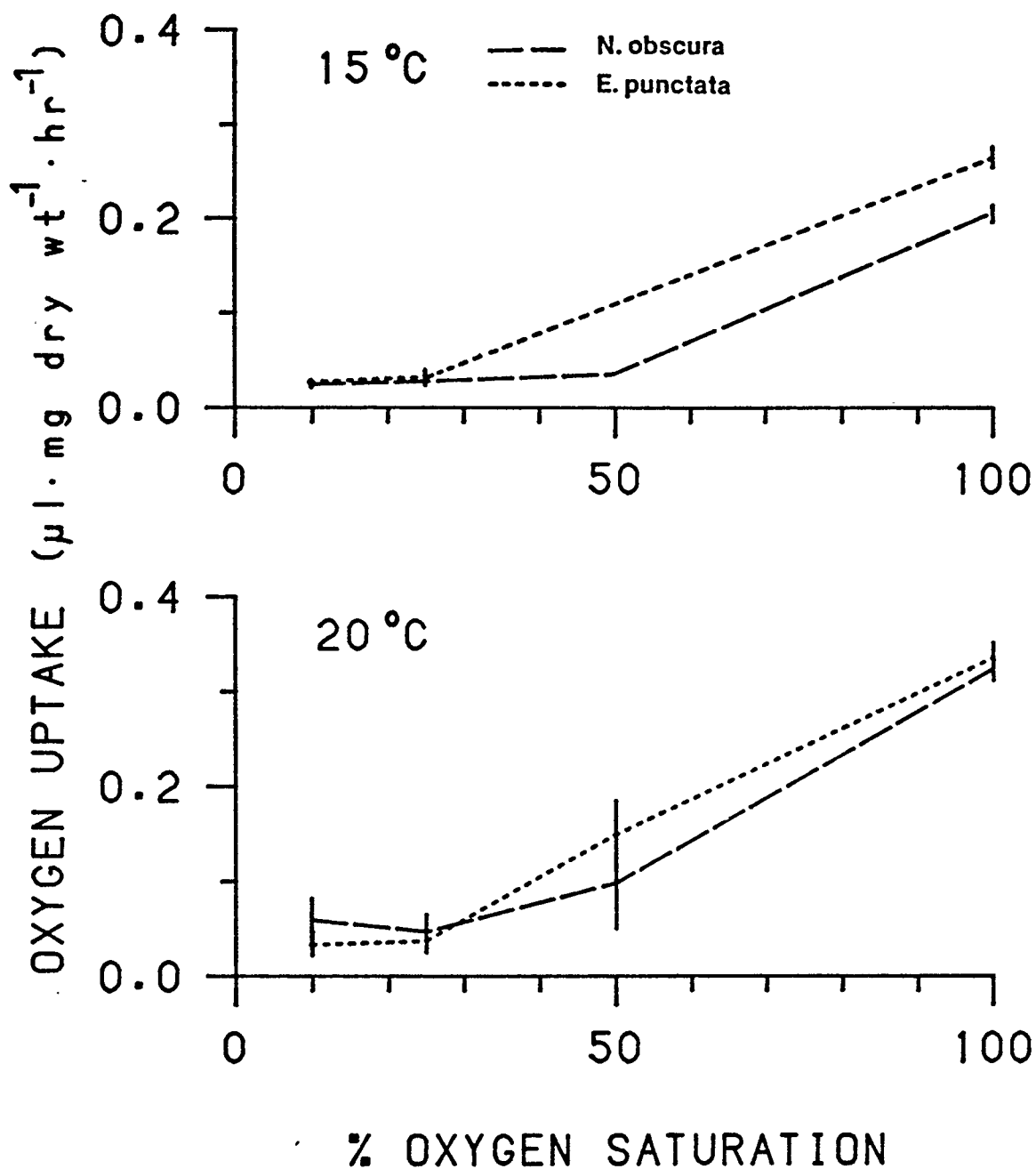
Figure 3.9: Mean oxygen uptake rates of resting, starved, non-reproductive N. obscura and E. punctata acclimated at 15 and 20°C when exposed to progressively increasing hypoxia over an 8 hour period (short term hypoxia). Vertical bars represent standard errors.

TABLE 3.3

Mean respiration rates ($\mu l \cdot$ mg dry wt $^{-1} \cdot$ hr $^{-1}$ ± SE) of randomly
moving starved, non-reproductive, N. obscura and E. punctata at different acclimation
temperatures when exposed to progressively increasing hypoxia over
an 8 hour period (short term hypoxia).

% Air-Saturation		N. obscura				E. punctata			
		100	50	25	10	100	50	25	10
T E M P (°C)	5	0.27 ± .05	0.07 ± .01	0.07 ± .03	0.06 ± .02	0.24 ± .04	0.06 ± .01	–	–
	10	0.30 ± .01	–	0.12 ± .07	0.05 ± .01	0.32 ± .03	–	0.13 ± .01	0.07 ± .01
	15	0.54 ± .09	0.36 ± .05	0.18 ± .01	0.03 ± .01	0.55 ± .10	0.35 ± .07	0.13 ± .04	0.06 ± .01
	20	0.60 ± .07	0.22 ± .05	0.10 ± .03	0.09 ± .01	0.58 ± .05	0.16 ± .04	0.27 ± .07	0.09 ± .08

TABLE 3.4

Mean respiration rates ($\mu l \cdot$ mg dry wt $^{-1} \cdot$ hr $^{-1} \pm$ SE) of ventilating, starved N. obscura and E. punctata at different acclimation temperatures when exposed to progressively increasing hypoxia over an 8 hour period (short term hypoxia).

% Air-Saturation	N. obscura				E. punctata			
	100	50	25	10	100	50	25	10
TEMP (°C) 10	−	−	0.16 ± .04	0.10 ± .01	−	−	0.25 ± .01	−
15	−	0.32 ± .04	0.10 ± .07	0.00 ± .02	0.44 ± .03	0.55 ± .05	−	−
20	0.73 ± .02	−	0.12 ± .05	−	0.55 ± .04	0.39 ± .05	0.18 ± .03	−

3.6.4 Long-term Hypoxia

Over the temperature range tested, no significant intra- or inter-specific differences in oxygen uptake were found for resting, starved N. obscura and E. punctata in response to exposure to long-term hypoxia of 10% air-saturated water over a five day period (Figures 3.10 and 3.11). Acclimation to reduced oxygen tension was not apparent in either species since the respiration rates at the end of the five day period were not significantly different from the rates at initial exposure (day 1), they at approximately an order of magnitude lower than the rates displayed under 100% air-saturated conditions.

