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A Comparison of Two Generations of Nephelopsis obscura Verrill: the Effect of Prehistory on Their Energetics and Lifehistory Traits

by

Yang Qian

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "A Comparison of Two Generations of Nephelopsis obscura Verrill: the Effect of Prehistory on Their Energetics and Lifehistory Traits" submitted by Yang Qian in partial fulfillment of the requirements for the degree of Master of Science.

Supervisor, Dr. R.W. Davies
Department of Biological Sciences

Dr. G.B. Bourne
Department of Biological Sciences

Dr. F.J. Wrona
Department of Biological Sciences

Dr. P.M. Boormen
Department of Chemistry

03 - 18 1992

ABSTRACT

Two generations of Nephelopsis obscura Verrill (Erpobdellidae) are produced in Alberta, one in early summer and the other in late summer. Although the basic biology, ecology, life-history and physiology of N. obscura have been studied extensively, no comparative studies on these two generations have been conducted.

This study compares, in the laboratory, the bioenergetics and life-history traits (growth rate, energy storage, size at maturity and age at maturity) between early summer and late summer N. obscura using population reared from cocoons collected from Stephenson's Pond, Alberta.

The objectives of this study were to: determine whether any differences in ecophysiology between the two generations can be attributed to the different environmental conditions experienced by the parents; describe differences between the two generations in terms of ecological fitness; determine if genetic differences, measured by the electropheretic patterns of twenty enzymes, occur between the two generations; and determine if the Stephenson's Pond population of N. obscura is genetically different from those in the Rocky Mountain foothills of Alberta and Utah, U.S.A..

It was concluded that the early summer and late summer generations of N. obscura from Stephenson's Pond are significantly different in terms of growth, energy acquisition, energy allocation, and life-history traits.

The genetic variation in both early summer and late summer N.

obscura was very low, only MDH locus being polymorphic. The genotypic frequency of the MDH locus was different between the two generations with more heterozygotes in late summer N. obscura than in early summer leeches.

Fixed genetic difference amongst populations from three localities were detected in this study at four enzymes indicated that the three populations are genetically distinct. The observed ecophysiological differences could be either due to phenotypic plasticity, i.e. due to the different prehistory their parents experienced or to genetic difference between the two generations. The genetic examination showed no fixed genetic difference and only the MDH locus showed difference in genotypic frequency. In addition, low genetic variation exhibited by N. obscura indicated its facultative adaptation. Therefore, the phenotypic plasticity hypothesis is strongly supported.

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1.0 GENERAL INTRODUCTION

Leeches often form an important component, in terms of numbers and / or biomass, of the benthos in lakes and ponds. They are, however, less abundant in streams and rivers. In North America 69 leech species in four families have been recorded (Davies 1991). Members of the family Glossiphoniidae are either predators of macroinvertebrates or temporary ectoparasites of freshwater fish, turtles, amphibians, or water birds; Piscicolidae are parasites of fishes and occasionally crustaceans; Erpobdellidae are primarily predators of macroinvertebrates and zooplankton; and Hirudinidae are predators of macroinvertebrates and / or sanguivorous (bloodsucking) temporary ectoparasites of amphibians and mammals.

In many small lentic ecosystems without fish, leeches are the top predators and in these localities are ideal for studying intra- and inter-specific resource competition, niche overlap, and predator-prey interactions. In large lakes and rivers, leeches form a component of the diet of fish and are grown commercially for fish bait in parts of the U. S. A.(Peterson 1983).

All leeches are hermaphrodites exhibiting protandry or cosexuality (Davies and Singhal 1988), with reciprocal cross-fertilization as the general rule. The life cycle of leeches consist of egg, hatchling, juvenile, and mature hermaphrodite adult. Predatory leech species usually have an annual or biannual life cycle, breeding once and then dying, i.e. exhibit semelparity. However, N. obscura has been shown to be

genetically capable of breeding several times, i.e. exhibits iteroparity although generally exhibiting semelparity in the field (Baird *et al.* 1986). While sanguivorous Hirudinidae are iteroparous, sanguivorous Glossiphoniidae (e.g. Theromyzon sp.) are semelparous.

Dispersal of freshwater leeches is generally assumed to be the result of passive transfer from water body to water body by other animals. Davies *et al.*(1982) examined passive transfer of two parasitic species (Theromuzon rude (Baird 1869) and Placobdella papillifera (Verrill 1872)) and two predatory species (Helobdella stagnalis (Linn. 1758) and Nephelopsis obscura Verrill (1872)) by ducks. Adult T. rude were transferred both in the nares, while taking a blood meal