Similar results were obtained for randomly moving (Figures 3.12 and 3.13) and ventilating (Figure 3.14) individuals of both species with no inter-specific differences in oxygen uptake or major ac-climatory responses being evident over the five day period.
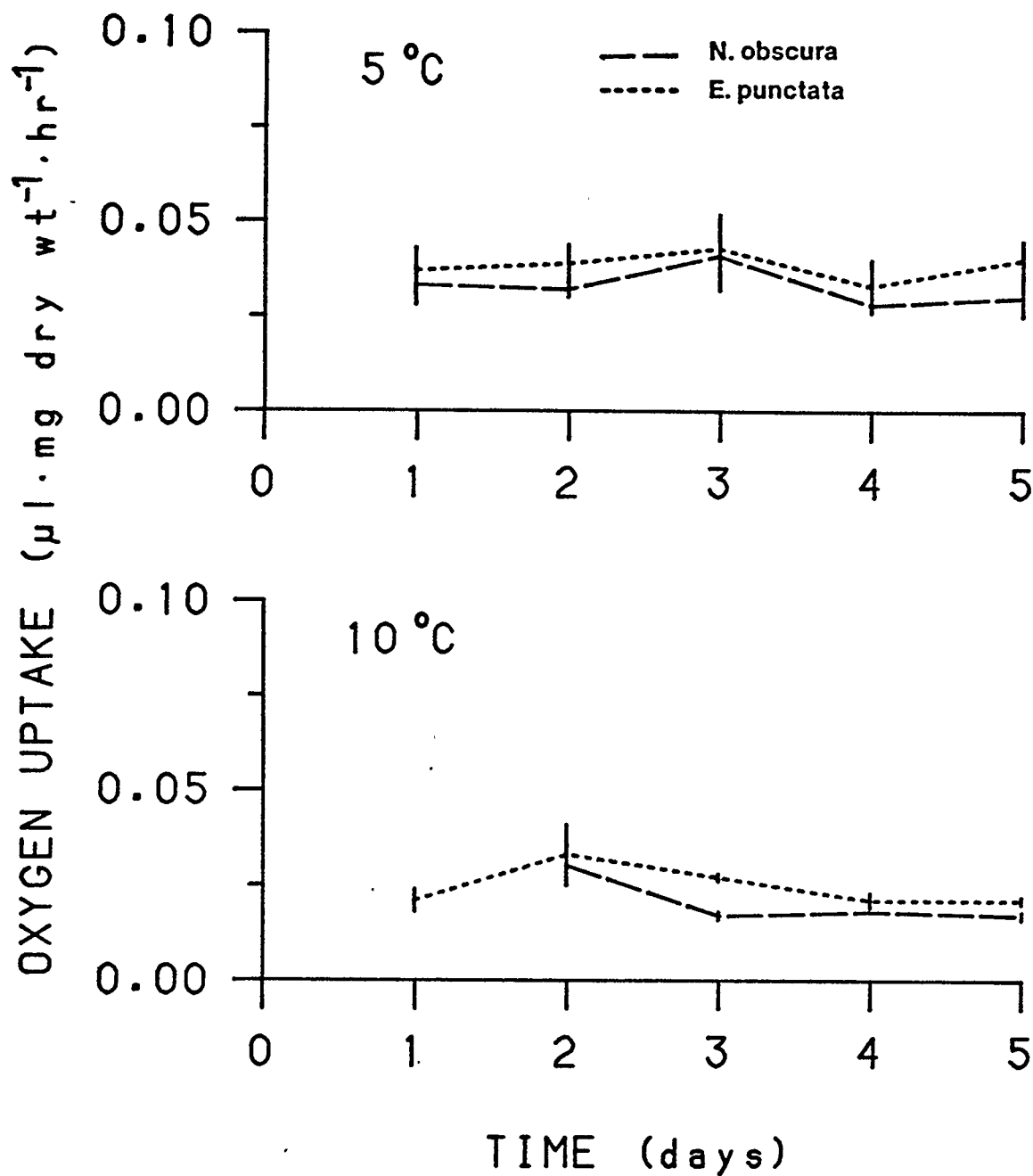
Figure 3.10: Mean oxygen uptake rates of resting, starved, non-reproductive N. obscura and E. punctata acclimated at 5 and 10°C when exposed to 10% air-saturated conditions over a 5 day period (long term hypoxia). Vertical bars represent standard errors.
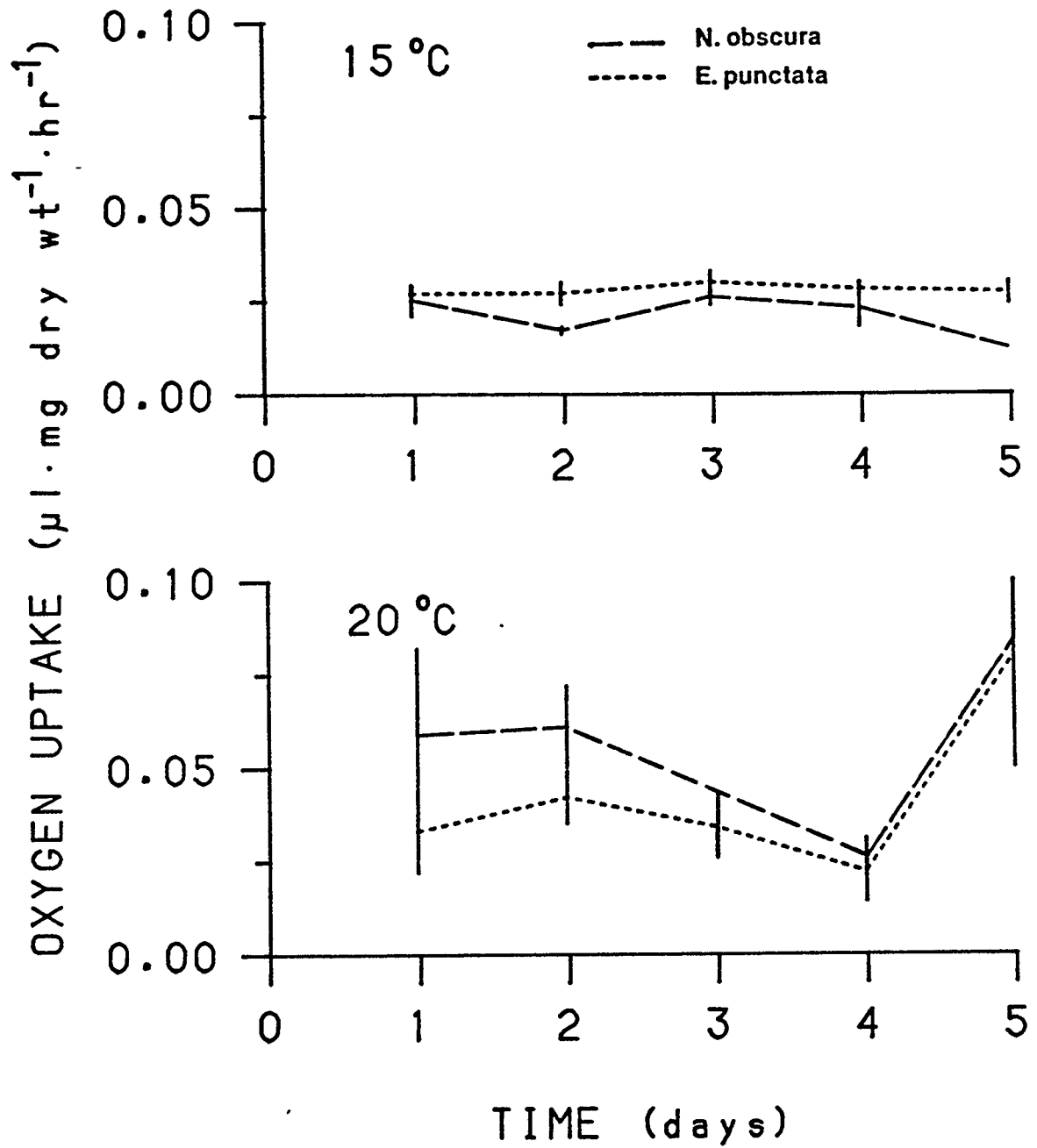
Figure 3.11: Mean oxygen uptake rates of resting, starved, non-reproductive N. obscura and E. punctata acclimated at 15 and 20°C when exposed to 10% air-satuarated conditions over a 5 day period (long term hypoxia). Vertical bars represent standard errors.
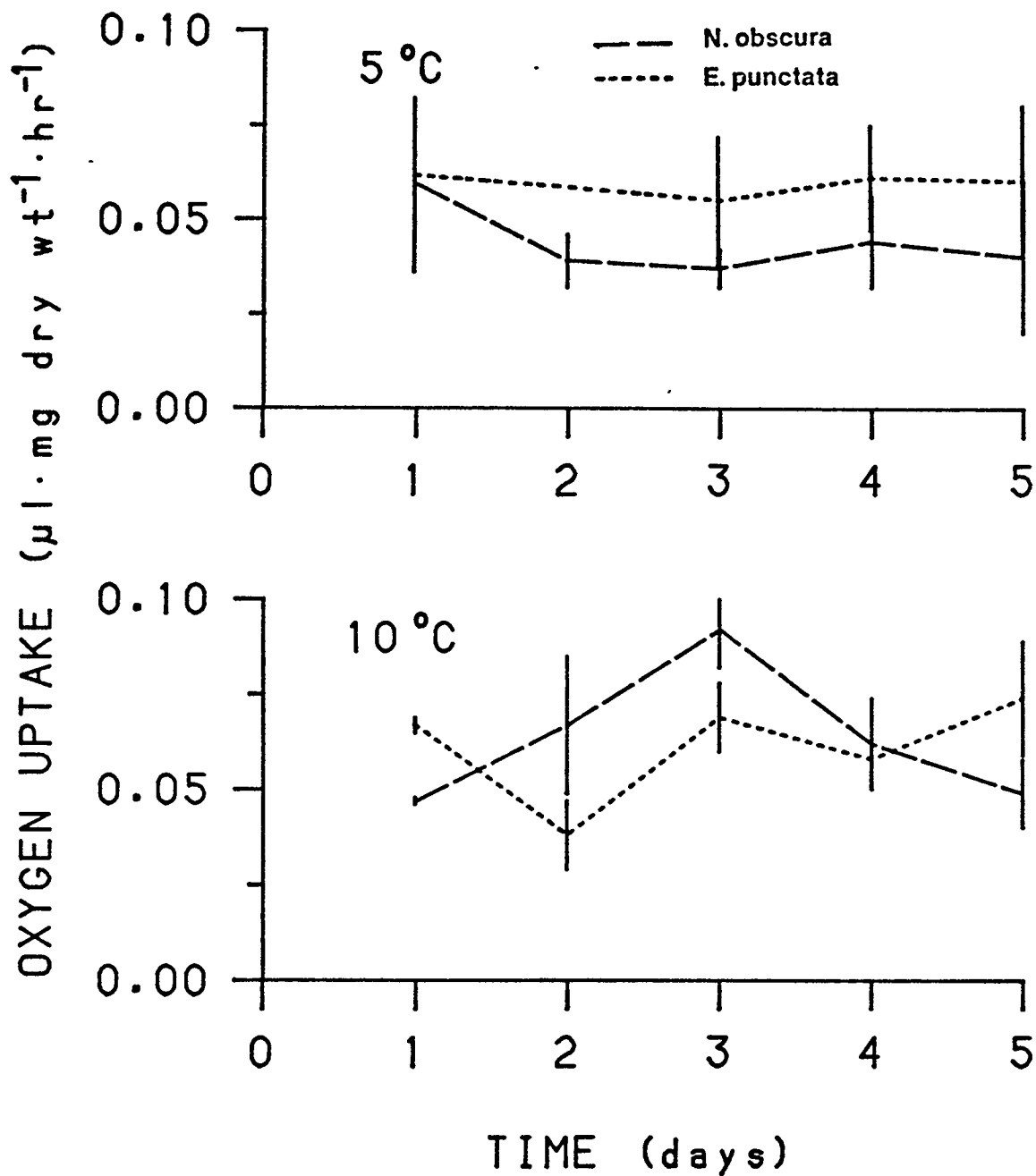
Figure 3.12: Mean oxygen uptake rates of randomly moving, starved, non-reproductive N. obscura and E. punctata acclimated at 5 and 10°C when exposed to 10% air-saturated conditons over a 5 day period (long term hypoxia). Vertical bars represent standard errors.
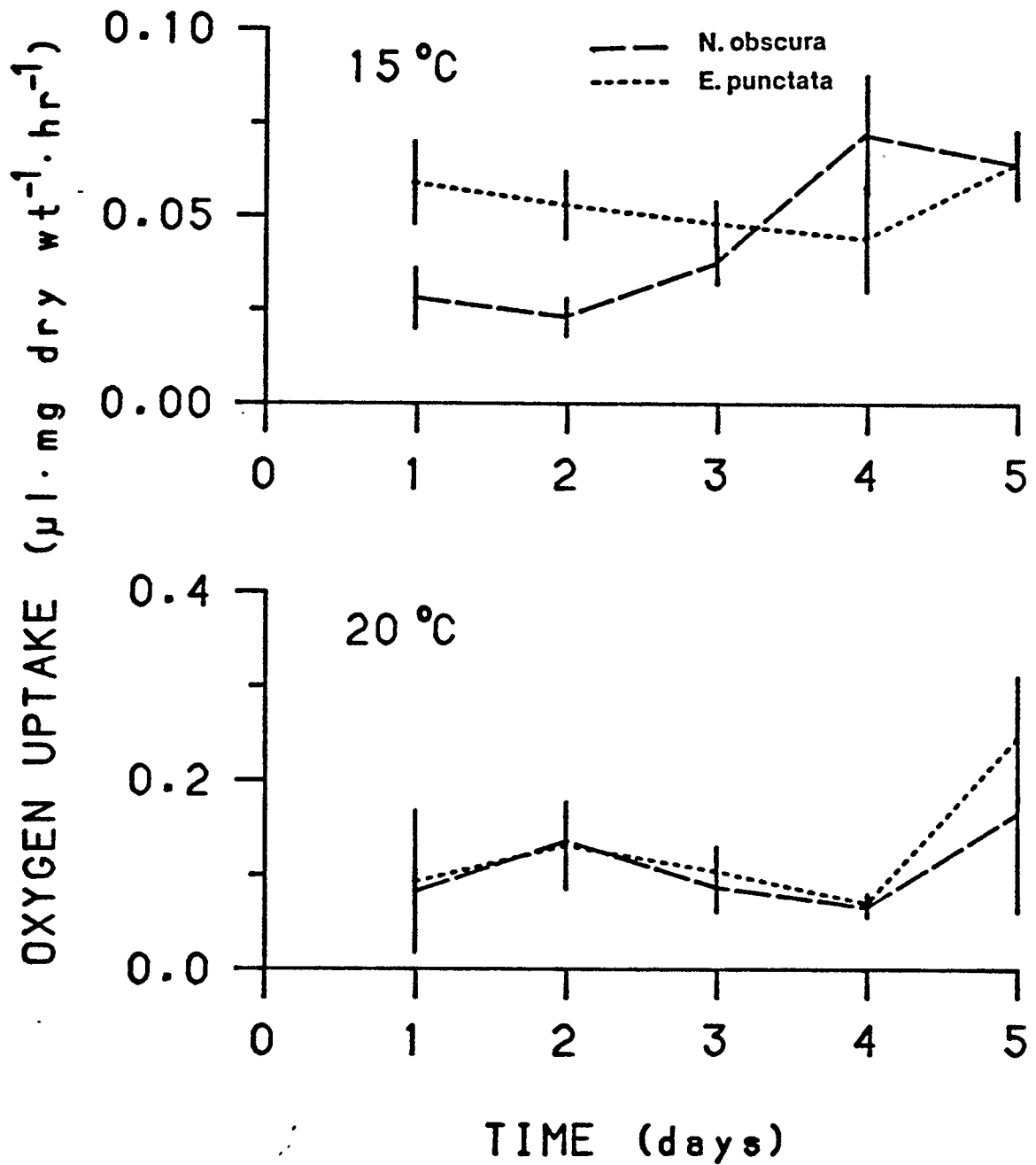
Figure 3.13: Mean oxygen uptake rates of randomly moving, starved, non-reproductive <u>N. obscura</u> and <u>E. punctata</u> acclimated at 15 and 20°C when exposed to 10% air-saturated conditions over a 5 day period (long term hypoxia). Vertical bars represent standard errors.
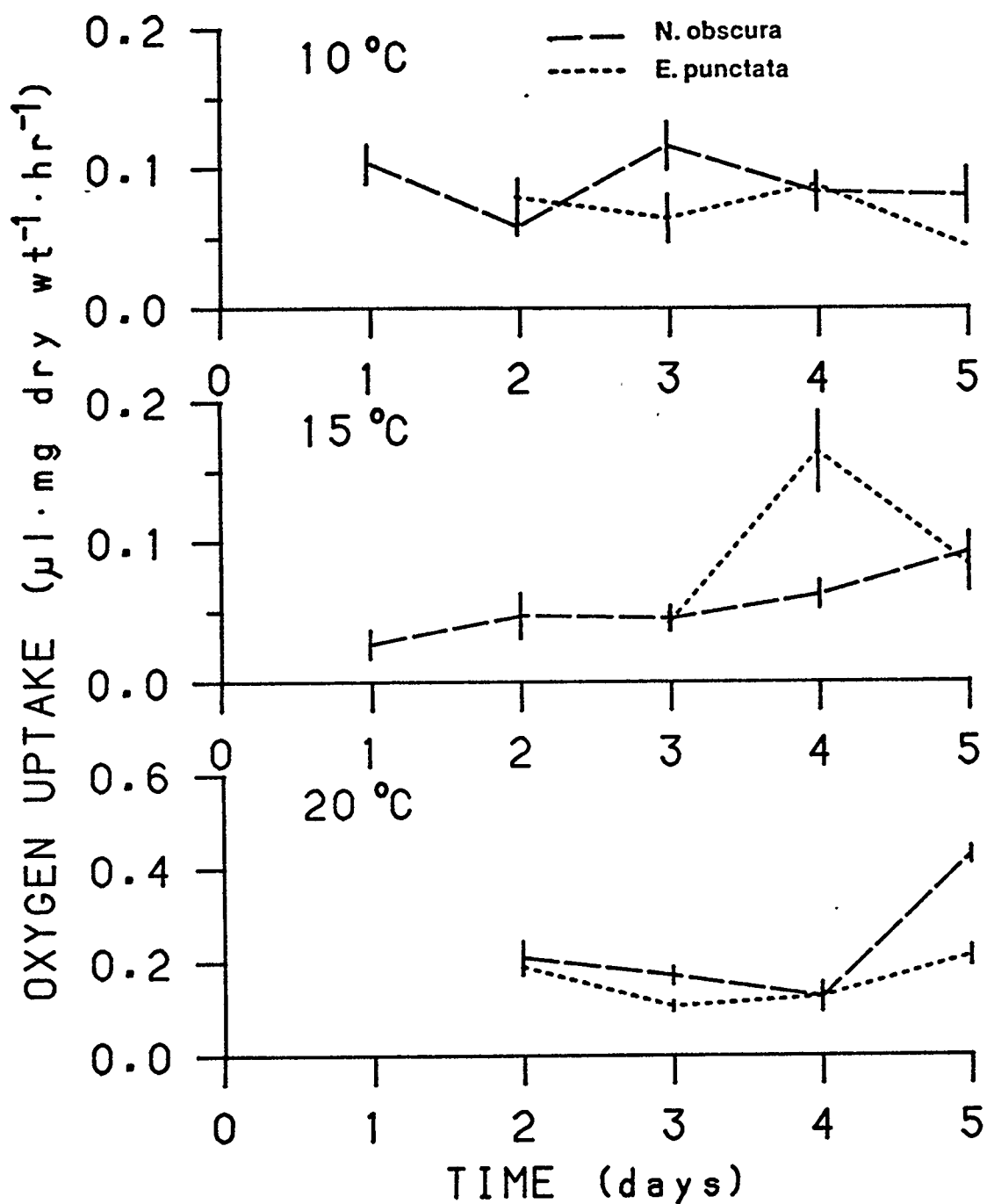
Figure 3.14: Mean oxygen uptake rates of ventilating, starved, non-reproductive N. obscura and E. punctata acclimated at 10, 15 and 20°C when exposed to 10% air-saturated conditons over a 5 day period (long term hypoxia). Vertical bars represent standard errors.

3.6.5 Anoxia Experiments

Differences were found between N. obscura and E. punctata with
respect to their ability to survive anoxia over the range of tem-
peratures examined (Figure 3.15). At 5 °C, 60% of N. obscura were
still alive at the end of a 30 day exposure period, with all mortality
occurring by day 12. In contrast, E. punctata had 100% mortality by
day 24. Survivorship under anaerobiosis was similar for both species
at 10, 15 and 20 °C, although survival time decreased with increasing
temperature (Figure 3.15).
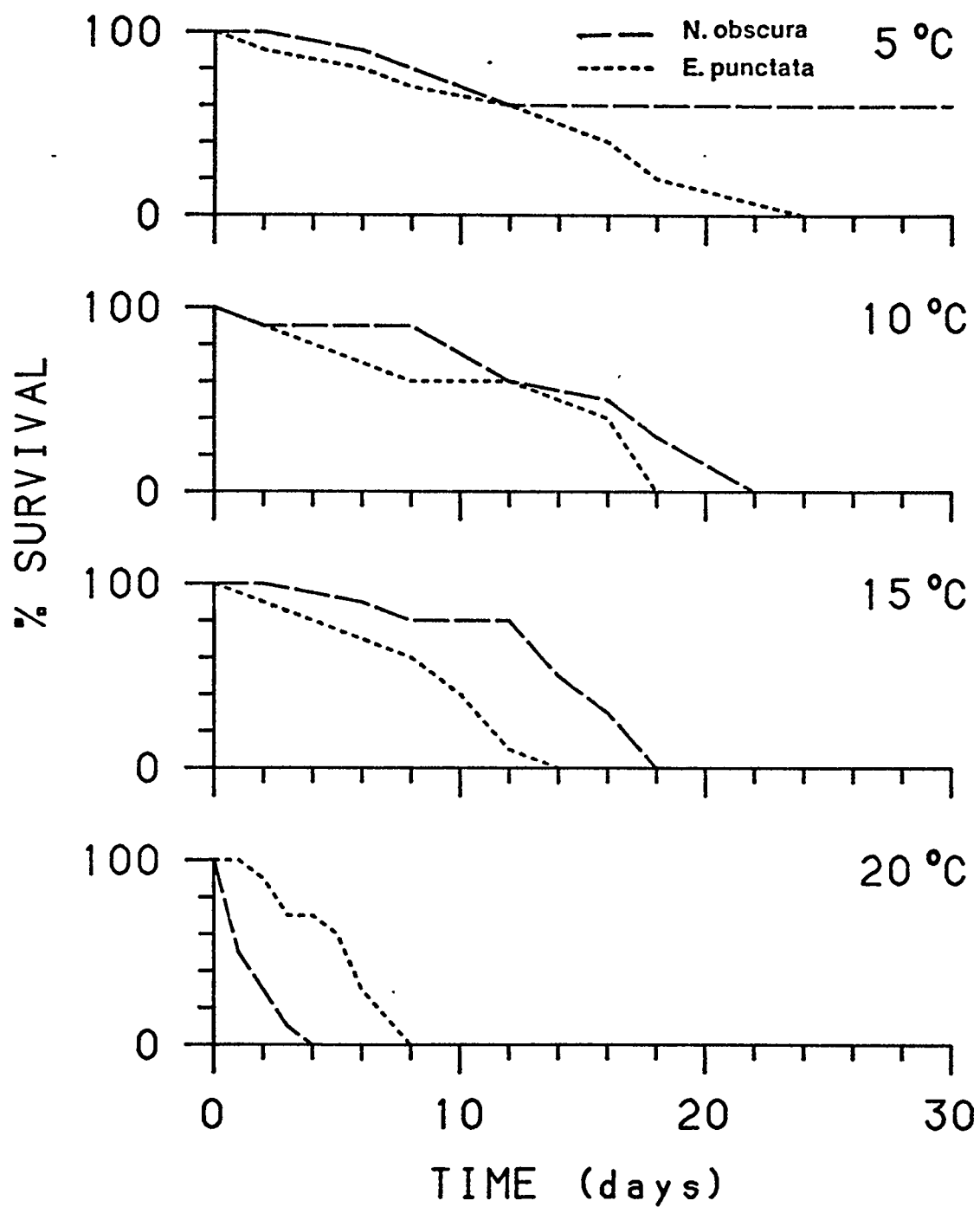
Figure 3.15: Survival time of starved, non-reproductive N. obscura and E. punctata under anoxia when acclimated at different temperatures.

3.6.6 Reproductive Condition

For both $\underline{N.\ obscura}$ and $\underline{E.\ punctata}$, the reproductive condition was found to have a marked influence on the measured oxygen uptake rates at all levels of activity at 15 °C (Table 3.5). Clitellate $\underline{N.\ obscura}$ and $\underline{E.\ punctata}$ at rest, randomly moving or ventilating, displayed higher rates of oxygen consumption than non-reproductive individuals with similar activity patterns. Additionally, resting, reproductive $\underline{E.\ punctata}$ were found to have oxygen uptake rates that were comparable to the values obtained for active, reproductive $\underline{N.\ obscura}$. Randomly moving and ventilating, reproductive $\underline{E.\ punctata}$ had significantly higher oxygen uptake rates than $\underline{N.\ obscura}$ under similar physiological states.

TABLE 3.5

Comparison of mean oxygen uptake rates ($\mu l \cdot$ mg dry wt $^{-1} \cdot$ hr $^{-1}$ ± SE) under different activity patterns of starved, reproductive (clitellate) vs. non-reproductive $\underline{N}$. $\underline{obscura}$ and $\underline{E}$. $\underline{punctata}$ at 15°C. R = resting, Rm = random movement, V = ventilating

	$\underline{N}$. $\underline{obscura}$			$\underline{E}$. $\underline{punctata}$		
	R	Rm	V	R	Rm	V
Reproductive	0.46 ± .02	0.86 ± .04	0.88 ± .08	0.73 ± .02	1.04 ±.04	1.09 ± .05
Non-Reproductive	0.21 ± .02	0.54 ± .09	–	0.27 ± .01	0.59 ± .10	0.44 ± .03

3.6.7 Ventilation Rates

3.6.7.1 100% Air-saturated Conditions

No significant intra-specific differences were found in the ventilation rates of starved, non-reproductive $N.$ obscura and $E.$ punctata at 15 and 20 ^{o}C (Table 3.6), but $E.$ punctata had higher ventilation rates than $N.$ obscura at 20 $^{o}C.$ Neither species were found to ventilate at 5 and 10 $^{o}C.$

Fed, non-reproductive $N.$ obscura showed no significant differences in ventilation rates from starved, non-reproductive individuals at 15 $^{o}C,$ but had significantly higher rates at 20 $^{o}C.$ Making the same comparison, $E.$ punctata displayed no significant differences at 15 or 20 $^{o}C.$ Both species when fed ventilated at 10 $^{o}C,$ with $E.$ punctata having rates significantly higher than $N.$ obscura.

Intra-specifically, fed, non-reproductive $N.$ obscura showed a significant functional relationship between ventilation rates and increasing temperatures. The rates were lowest at 10 ^{o}C and highest at 20 $^{o}C.$ In contrast, fed non-reproductive $E.$ punctata displayed no significant differences in ventilation rate over the temperature range of 10 - 20 ^{o}C (Table 3.6).

At 15 $^{o}C,$ reproductive, starved $N.$ obscura displayed no differences in ventilation rates to similar sized non-reproductive individuals. However, starved, reproductive $E.$ punctata showed significantly higher ventilation rates than comparable non-reproductive animals and had significantly higher values than reproductive $N.$ obscura.

TABLE 3.6

Mean ventilation rates (undulations $\cdot$ min $^{-1}$ $\pm$ SE) of
N. obscura and E. punctata under different reproductive conditions
when acclimated to different temperatures and feeding histories in
100% air-saturated water.

		Species	
Feeding History	Temperature (°C)	N. obscura	E. punctata
Starved: Non-reproductive	5	–	–
	10	–	–
	15	31 $\pm$ 2	40 $\pm$ 3
	20	31 $\pm$ 1	53 $\pm$ 4
Starved: Reproductive	15	28 $\pm$ 2	53 $\pm$ 2
Fed: Non-reproductive	5	–	–
	10	23 $\pm$ 1	34 $\pm$ 1
	15	32 $\pm$ 2	30 $\pm$ 2
	20	50 $\pm$ 2	40 $\pm$ 2

3.6.7.2 Hypoxic Conditions

Under short-term hypoxia, both N. obscura and E. punctata displayed no functional relationship between ventilation rates and partial pressure of oxygen at a given temperature (Table 3.7). However, the ventilation rates of E. punctata tended to be higher than N. obscura at 15 and 20 $^{\circ}$C.

During long-term hypoxia, no significant differences were found in mean ventilation rates of N. obscura between 10 and 15 $^{\circ}$C, but ventilation at 20 $^{\circ}$C was significantly higher (Table 3.6). The ventilation rate of E. punctata was found to significantly increase as a function of increasing temperature. No significant inter-specific differences were found between the ventilation rates of N. obscura and E. punctata under long-term hypoxia at 10 and 15 $^{\circ}$C, however significant differences occurred at 20 $^{\circ}$C, with E. punctata displaying a higher mean rate.

No ventilatory behavior was observed for either species at 5 $^{\circ}$C during short or long-term hypoxia.

TABLE 3.7

Mean ventilation rates (undulations $\cdot$ min^{-1} $\pm$ SE) of starved N. obscura and E. punctata at different temperatures when exposed to progressively increasing hypoxia over an 8 hour period (short term hypoxia) and over a 4 day period at 10% air-saturation (long term hypoxia). 10_S = 10% air-saturation-short term hypoxia, 10_L = 10% air-saturation-long term hypoxia.

% Air-Saturation	N. obscura					E. punctata				
	100	50	25	10 S	10 L	100	50	25	10 S	10 L
TEMP 10	–	–	33 ± 1	25 ± 1	27 ± 2	–	–	30 ± 1	–	28 ± 2
(°C) 15	31 ± 2	20 ± 7	29 ± 2	26 ± 3	29 ± 2	40 ± 5	56 ± 2	–	–	37 ± 2
20	31 ± 1	–	38 ± 2	–	43 ± 2	53 ± 4	42 ± 2	65 ± 7	–	53 ± 3

3.7 Discussion

The existence of differing respiratory strategies between
N. obscura and E. punctata could account for the apparent ecological
advantage one species has over the other in certain types of habitats.
Linton, Davies and Wrona (1982) found no inter-specific differences in
the respiratory strategies of N. obscura and E. punctata in response
to ionic composition, total dissolved solids and temperature, and
concluded that the observed differences in distribution and abundance
of these two species between lentic and lotic habitats could not be
explained on the basis of differences in respiratory physiologies.
This study reveals that the respiratory strategy displayed by
N. obscura would give it an ecological advantage over E. punctata in
warmer habitats and the respiratory strategy displayed by E. punctata
an advantage in colder situations. The contradiction between these
two studies is probably a function of Linton, Davies and Wrona (1982)
employing a Gilson respirometer, an inadequate technique for
monitoring leech respiration, and their inability to monitor the
activity patterns of the leeches during measurement of oxygen uptake.
Calow and Riley (1980) have also reported the difficulty in measuring
leech respiration using systems based on closed chambers and advocated
the use of a continuous flow-through respirometer of the type
developed in this study.

In this study, the development of a continuous flow-through
respirometer system in conjunction with an impedance system for
monitoring animal movement has allowed the oxidative metabolism of

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both species to be examined in relation to different environmental and
physiological states while under different activity patterns. Three
distinct activity patterns were defined for N. obscura and E. punctata
these being rest, random movement and ventilation (Figure 3.3).

During respiratory analysis of aquatic poikilotherms, it is
important to distinguish between active and quiescent rates of oxygen
consumption since experimental temperature may affect the two rates
differently (Newell, 1973). Although temperature has been recognized
to be one of the major environmental determinants of the rate of
oxidative metabolism and activity levels of aquatic poikilotherms,
many invertebrates when quiescent and under starvation have shown the
ability to maintain oxygen uptake rates relatively independent of
environmental temperature (Bayne, Thompson and Widdows, 1976; Newell,
1969,1973). In this study, the respiration rates of resting, starved
N. obscura were found to remain relatively independent of temperature
over the range of 5 - 15 ^{o}C, with a marked increase occurring only at
20 ^{o}C (Figure 3.4). Starved, quiescent E. punctata displayed similar
trends between 10 - 20 ^{o}C, but had higher rates than N. obscura at 10
and 15 ^{o}C (Figure 3.4). In contrast, starved, active N. obscura
displayed temperature-dependent oxygen uptake rates over the tem-
perature range of 5 - 20 ^{o}C, while E. punctata of similar physio-
logical state showed temperature-dependent metabolism between 10 -
15 ^{o}C (Figure 3.4).

Newell (1973) suggested that the variations in the level and
temperature-dependence of respiration in poikilotherms is associated
with mobilization or reduction in enzyme substrate availability to the

mitochondria. Starvation was found to deplete metabolic reserves thereby making substrate availability to the mitochondria the rate limiting process. Both N. obscura and E. punctata displayed lowest temperature-dependence in oxygen consumption when starved and at rest (Figure 3.4). The maintenance of thermal-independence in metabolism under starvation would be adaptive to both leech species in that it would allow them to cope with times of low food availability over a relatively wide range of temperatures. N. obscura may be slightly more successful at this than E. punctata at 10 and 15 ^{o}C since it maintains lower oxygen consumption rates over this range. However, the metabolic costs associated with maintenance are similar for both species at 5 and 20 ^{o}C.

Because the rate of movement of aquatic poikilotherms is generally temperature dependent, their measured oxygen uptake rates also tend to be temperature dependent since activity is an energy consuming process that dominates the energy requirements of an organism (Newell, 1973; Bayne, Thompson and Widdows, 1976).

The increase of thermal-dependence in the respiration rate of active animals, displayed by N. obscura and E. punctata (Figure 3.4), may be attributable to the fact that food reserves are biochemically being made available for the energy demanding processes involved with locomotion. This would result in enzyme-substrate concentrations being at or near saturation levels, resulting in the enzyme reaction rate now being temperature limited rather than limited by substrate availability (Newell, 1973).

The role of the nutritional condition of the animal in affecting both oxygen consumption and the relationship of oxygen consumption to temperature change is an important factor in determining the overall respiratory strategy of a species (Feder, 1981; Bayne, Thompson and Widdows, 1973, 1976).

The influence of temperature on the respiration rate of fed, resting N. obscura and E. punctata (Figure 3.6) was greater than that observed for starved individuals (Figure 3.4). Except at 5 ^{o}C, satiated, resting N. obscura displayed higher oxygen consumption rates than E. punctata (Figure 3.6). Significantly different trends were found between active, satiated N. obscura and E. punctata with N. obscura displaying temperature-dependent uptake rates from 5 - 15 ^{o}C, where it reached an asymptote, and E. punctata showing temperature-independent respiration rates between 10 - 20 ^{o}C. Similar comparative results were obtained on Mytilus edulis (L.), where starved, inactive individuals were found to have oxygen consumption rates that were insensitive to temperature, while fed animals displayed temperature-dependent metabolic rates (Bayne, Thompson and Widdows, 1973).

It is evident that the combined influence of hunger and activity levels and temperature on the observed oxygen uptake of N. obscura and E. punctata are complex and different. It is therefore imperative to have knowledge of the nutritional condition and activity of the animal before the physiological effects of temperature can be ascertained.

One of the most promising methods for physiologically assessing the influence of environmental factors and physiological states on the

survival of a species is the measurement of scope for activity (Rutledge and Pritchard, 1981). The scope for activity, which is the capacity to which activity can be aerobically supported, is calculated from the difference between the maximal (active metabolism) and the minimal (standard metabolism) levels of oxygen consumption in any particular physiological state (Bennett, 1978). Standard metabolism is defined as "the rate of oxygen consumption by an animal incurring minimal physiological and mechanical costs of metabolism", while active metabolism is "the rate of oxygen consumption by an animal incurring the maximal physiological and mechanical metabolic costs" (Bayne, Thompson and Widdows, 1973).

Fry (1947, 1957) originally formulated the term scope of activity, and used it as a physiological index of muscular work capacity. However, recently the recognition of the importance of the role of anaerobic metabolism to the activity energetics of organisms has resulted in renaming the measurement as aerobic metabolic scope (Bennett, 1978).

From an ecological perspective, the aerobic metabolic scope of a species represents the capacity to which activities such as locomotion involved with foraging and reproduction can be aerobically supported, which are energy-expenditures above the short-term maintenance requirements. A species displaying a higher aerobic metabolic scope compared to another under similar environmental conditions and physiological states will potentially have an ecological advantage (Figure 3.1). Since temperature is of major importance to the overall physiology of aquatic poikilotherms, aerobic metabolic scope should be

measured for a series of temperatures within the range of temperature tolerances for the species in order to elucidate its overall ecological importance (Rutledge and Pritchard, 1981).

Starved E. punctata were found to display no correlation between aerobic metabolic scope and temperature (Figure 3.6), which indicates that over the temperature range 5 - 20 °C, starved E. punctata have relatively the same potential to support aerobic work. In contrast, N. obscura showed temperature-dependent aerobic metabolic scope, having low values at colder temperatures (5 and 10 °C) and higher values in warmer conditions (15 and 20 °C) Therefore at warmer temperatures and under saturated oxygen conditions, N. obscura would have a potential ecological advantage over E. punctata in that it has a higher physiological capacity to aerobically support energy demanding processes such as foraging, reproduction, swimming and ventilation. However, the reverse situation occurs at colder temperatures (5 °C) with saturated oxygen conditions where E. punctata possesses the higher potential capacity for aerobic work.

This relationship is reinforced when the aerobic metabolic scope of fed individuals is examined (Figure 3.7). Again N. obscura has potentially more energy available to perform aerobic physiological activities above the costs of normal maintenance at warmer temperatures (15 and 20 °C), while the same is true for E. punctata under colder conditions (<10 °C).

It is evident that the differing respiratory strategies displayed by fed and starved N. obscura and E. punctata at the various temperatures would potentially have a profound influence on their

ecological distribution and success, and be directly dependent on the temperature regimes of the habitat. On the basis of these results, it would be predicted that N. obscura would be ecologically more successful in warmer habitats, and E. punctata in colder. This validates the field distribution data where N. obscura is found to be numerically dominant in lentic habitats and E. punctata in lotic (Davies, Reynoldson and Everett, 1977; Linton, Davies and Wrona, 1982).

Over the range of temperatures tested, irrespective of activity level, both species displayed similar trends in oxygen consumption when exposed to short-term hypoxia (Figures 3.8 and 3.9, Tables 3.3 and 3.4). Starved N. obscura and E. punctata behaved as oxy-conformers, displaying oxygen uptake rates that were functionally dependent on the oxygen concentration of the medium. It should be noted that categorizing N. obscura and E. punctata as oxy-conformers is of heuristic value only, since classification of animals as metabolic conformers or regulators to external oxygen concentrations is usually an oversimplification. Mangum (1970) and Mangum and Van Winkle (1973) have demonstrated for a wide variety of aquatic poikilotherms that regulation of oxygen uptake is often imperfect and is influenced by other environmental factors (i.e. temperature) over the entire range of oxygen concentrations. A number of additional physiological factors, including body size, efficiency of the circulatory system, activity, presence of respiratory pigments and diffusion distances (Nicol, 1967; Davis, 1975) have been suggested as determinants of whether an animal displays oxy-comformity or

oxy-regulation. Although oxy-conformity tends to be depicted by organisms living in relatively well oxygenated habitats, animals that live in low oxygen conditions and can rely on some form of anaerobic metabolism for energy also tend to be oxy-conformers (Prosser and Brown, 1962). Weber (1978) found that anaerobiosis in aquatic annelids is not limited to conditions of complete anoxia, but the oxy-conformity displayed by many species may in part result from progressive increase in the participation of anaerobic metabolism as oxygen tensions decline. Both N. obscura and E. punctata displayed differential abilities to survive anoxic conditions (Figure 3.15), therefore raising the possibility that contribution from anaerobic metabolism allows them to be oxy-conformers.

The time period allowed for acclimation to low oxygen conditions may be critical in influencing the degree of regulation of oxygen uptake displayed by a species. Chironomidae larvae have been found to have oxygen consumption rates that are more independent of environmental oxygen after being allowed to acclimate to low oxygen tensions for several hours and Daphnia sp. were found to produce more haemoglobin after exposure to low oxygen concentrations for several days (Prosser and Brown, 1962). While it may be postulated that the observed oxy-conformity of N. obscura and E. punctata was a result of the acclimation to 100% air-saturation at the various temperatures, the results from the long-term hypoxia experiments indicate no inter-specific differences in oxygen uptake at any activity level over the range of experimental temperatures and showed no acclimation to low oxygen tensions (Figures 3.10 - 3.14). Over the five day exposure

to 10% oxygen saturation, N. obscura and E. punctata maintained
relatively constant and suppressed levels of aerobic metabolism.
Thus, starved N. obscura and E. punctata appear to have similar
abilities to aerobically cope with declining oxygen conditions and no
clear ecological advantage is apparent for either species under
hypoxic conditions. However, inter-specific differences were found
with respect to their abilities to survive environmental anoxia
(Figure 3.15).

Between 5 - 15 °C, N. obscura survived anaerobic conditions for a
longer period of time than E. punctata, especially at 5 °C where 60%
of N. obscura were still alive at the end of 30 days. Survival time
of both species under anoxic conditions tended to decrease as a
function of increasing temperature. In contrast, E. punctata showed
slightly longer survivorship at 20 °C. These results refute the
hypothesis forwarded by Linton (1980), who suggested that since the
two species are phylogenetically closely related, they would display
similar anaerobiosis.

It is evident that the degree to which anaerobic metabolism
contributes to the overall respiratory strategy of each species is of
major importance to their ecological success within a given habitat.
N. obscura appears to be superior in withstanding anaerobic conditions
at low temperatures, possibly allowing it to better survive the
oxygen-poor conditions typical of lentic habitats during the fall and
winter. In contrast, E. punctata would not be expected to be exposed
to low oxygen conditions at low temperatures in the winter in lotic
situations, which may be reflected by its inability to survive anoxia

at 5 °C. The shift in responses between N. obscura and E. punctata at
20 °C may be indicative of inter-specific differences in anaerobic
pathways. Schottler and Weinhauser (1981) found inter-specific
differences to exist in the anaerobic pathways of two marine
polychaetes, Nereis virens (L.) and Arenicola marina (L.). A more
closely related species to N. obscura and E. punctata, Hirudo
medicinalis utilized different anaerobic biochemical pathways as a
function of varying environmental and physiological conditions (Zebe,
Salge, Wiemann and Wilps, 1982). Based on these studies, elucidation
of the contribution of anaerobic metabolism to the respiratory
strategies of N. obscura and E. punctata may be a complex problem.

The stage of development of an individual has a great influence
on the measured rate of oxygen uptake in aquatic poikilotherms
(Prosser and Brown, 1962; Calow, 1975; Bayne, Thompson and Widdows,
1976; Weber, 1978). At 15 °C, starved, reproductive N. obscura and
E. punctata were found to have significantly higher rates of oxygen
uptake than similar sized non-reproductive individuals performing
identical activities (Table 3.5). However, reproductive E. punctata
had significantly higher respiration rates than N. obscura under
similar activity patterns. Therefore at 15 °C, being in a repro-
ductive condition appears to be energetically more costly to
E. punctata than N. obscura, which may express itself in inter-specifc
differences in reproductive output.

Both N. obscura and E. punctata ventilated under 100% saturation
and hypoxic conditions (Tables 3.6 and 3.7). Since oxygen intake in
most Hirudinoidea is via diffussion gradients through the general body

surface, ventilation is believed to supplement oxygen uptake by irrigating the body surface with a renewed supply of water and oxygen (Mann, 1961c). The rate of ventilation has been found in other species of Hirudinoidea to be functionally related to temperature (Herter, 1936; Mann, 1961c) and oxygen tension (Mann, 1956). Under 100% air-saturated conditions, temperature, nutritional condition and reproductive state were found to differentially influence the ventilation rates of N. obscura and E. punctata (Table 3.6). Starved individuals of both species were found to ventilate only at warmer temperature (15 and 20 °C) while fed animals ventilated over a wider range of temperatures (10 - 20 °C), with ventilation rates higher at the warmer temperatures. The occurrence of elevated ventilation rates in both species at higher temperatures is probably a physiological response to declining oxygen availability, since oxygen concentration decreases with increasing temperature. Reproductive condition did not influence the ventilation rates of N. obscura, but reproductive E. punctata had higher ventilation rates than non-reproductive individuals displaying a similar activity. In contrast, nutritional state affected the ventilatory rate of N. obscura but not of E. punctata, with the ventilation rate of N. obscura increasing with increasing temperature.

Under declining oxygen conditions, N. obscura and E. punctata displayed no functional relationship between ventilation rate and partial pressure of oxygen over the range of temperatures examined, with the ventilation rates of E. punctata tending to be higher than N. obscura. Therefore, neither species appeared to use oxygen tension

as an environmental cue for determining the rate of ventilation.

Under long-term hypoxia, inter-specific differences occurred only at 20 °C, with E. punctata displaying higher mean ventilation rates. Temperature was the most important environmental factor influencing ventilation rates under long-term exposure to low oxygen concentrations since E. punctata was found to significantly increase its ventilation rate with increasing temperature and N. obscura displayed its highest rates at 20 °C.

Although the ecological ramifications of these many complex relationships are difficult to ascertain, they do reveal the importance of considering the influences of the physiological state as well as the environmental parameters (Table 3.1) when performing comparative respiratory studies.

4.0 GROWTH AND REPRODUCTION

4.1 Introduction

The ecological success of a species is in part dependent upon

each individual's longevity, speed of development to reproductive age

and production of viable young (progeny which themselves successfully

reproduce). Since fitness is positively correlated with survivorship

and fecundity and negatively correlated with generation time, under

the maximization principle (Calow, 1978; Calow and Townsend, 1981),

natural selection would tend to maximize the energy available for

growth (Calow, 1978). Thus, the measurement of growth rates under

defined biotic and abiotic conditions provides a partial measurement

of fitness and potential ecological success of a species at those

conditions. Also, as fitness is usually measured in terms of

differential production of viable offspring, examination of cocoon

production provides an additional more direct measure of fitness.

Water temperature has been suggested by many authors to be a

major environmental factor in controlling growth and reproductive

activity of leeches. The onset of cocoon production by _Helobdella_

stagnalis and _Glossiphonia_ _complanata_ has been assumed to be related

to water temperature increases coincident with the onset of spring

(Castle, 1900; Bennike, 1943; Mann, 1957a, b, 1961c; Sawyer, 1972;

Tillman and Barnes, 1973; Learner and Potter, 1974). Davies and

Reynoldson (1976), comparing the life history and growth of

_H._ _stagnalis_ between populations in British Columbia and Alberta,

found the temperature regime at each location to be important in

determining the number of generations produced per year, and attributed the absence of H. stagnalis from smaller lentic habitats in Alberta to an insufficient number of degree-days to successfully complete its life cycle. However, Young and Ironmonger (1981) studying the influence of temperature on the egg production, speed of egg development, growth and maturity of H. stagnalis, G. complanata and Erpobdella octoculata, concluded that differences in thermal ecologies could not account for the observed distribution and abundance of these species in British lakes.

Limited information is available on the influence of water temperature regime on the growth and reproductive activities of N. obscura and E. punctata. N. obscura always had two distinct breeding periods (May - June and August - September) which occurred irrespective of any fluctuations in water temperature and Davies and Everett (1977) showed that the primary prerequisite for cocoon production by N. obscura was the attainment of a critical adult size (>150 mg wet weight) before the start of the cocoon producing period. If an individual had not reached the critical reproductive weight before the onset of one of the reproductive periods (May - June, August - September), it did not reproduce until the next reproductive period, even though the individual grew beyond the critical reproductive weight in the meanwhile.

In a laboratory study of N. obscura cocoon production in relation to water temperature (4, 15 °C), ionic content (hard versus soft water) and T.D.S. (25 - 2000 mg·l^{-1}), Linton (1980) concluded that

both the initiation and continuation of cocoon production by mature

sized N. obscura was temperature related, while water type and ionic

concentration had minimal influence.

Water temperature has also been assumed to influence the onset of

reproduction in field populations of E. punctata. In Michigan,

E. punctata displayed a correlation between the initiation of cocoon

production and the increasing water temperature in the spring (Sawyer,

1970, 1972). However, Davies, Reynoldson and Everett (1977) found the

reproductive strategy of E. punctata in Alberta not directly or solely

correlated with water temperature as the timing of cocoon production,

speed of development to reproductive maturity, weight at reproductive

maturity, and duration of the reproductive period was related to the

dominance or sub-dominance of sympatric N. obscura.

One of the major abiotic differences between lentic and lotic

habitats in southern Alberta where N. obscura and E. punctata are

abundant is the thermal regime (Figure 1.1). Thus, the observed

differences in numerical dominance of these two species in lentic and

lotic habitats could possibly be explained on the basis of

species-specific differences in population fitness, as measured by

growth and production of viable offspring, in relation to the water

temperature regime.

4.2 Objectives

The objective of the study was to examine quantitatively the hypothesis that the numerical dominance of N. obscura in lentic and E. punctata in lotic habitats of southern Alberta is in part a function of species-specific differences in growth and reproductive success at the thermal regimes experienced.

4.3 Materials and Methods

4.3.1 Cocoon Production

Pre-reproductive individuals (clitellate, >150 mg wet weight) of N. obscura and E. punctata were collected from Stephenson Pond and the Bow River respectively in the last two weeks of April. At least thirty specimens of each species were acclimated for a minimum of 14 days at 5, 10, 15, 20 °C in individual containers filled with de-chlorinated Bow River water. The total number of cocoons deposited by each individual was recorded and when the chitin of the cocoons was sufficiently tanned to allow handling, the number of viable (i.e. cleaved) eggs was determined using a stereo dissecting microscope. Cocoons were subsequently incubated at the appropriate test temperature to determine hatching success.

4.3.2 Growth

Fifty young (initially 5 - 15 mg wet weight) N. obscura and E. punctata were maintained for 12 weeks in de-chlorinated, air-saturated, Bow River water at 5, 10, 15 and 20 °C with the water changed bi-weekly to eliminate fouling. The leeches were fed daily ad libitum on Enchytraeus sp. and growth rates determined by weighing each population weekly to the nearest 0.1 mg on a Sartorius analytical balance (Model 1602 MP).

4.4 Results

4.4.1 Cocoon Production

The onset of cocoon production was temperature related for both
N. obscura and E. punctata, although species-specific differences were
also observed (Figure 4.1). N. obscura laid no cocoons at 5 °C and
only 38% of the population produced cocoons at 10 °C; at 15 and 20 °C
over 80% of the individuals produced cocoons. One-way ANOVA with SNK
analysis showed a significant difference in the mean number of cocoons
produced per individual N. obscura between experimental temperatures,
with the lowest number of cocoons produced at 10 °C, and the highest
at 20 °C (Table 4.1). In contrast, 40% of the E. punctata produced
cocoons at 5 °C, 65% at 10 °C, and over 90% of the population produced
cocoons at 15 and 20 °C (Table 4.1). At 5 °C, cocoon production by
E. punctata was significantly lower than at 10, 15 and 20 °C (Table
4.1). No inter-specific differences occurred in the mean number of
cocoons laid at 15 and 20 °C, however E. punctata produced signif-
icantly more than N. obscura at 5 and 10 °C.

One-way ANOVA showed a significant temperature-dependent effect
on the mean number of eggs per cocoon produced by N. obscura with the
number of eggs positively correlated with increasing temperature
(Table 4.1). E. punctata produced a similar number of eggs per cocoon
at 5, 10 and 15 °C, but a lower number of eggs per cocoon at 20 °C.
At 5, 10, 15 °C, E. punctata produced significantly more eggs per
cocoon than N. obscura, however no inter-specific differences occurred
at 20 °C.

Inter-specific differences were also found in cocoon hatching success at each experimental temperature. Only <u>N. obscura</u> cocoons laid at 15 and 20 °C successfully hatched at these temperatures, whereas <u>E. punctata</u> cocoons hatched at 10, 15 and 20 °C (Table 4.1).

The range of the average expected number of young produced per individual was obtained by multiplying the lower and upper mean number of cocoons produced per individual (based on 95% confidence limits) by the lower and upper values for the mean number of eggs per cocoon. <u>E. punctata</u> would be expected to produce more young per individual than <u>N. obscura</u> at 10 and 15 °C, while at 20 °C, there is no significant difference between the two species (Table 4.1).
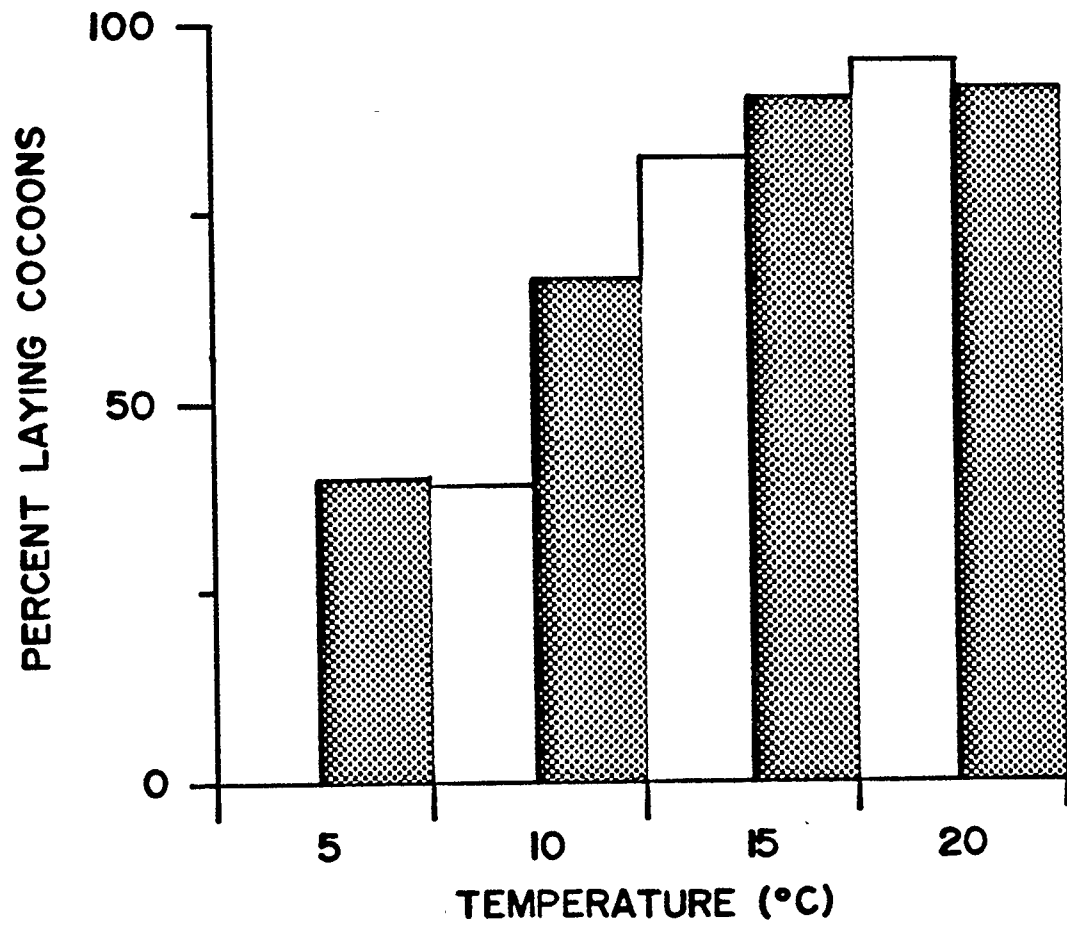
Figure 4.1:   Proportion of populations of N. obscura and
E. punctata found to lay cocoons at 5, 10, 15,
20°C.
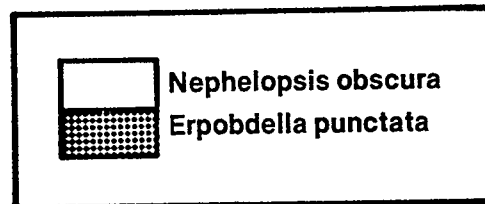
TABLE 4.1

Mean number of cocoons/individual (± SE), mean number of
eggs/cocoon (± SE), the occurrence of successful hatching
of young, and the expected number of young/individual for
N. obscura and E. punctata acclimated at 5, 10, 15, 20°C.

	Temperature (°C)	Mean No. Cocoons/Indiv.	Mean No. Eggs/Cocoon	Successful Hatching	Expected Range of Young/Indiv.
N.obscura	5	0	0	NO	0
	10	1.78 ± 0.26	1.96 ± 0.29	NO	0
	15	4.14 ± 0.31	3.02 ± 0.10	YES	10 − 15
	20	7.29 ± 0.38	3.64 ± 0.06	YES	23 − 30
E. punctata	5	1.75 ± 0.41	6.67 ± 1.20	NO	0
	10	6.00 ± 1.19	6.27 ± 0.29	YES	21 − 57
	15	5.74 ± 0.92	5.04 ± 0.60	YES	15 − 47
	20	6.24 ± 1.15	3.72 ± 0.39	YES	22 − 45

4.4.2 Growth

The growth rates of young $N.$ obscura and $E.$ punctata under ad libitum food conditions followed an exponential function, with linear relationships between log wet weight (WWT) and time (t) as expressed in the following equations:

$N.$ obscura –

$$5 \ ^oC - \log_e WWT = 1.64 + 0.02 \ t \ (r^2 = 0.71)$$

$$10 \ ^oC - \log_e WWT = 1.46 + 0.14 \ t \ (r^2 = 0.97)$$

$$15 \ ^oC - \log_e WWT = 2.17 + 0.26 \ t \ (r^2 = 0.98)$$

$$20 \ ^oC - \log_e WWT = 2.42 + 0.25 \ t \ (r^2 = 0.98)$$

$E.$ punctata –

$$5 \ ^oC - \log_e WWT = 2.18 + 0.05 \ t \ (r^2 = 0.97)$$

$$10 \ ^oC - \log_e WWT = 2.40 + 0.17 \ t \ (r^2 = 0.99)$$

$$15 \ ^oC - \log_e WWT = 2.72 + 0.26 \ t \ (r^2 = 0.96)$$

$$20 \ ^oC - \log_e WWT = 2.95 + 0.34 \ t \ (r^2 = 0.93)$$

With the exception of $N.$ obscura at 5 ^oC, the regression slopes (coefficients of growth) all differed significantly from zero indicating some growth occurred over the 12 week period (Figure 4.2).

Analysis of covariance showed $N.$ obscura to have significant differences in growth rates between 5, 10 and 15 ^oC, but no significant difference between 15 and 20 ^oC. The growth rates were positively correlated with increasing temperature, with no growth occurring at 5 ^oC and highest growth rates at 15 and 20 ^oC (Figure 4.2).

E. punctata displayed a similar temperature-dependent pattern with highest growth rates at 15 and 20 °C, and significant differences in the growth coefficient occurring between 5, 10 and 15 °C. Inter-specific comparisons of growth rates show no significant differences at 10 and 15 °C, however E. punctata displayed significantly higher growth rates than N. obscura at 5 and 20 °C (Figure 4.2).
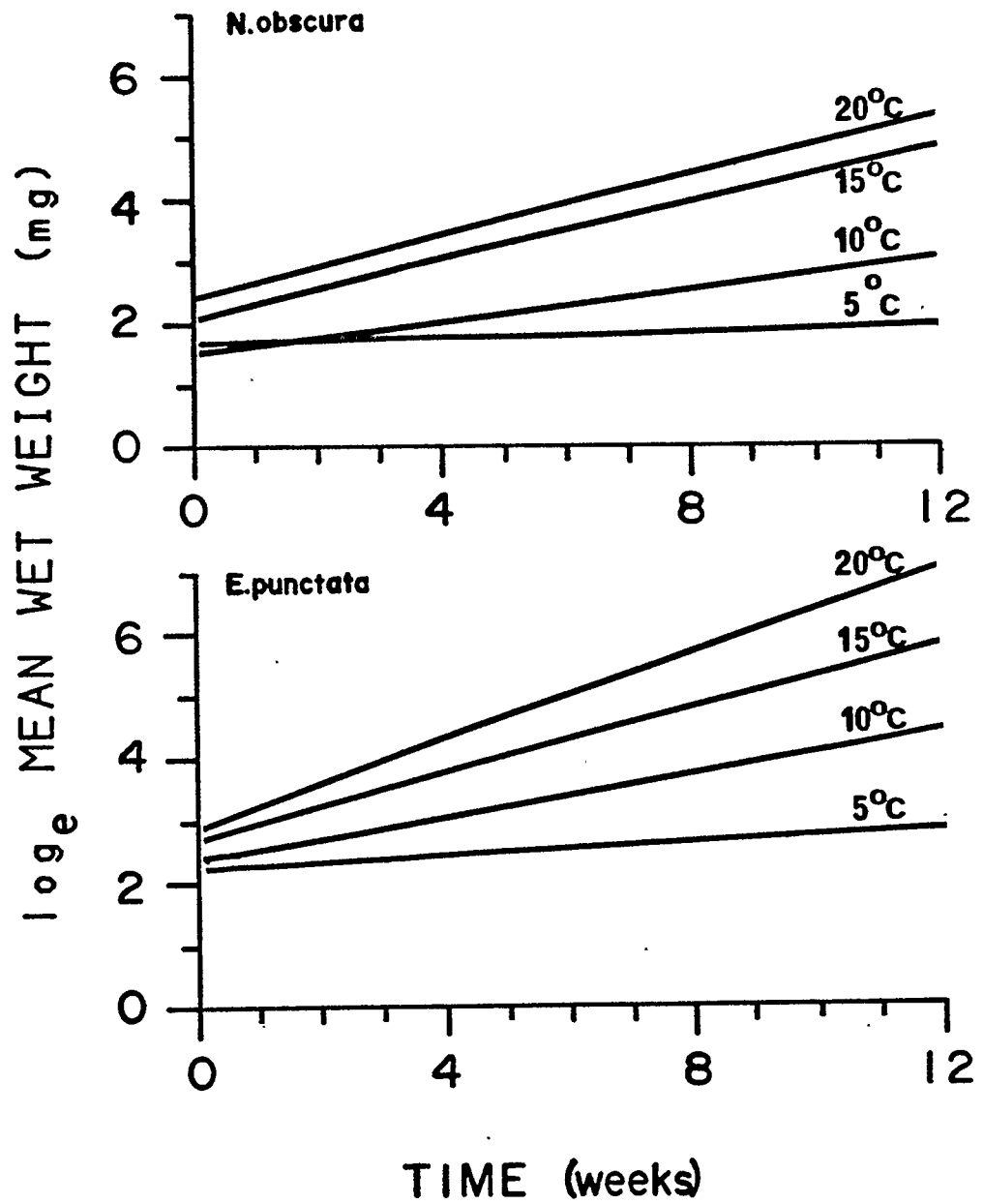
Figure 4.2:   Growth rates of N. obscura and E. punctata
              under ad libitum food conditions at 5, 10, 15,
              20°C.

4.5 Discussion

The species-specific differences in reproduction and growth in relation to water temperature displayed by N. obscura and E. punctata may account in part for the observed differences in their distribution and abundance in lentic and lotic habitats. In general, the lotic habitats in southern Alberta (i.e. Bow River) are colder than the lentic habitats (i.e. Cairn Pond, Stephenson Pond) having 2500 and 2900 average annual degree-days respectively (Figure 1.1). For both N. obscura and E. punctata, May - September is the period of cocoon and young production, highest feeding activity (Figure 2.7) and growth rates. (Davies and Everett, 1977; Davies, Reynoldson and Everett, 1977) and during this period, there is a 600 degree-day difference between the warmer lentic and colder lotic habitats (Figure 1.1).

The onset of cocoon production by mature pre-reproductive (>150 mg) N. obscura and E. punctata collected in April is temperature related, with lowest production occurring at 5 and 10 °C and highest at 15 and 20 °C (Figure 4.1). This is in general agreement with the results obtained by Linton (1980), who recorded spring collected mature pre-reproductive individuals of both species producing cocoons in highest proportion at 15 °C and lowest proportion at 4 °C. At 5 °C N. obscura displayed no cocoon production, while at 10 °C, only 38% of the population produced cocoons (Figure 4.1). E. punctata displayed a greater ability to initiate cocoon production at colder temperatures than N. obscura, having 40% and 65% of its population producing cocoons at 5 and 10 °C respectively. This would give E. punctata,

when sympatric with N. obscura, an ecological advantage by allowing it
to reproduce earlier in the spring in colder water temperatures.
Indeed, Davies, Reynoldson and Everett (1977), found E. punctata when
sub-dominant producing cocoons earlier in the spring in colder water
temperatures than when it was dominant, when it started cocoon
production later in warmer water. At 15 and 20 °C, over 80% of
N. obscura and E. punctata produced cocoons, indicating these higher
temperatures to be the most suitable for cocoon production.

Based on the mean number of cocoons produced per individual, the
mean number of eggs per cocoon, and the hatching success of the
cocoons, E. punctata would be expected to produce more young than
N. obscura at 10 and 15 oC (Table 4.1). Although the cocoons laid by
E. punctata at 5 °C did not hatch, at this temperature they did
constitute a potential recruitment since under field conditions, with
the onset of spring, the cocoons would soon be exposed to the 10 °C
minimum temperature required for successful hatching (Figure 1.1).
Although N. obscura produced a low number of cocoons at 10 °C,
successful hatching of young did not occur until 15 °C. The expected
fecundity of N. obscura at 15 °C was significantly lower than that
calculated for E. punctata and only at 20 °C did N. obscura and
E. punctata display no significant inter-specific differences in
production of viable offspring (Table 4.1).

Clearly, the low abundance of N. obscura compared to E. punctata
in lotic habitats in western Canada could be the result of it being at
an ecological disadvantage with respect to growth and production of
young in colder water regimes. Since N. obscura can only successfully

offspring over a restricted, warm temperature range (15 - 20 °C), this would reduce its abundance in colder, lotic habitats, which over the breeding and growing season have approximately 600 degree-days less than the lentic habitats (Figure 1.1), where it is usually abundant and dominant. E. punctata however has the capability to both grow and produce more viable young than N. obscura at cold temperature regimes, which at least partially explains its relative success in lotic habitats.

In the warmer temperature regimes more typical of lentic habitats, neither species shows a clear ecological advantage over the other in relation to growth or production of young. No significant differences occurred between N. obscura and E. punctata at 20 °C in the production of young although E. punctata had higher growth rates. At 15 °C, E. punctata produces more viable young but has a similar growth rate as N. obscura. Since N. obscura is usually numerically dominant in lentic habitats, its ecological advantage must be related to other abiotic or biotic parameters and as E. punctata changes its reproductive strategy in relation to the abundance of N. obscura (Davies, Reynoldson and Everett, 1977) and shows food resource overlap (Davies, Wrona, Linton and Wilkialis, 1981), the dominance of N. obscura is probably due to its inter-specific competitive abilities. The plasticity of E. punctata in its ability to grow and produce young over a wide temperature range (5 - 20 °C) appears to be partially responsible for its co-existence with N. obscura in lentic habitats.

5.0 SUBSTRATE PREFERENCE

5.1 Introduction

In general, the Hirudinoidea are scarce in aquatic habitats with
a primarily muddy substrate, but are relatively abundant in habitats
with solid substrates such as rocks or macrophytes (Elliott and Mann,
1979). Similarly, Sawyer's (1974) review of the literature showed
predaceous leeches to be most abundant in habitats containing rock,
gravel, and mud with macrophytes, and least abundant in areas with
sand and soft mud. The correlation between leech distribution and
solid substrates has been attributed to the necessity of a solid
substrate for the efficient functioning of the leech suckers, es-
pecially the posterior sucker (Sawyer, 1974). Members of the family
Erpobdellidae use their suckers for both locomotion and feeding and
additionally requiring a solid substrate for cocoon deposition
(Elliott and Mann, 1979). Thus, the ecological success of erpob-
dellids within a habitat could be dependent on the availability of
suitable substrate.

Aside from the obvious difference of standing water versus
running water, a major physical abiotic dissimilarity between lentic
and lotic habitats in southern Alberta is the type of substrate
present. Smaller lentic habitats (ponds, sloughs), where N. obscura
is generally numerically dominant, characteristically have a soft
mud-detritus bottom containing dense growths of aquatic macrophytes
during ice-free periods. A rock-gravel littoral substrate occurs
infrequently in these habitats and when present, generally constitutes

only a small proportion of the littoral zone. In contrast, the lotic

habitats where E. punctata is usually numerically dominant, have

predominately a stone-gravel substrate. Thus, the observed differ-

ences in the distribution and abundance of N. obscura and E. punctata

between lentic and lotic ecosystems may be related to differences in

substrate preference.

5.2 Objectives

The objectives of these experiments were to:

1) Determine whether N. obscura and E. punctata display intra-specific differences in substrate preference in response to water temperature, light regime and their nutritional condition.

2) Determine whether N. obscura and E. punctata display inter-specific differences in substrate preference in response to water temperature, light regime and their nutritional condition.

3) To provide a possible explanation for the observed species differences in distribution and abundance of lentic and lotic populations of N. obscura and E. punctata.

5.3 Materials and Methods

Experiments to determine the substrate preference of N. obscura
and E. punctata were conducted in a circular (75 cm diameter),
plexi-glass substrate apparatus (adopted from Gale, 1971), containing
36 compartment (7 cm diameter by 8 cm deep) of equal area and volume
each filled with one substrate type. The circular shape of the
apparatus prevented the leeches from congregating in corners and
enabled dispersal tendencies (row and column effects) to be measured
simultaneously with substrate selection (treatment differences) (Gale,
1971).

The substrate types used were 1) rock, 2) mud, and 3) Potamogeton
richardsonii anchored in mud. Rocks were obtained from a gravel bed
in the Bow River, near Calgary, and mud and P. richardsonii were
collected from Stephenson Pond. Before use, the rocks and mud were
autoclaved to ensure removal of all living material, and the plants
visually inspected for macro-invertebrate epifauna, which was removed.
The substrates were arranged in the apparatus with four replicates of
a modified 3 X 3 Latin square experimental design (Figure 5.1), which
permitted each substrate type to be proceeded four times by the other
two substrates. This allowed the measurement of carry over effects
(see Section 1.2.1) between substrate treatments (Cochran and Cox,
1957; Gale, 1971). During the experimental runs, two replicate
substrate apparati were placed in a water table filled with enough
de-chlorinated Bow River water to cover the substrates to a depth of
approximately 15 cm.

Unfed adult N. obscura and E. punctata (>150 mg wet weight) used
in the experiments were first acclimated in de-chlorinated Bow River
water at the appropriate experimental temperature with a 12 hour light
and 12 hour dark photo-period for a minimum of 14 days. The influence
of feeding on substrate preference was determined using acclimated
individuals subsequently fed to satiation on Enchytraeus sp. 48 hours
prior to the experimental run.

In the single species runs, 50 individuals were placed in each of
the two replicate experimental tanks, while in the combined species
runs, 25 individuals of each species were used.

At the start of each experiment, the leeches were placed in the
center of the apparatus and subsequently allowed to distribute
themselves into the various substrates. Each experimental run lasted
24 hours, after which the substrates were removed from the com-
partments and the leeches present enumerated noting the column, row
and substrate type. Leeches not found within a substrate compartment
were not enumerated.

The influence of the experimental treatments (temperature,
photo-period, nutritional state) on substrate selection by N. obscura
and E. punctata were examined in single and combined species runs
using the following combinations:

      1) 10 °C; 24 hr light; fed individuals

      2) 10 °C; 24 hr dark ; fed individuals

      3) 20 °C; 24 hr light; starved individuals

      4) 20 °C; 24 hr dark ; starved individuals

5) 20 ºC; 24 hr light; fed individuals

6) 20 ºC; 24 hr dark ; fed individuals

As leeches are more active at 20 ºC, starved <u>N. obscura</u> and
<u>E. punctata</u> were not tested at 10 ºC on the assumption that the
effects of nutritional condition, if any, would be more apparent at
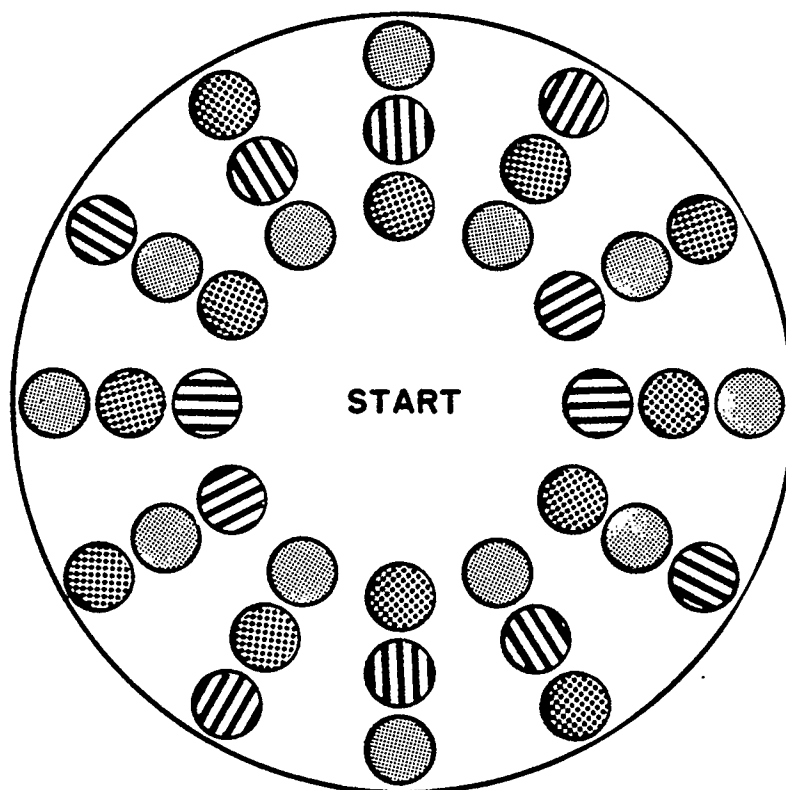the higher temperature.

Figure 5.1:   Plexi-glass substrate apparatus with substrates arranged according to 4 replicates of a 3 X 3 Latin square.   ▨ = rocks,   ▦ = plants, ▨ = mud.

5.4 Results

In all the single and combined species experiments, no significant row or columns effects were found, indicating unbiased equal dispersion in all directions. However, analysis of variance showed significant differences with respect to the substrate treatments. In all experiments, a minimum of 76% of the leeches tested were found in one of the substrate types (i.e. a maximum of 24 out of 100 individuals tested were non-selectors).

In the single species experiments, $\underline{N.}$ $\underline{obscura}$, irrespective of temperature (10, 20 ^{o}C), photo-period (24 hr light, dark) or nutritional condition (starved, fed), displayed the same trends in substrate preference with rocks selected significantly more than plants (Figures 5.2 - 5.4). The mud substrate was used only once by starved $\underline{N.}$ $\underline{obscura}$ at 20 ^{o}C with 24 hour light (Figure 5.3). However, this did not cause the overall distribution of substrate selection in 24 hour light to significantly differ from the 24 hour dark treatment (Figure 5.3).

Using the Kolmogorov-Smirnov two sample test, $\underline{E.}$ $\underline{punctata}$ which with only one exception (Figure 5.4) showed no preference between plants and rocks and very low utilization of mud (Figures 5.2 - 5.4), displayed a significant difference in substrate preference from $\underline{N.}$ $\underline{obscura}$.

In the combined species experiments, $\underline{N.}$ $\underline{obscura}$ generally displayed a similar pattern in substrate selection to that exhibited by it in the the single species experiments, with rock preferred as a

substrate significantly more than plants (Figures 5.5 - 5.7). Although starved N. obscura at 20 °C in 24 hour dark showed a higher proportion of individuals on plants than on rocks, the Kolmogorov-Smirnov test showed this to be not significantly different from the distribution in the 24 hour light experiment where rocks were selected in higher proportion than plants (Figure 5.6). Although N. obscura occurred slightly more frequently in mud in the presence of E. punctata than when alone, this difference was not significant.

E. punctata in the presence of N. obscura was found to significantly change its pattern of substrate selection compared to the single species experiments. In the combined species experiments, irrespective of the experimental treatment combinations, E. punctata showed a similar substrate preference to N. obscura and consistently selected rocks compared to plants and infrequently used mud (Figures 5.5 - 5.7).

Figure 5.2: Substrate preference of fed N. obscura (clear) and E. punctata (hatched) in single species experiments at 10°C and 24 hour light and dark photo-period.
M = mud, R = rock, P = plants.

Figure 5.3: Substrate preference of starved N. obscura (clear) and E. punctata (hatched) in single species experiments at 20°C and 24 hour light and dark photo-period.
M = mud, R = rock, P = plants.

Figure 5.4:   Substrate preference of fed N. obscura (clear) and E. punctata (hatched) in single species experiments at 20°C and 24 hour light and dark photo-period.
M = mud, R = rock, P = plants.
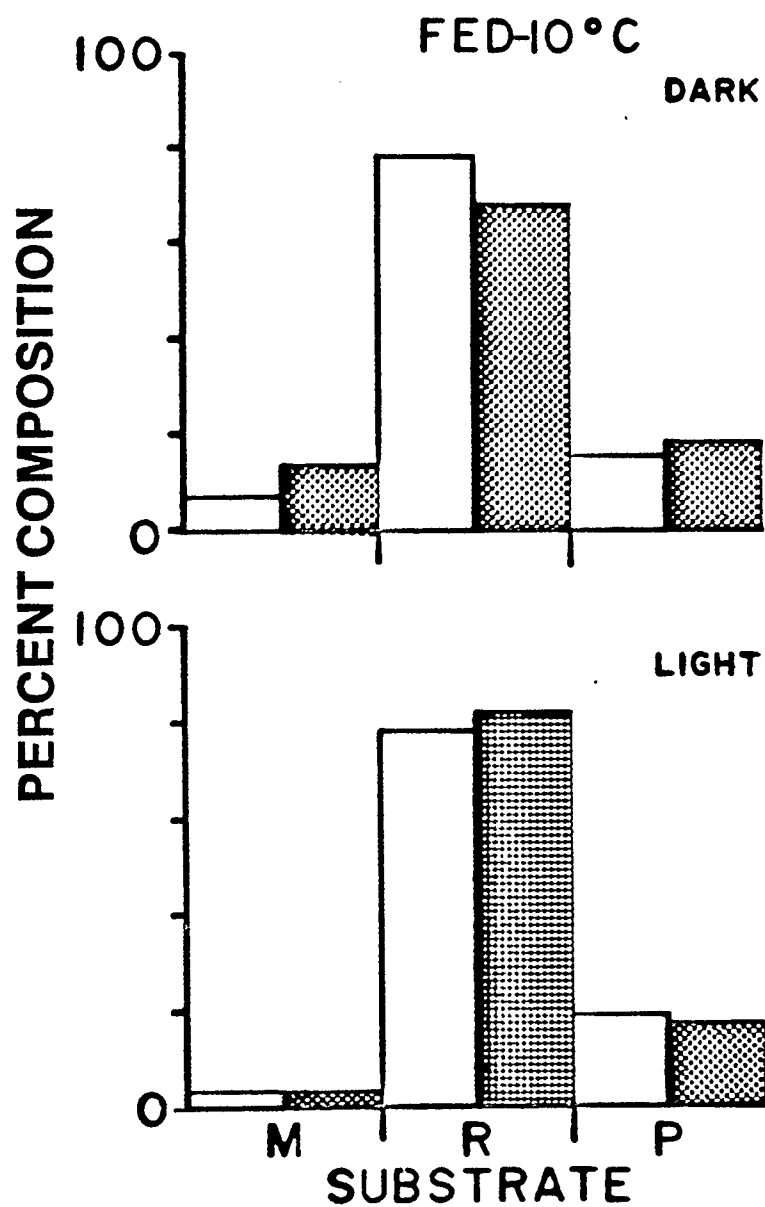
Figure 5.5:   Substrate preference of fed N. obscura (clear)
and E. punctata (hatched) in combined species
experiments at 10°C and 24 hour light and dark
photo-period.
M = mud, R = rock, P = plants.
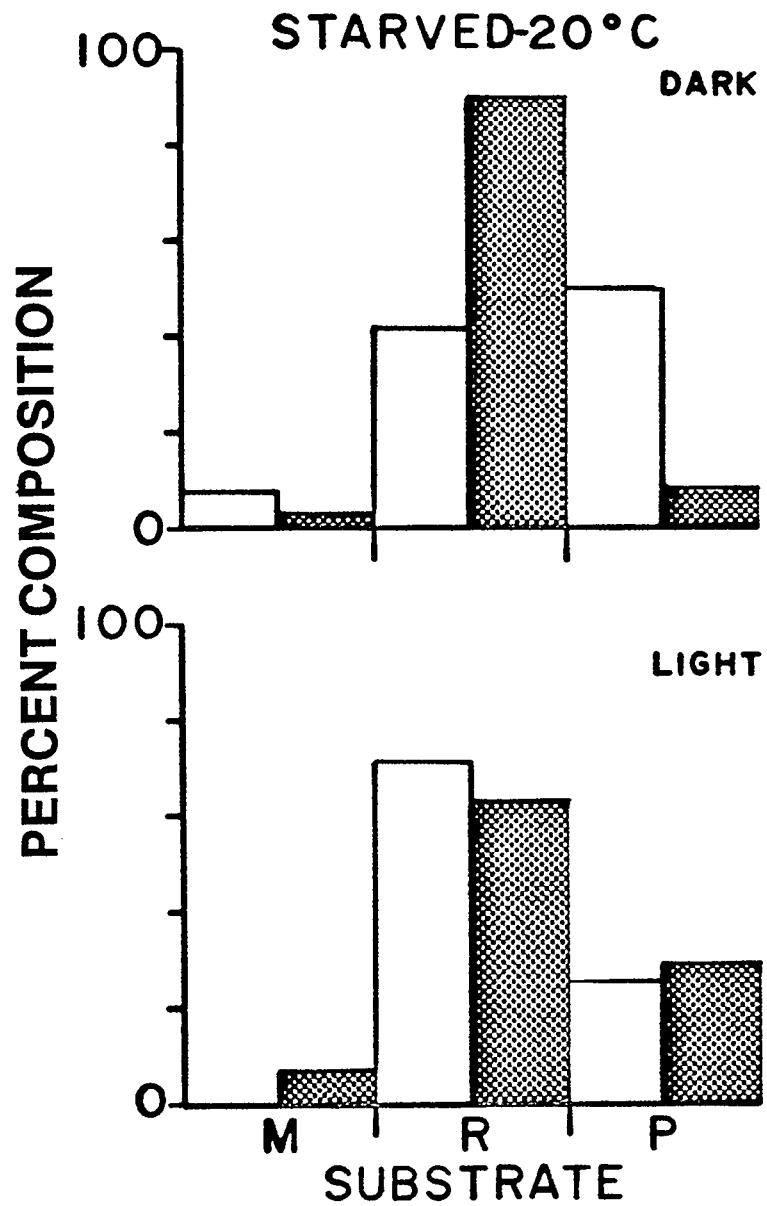
Figure 5.6: Substrate preference of starved N. obscura
(clear) and E. punctata (hatched) in combined
species experiments at 20°C and 24 hour light
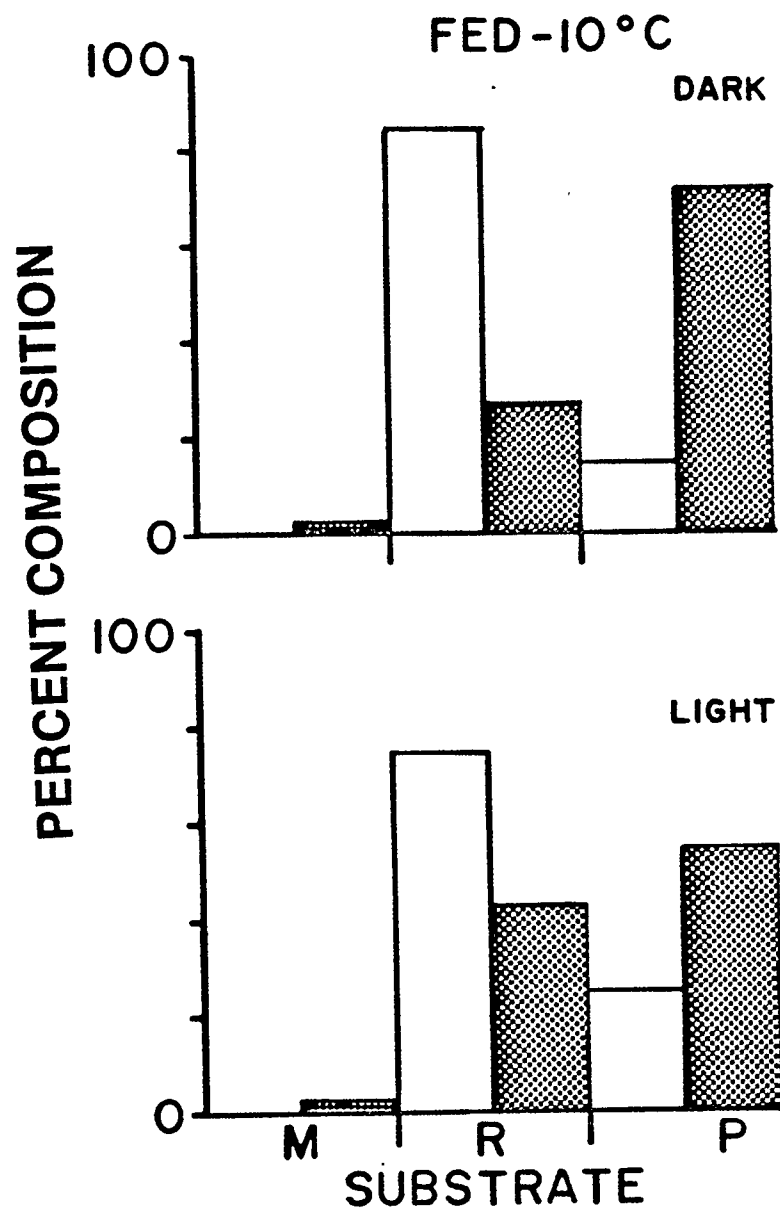and dark photo-period.
M = mud, R = rock, P = plants.
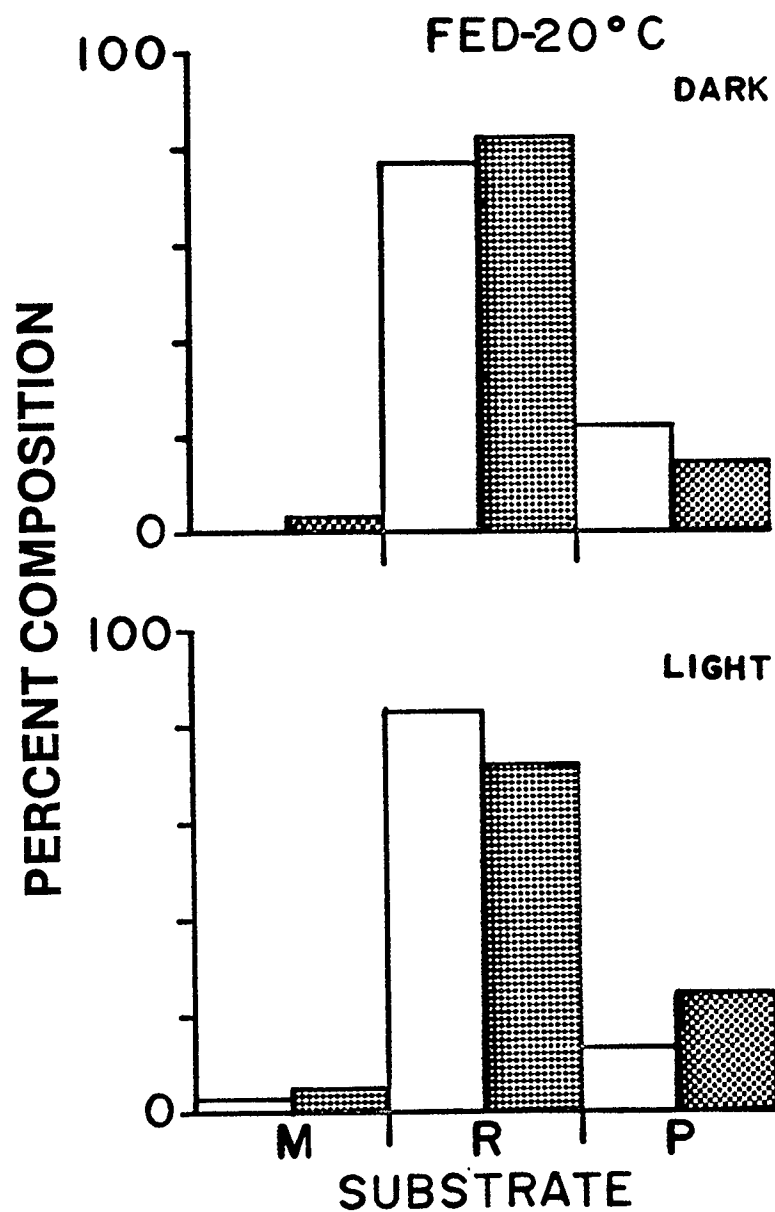
Figure 5.7: Substrate preference of fed N. obscura (clear) and E. punctata (hatched) in combined species experiments at 20°C and 24 hour light and dark photo-period.
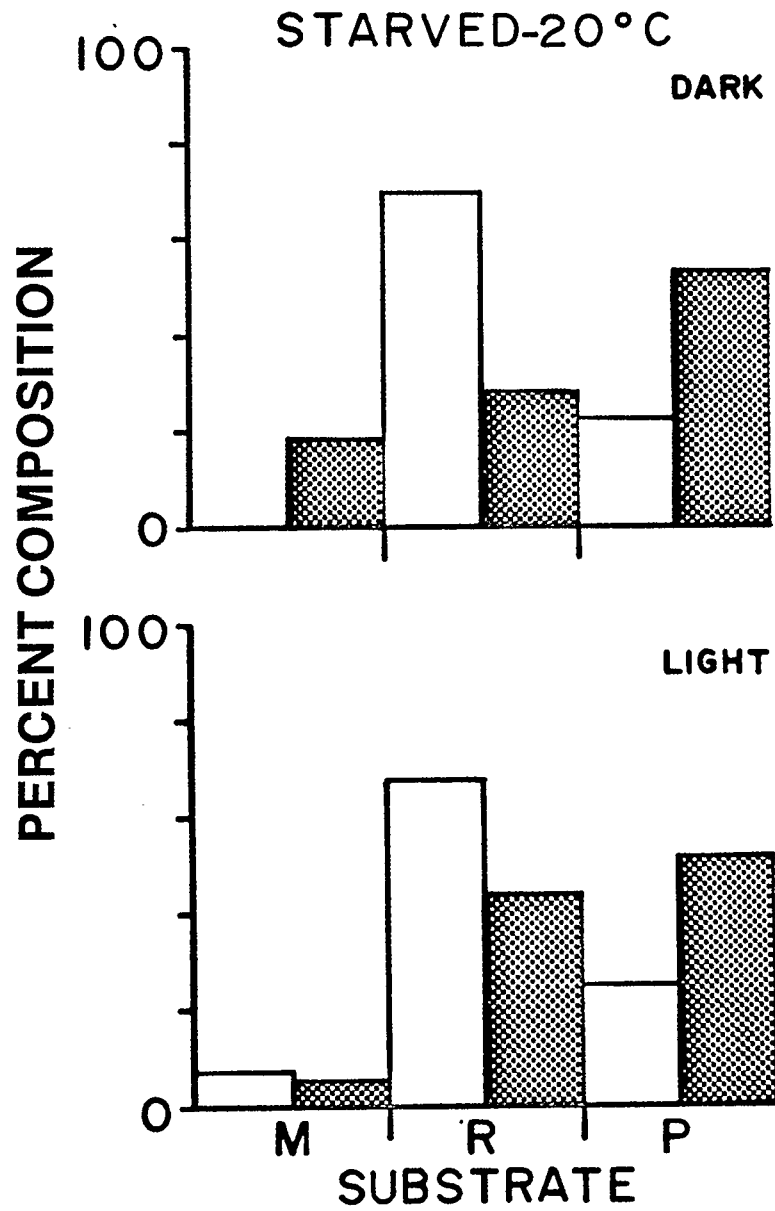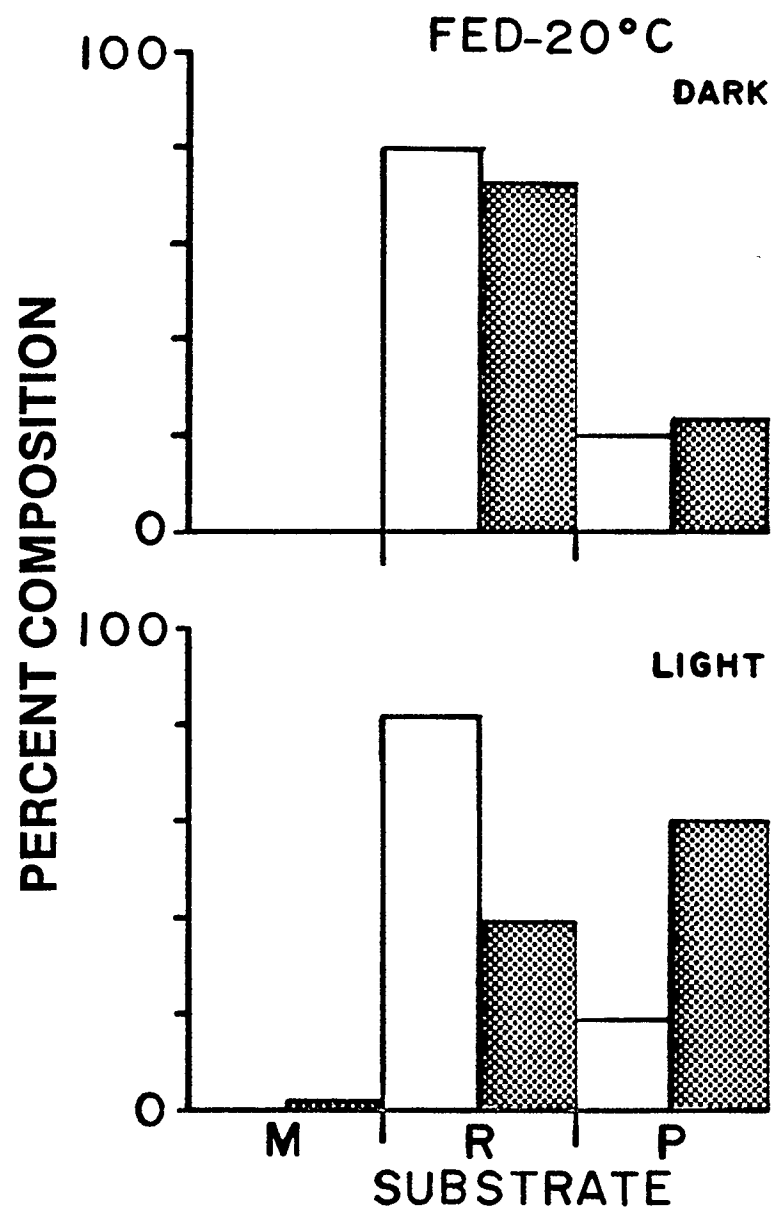M = mud, R = rock, P = plants.

5.5 Discussion

In both the single and combined species experiments, temperature, photo-period and nutritional condition of the leeches did not significantly influence the patterns of substrate selection displayed by either N. obscura or E. punctata (Figures 5.2 - 5.7).

Under all experimental conditions, N. obscura selected rocks in highest proportion, followed by plants and rarely mud. Although mud and macrophytes are the prevalent substrate types in the lentic habitats where N. obscura is dominant, it is evident it preferentially selects a rock substrate if available. In contrast, when alone, E. punctata generally displayed no significant preference between the broad-leaved P. richardsonii and rocks, but rarely chose mud. This indicates E. punctata also prefers solid substrates, but not necessarily rocks which are the most abundant substrate type in lotic habitats. A substrate additionally available to E. punctata is the dense growth of Potamogeton filiformis which occurs in the Bow River for a short period of time in late summer. However, adult E. punctata have rarely been found associated with P. filiformis, which is most likely a result of the slender, circular leaf morphology of this macrophyte being unsuitable for sucker attachment.

E. punctata changed its pattern of substrate preference in response to the presence of N. obscura. In the combined species experiments, E. punctata displayed a substrate selection pattern similar to N. obscura, choosing rocks and plants and rarely mud (Figures 5.5 - 5.7). This indicates an inter-specific interaction

which modifies the substrate selection behavior of E. punctata, but not of N. obscura. Two hypotheses can explain this inter-specific interaction.

1) E. punctata is in some manner attracted to the presence of N. obscura, and are thus found to be associated more with a rock substrate. This would be counter-adaptive with respect to aggregation for mating and reproductive success, and because it decreases the probability for spatial segregation within a habitat, would increase the potential for inter-specific competition for food and/or space resources.

2) N. obscura in some way displaces E. punctata from the plant substrate. It appears that the presence of N. obscura on the plant substrates either physically or chemically causes E. punctata to shift to the rocks. Possibly the number of suitable attachment sites on the plants is limited and N. obscura has a better ability to attain or maintain them. Since the total proportional use of plants does not differ between the single and combined species experiments, the decrease in preference for the plant substrate by E. punctata appears to be due to a decrease in the availability of attachment sites resulting from displacement by N. obscura.

The substrate preference patterns shown by both species cannot be used as an ecological explanation for the observed numerical dominance of N. obscura in lentic and E. punctata in lotic habitats. Both species showed very low preference for a mud substrate and were significantly more abundant on rocks and plants. N. obscura selected rock over plants, but in most lentic habitats in Alberta, a rocky

littoral substrate rarely occurs and mud and plants constitute the major substrates types available. _E. punctata_ also selected for solid substrates, utilizing rocks and plants almost equally, although the lotic habitats where _E. punctata_ is most abundant primarily have rock substrates. Clearly, the observed differences in distribution and abundance between _N. obscura_ and _E. punctata_ are not primarily the result of substrate preference, but must be the result of responses to other environmental parameters.

6.0 CONCLUSIONS

In southern Alberta, N. obscura is generally the numerically dominant erpobdellid in warmer lentic habitats, while E. punctata is most abundant in colder lotic ecosystems. Davies, Reynoldson and Everett (1977) hypothesized that inter-specific competition for food resources was responsible for the dominance of N. obscura in lentic habitats of Alberta. The hypothesis primarily stemmed from the finding that in two limnologically similar ponds, E. punctata displayed different reproductive strategies depending on whether it was numerically dominant or sub-dominant and high dietary overlap (Davies and Everett, 1975). When sub-dominant, E. punctata had a simple annual life cycle, reproducing at a small size, after a short maturation period, early in the breeding season, and over a longer period, thereby resembling the life history correlates of r-selection (Pianka, 1970, 1972). However, when dominant, E. punctata had life history attributes resembling those of K-selection (Pianka, 1970, 1972), reproducing at a larger size, later in the breeding season, and over a more restricted breeding season. In contrast, irrespective of whether numerically dominant or sub-dominant, N. obscura populations always produced two cohorts of young annually, one in the spring and the other in the fall (Davies and Everett, 1977). Thus, the differences in reproductive strategies shown by E. punctata, coupled with an observed high dietary overlap with N. obscura (Davies and Everett, 1975), made the competition hypothesis a viable explanation for their observed abundance differences in lentic habitats. The hypothesis erected by Davies, Reynoldson and Everett (1977) was based on the

differences in distribution and abundance of N. obscura and

E. punctata in lentic ecosystems, and no hypotheses were forwarded to

explain their differences in distribution and abundance between lentic

and lotic habitats.

The objective of this study was to determine whether the observed

differences in distribution and abundance of N. obscura and

E. punctata in lentic and lotic habitats could be explained by: 1)

providing further evidence for inter-specific competition operating in

lentic habitats, 2) differing physiological responses to temperature

and/or oxygen concentration as measured by respiration, growth and

reproduction, and 3) differences in substrate preference.

The competition hypothesis was tested by quantitatively examining

two of seven criteria (Reynoldson and Bellamy, 1970; Williamson, 1972;

Lock and Reynoldson, 1976), suggested necessary to demonstrate that

inter-specific competition is or was occurring. The criteria examined

were: a) to determine whether morphological character divergence

existed between N. obscura and E. punctata, and b) determine whether

the feeding strategies of N. obscura and E. punctata differed fol-

lowing the introduction of a potentially competing species.

The feeding ecologies of N. obscura and E. punctata was

serologically examined in Cairn Pond when E. punctata was numerically

dominant (1976) and during the immediate transition period to dom-

inance by N. obscura (1978). Compared at the species level, no

significant differences were found in prey utilization between 1976

and 1978. In both years, Chironomidae were consumed most heavily by

E. punctata in the late-winter, early-spring and by N. obscura

throughout the remainder of the year. On a monthly basis, Amphipoda were more heavily utilized by E. punctata and Oligochaeta by N. obscura. Both species utilized Copepoda/Cladocera to a limited extent during the spring and summer. Food niche overlap between N. obscura and E. punctata was found to decrease as E. punctata changed from dominant (1976) to sub-dominant (1978). The decrease in overlap was consistent with the proposal that maximal tolerable overlap should be lower in communities where inter-specific competition is intense than in environments where it is weak (Pianka, 1972). Thus, when N. obscura established dominance in 1978, E. punctata if competitively inferior was expected to decrease its overlap in utilization of food resources in order to accommodate co-existence. An explanation for the decrease in niche overlap between the two species without an apparent change in feeding at the species level became evident when the data was further analyzed with respect to intra-specific differences in prey utilization.

Intra-specifically, E. punctata was found to partition food resources on both a temporal and weight-class basis. Temporal differences in prey utilization by E. punctata were found to be greater when it was sub-dominant than when dominant. Although E. punctata showed weight-class partitioning of food resources when dominant and sub-dominant, the weight-range groups were less cohesive when it was sub-dominant and E. punctata displayed greater specialization in prey utilization as confirmed by lower niche breadth. In contrast, N. obscura showed no temporal differences, but did show weight-class utilization of prey, with the weight groups defined

remaining consistent in composition regardless of whether N. obscura was dominant or sub-dominant. Additionally, the decrease in overlap between N. obscura and E. punctata could not be explained on the basis of morphological character displacement.

It appears that N. obscura is competitively superior in relation to food resource utilization than E. punctata since irrespective of whether dominant or sub-dominant it displays a similar pattern in feeding and reproduction, while E. punctata is found to alter its pattern of feeding and reproduction in relation to the presence or absence of N. obscura. It is suggested that the changes in feeding and reproductive strategy displayed by E. punctata allows co-existence with N. obscura. The results obtained in this study, combined with those obtained from previous research support the competition hypothesis in that in lentic habitats, E. punctata and N. obscura share common resources, display different reproductive and/or feeding strategies when dominant and sub-dominant, partition food resources on a temporal and weight (size) basis, and reduce inter-specific overlap in potentially competitively intense situations as theory predicts.

Since the temperature and oxygen regimes differed between the lentic and lotic habitats in which N. obscura and E. punctata occurred, it was hypothesized that the respiratory strategy adopted by E. punctata gives it an ecological advantage over N. obscura in lotic habitats, which would provide a possible physiological explanation for the observed field distribution differences between these two species. The respiratory strategies of N. obscura and E. punctata were examined in response to different environmental factors (temperature, oxygen

concentration), while under different physiological states (fed, starved, reproductive, non-reproductive, active, resting) using a flow-through respiration system.

All of the environmental and physiological factors listed were found to differentially influence oxygen uptake of both species. N. obscura was found to display higher aerobic metabolic scope than E. punctata at warmer temperatures, while the converse was true at colder water temperatures. Thus, N. obscura has potentially more energy available to perform aerobic activities (i.e. locomotion, foraging, reproduction) above the costs of normal maintenance at 15 and 20 °C, while the same was true for E. punctata under colder conditions (<10 °C). On the basis of these results, N. obscura would be predicted to be ecologically more successful in warmer (lentic) habitats and E. punctata in colder (lotic), which agrees with the field distributional data. Over the range of temperatures tested (5 - 20 °C), and irrespective of activity level, both species displayed similar trends in oxygen consumption when exposed to short-term and long-term hypoxia. Starved, N. obscura and E. punctata behaved as oxy-conformers, displaying oxygen uptake rates that were functionally dependent on the oxygen concentration of the water. Thus, N. obscura and E. punctata appear to have similar abilities to aerobically cope with declining oxygen conditions and no clear ecological advantage is apparent for either species in hypoxic conditions. However, inter-specific differences were found with respect to their abilities to survive environmental anoxia. Between 5 - 15 °C, N. obscura survived anaerobic conditions longer than E. punctata, especially at 5 °C, where 60% of N. obscura were still

alive after exposure for 30 days. However, survival time of both species declined with increasing temperature. Thus, it appears that N. obscura is superior in withstanding anaerobic conditions at low temperatures, possibly allowing it to better survive the oxygen-poor conditions typical of lentic habitats during the fall and winter. In contrast, E. punctata would not be exposed to such low oxygen conditions at low temperatures in the winter in lotic situations. The high mortality of E. punctata under anoxia at low temperatures provides an additional explanation for its low abundance in lentic habitats.

As the lentic habitats where N. obscura is abundant are approximately 600 degree-days warmer over the period suitable for growth and reproduction than the lotic habitats, species-specific differences in growth and/or reproduction in relation to water temperature could provide an additional basis for their observed distributional differences. The onset of cocoon production of both species was temperature related, with the lowest proportion of individuals laying cocoons at 5 and 10 °C, and the highest proportion at 15 and 20 °C. Based on the mean number of cocoons/individual, the mean number of eggs/cocoon, the hatching success of the cocoons, and the growth rates of young leeches, E. punctata was found to more successful over a wide temperature range. E. punctata displayed growth at 5 - 20 °C and successfully produced viable offspring at 10 - 20 °C. In contrast, N. obscura showed growth at 10 - 20 °C and only successfully produced offspring over the restricted warmer temperature range of 15 - 20 °C.

Thus, the low abundance of <u>N. obscura</u> compared to <u>E. punctata</u> in lotic habitats appears to be at least partially a result of it being at an ecological disadvantage with respect to growth and production of young in colder water regimes. <u>E. punctata</u> however, has the capabilities to both grow and produce more viable young than <u>N. obscura</u> at colder water temperatures, giving it an advantage in lotic habitats.

However, in the warmer temperature regimes more typical of lentic habitats, neither species showed a clear ecological advantage over the other in relation to growth and production of young. Since <u>N. obscura</u> is usually numerically dominant in lentic habitats, its ecological advantage must be related to other biotic or abiotic factors. Evidence suggests that <u>N. obscura</u> is competitively superior to <u>E. punctata</u>. Co-existence of <u>E. punctata</u> with <u>N. obscura</u> in lentic habitats appears to be partially accommodated by its physiological plasticity in growth and reproduction over a wide temperature range.

Another major physical abiotic dissimilarity between the lentic and lotic habitats is the type of substrate present. As the lentic habitats characteristically have a soft mud-detritus bottom containing dense growth of macrophytes during the summer, with a rock-gravel littoral substrate occurring infrequently and the lotic habitats have predominately a rock-gravel substrate, the observed differences in the distribution and abundance of <u>N. obscura</u> and <u>E. punctata</u> between lentic and lotic habitats could be related to differences in substrate preference. However, irrespective of water temperature, photo-period and nutritional condition of the leeches, both species were found to preferentially select solid substrates (rock and macrophytes) over

mud. Thus, differences in substrate preferences could not explain the observed differences in distribution. As both species require a solid substrate for laying cocoons and attachment, the presence of a solid substratum will affect the ecological success of both species. Additionally, when sympatric, inter-specific interaction is potentially important in determining the final substrate preference of _E. punctata_ since an altered pattern of substrate choice was observed in the presence of _N. obscura_.

It can be concluded that the differential physiological responses as measured by oxygen uptake, growth, and cocoon production of _N. obscura_ and _E. punctata_ in relation to temperature and oxygen concentration can explain the dominance of _E. punctata_ in lotic habitats. However, at warmer water temperatures, _E. punctata_ displayed comparable capabilities to _N. obscura_ with respect to successful production of young and growth, although it had lower metabolic scope, indicating it to be more energetically costly for _E. punctata_ to perform physiological work at these temperatures. Thus, combined with the additional evidence supporting inter-specific competition, it is concluded that biotic factors are primarily responsible for the dominance of _N. obscura_ in lentic habitats of southern Alberta.

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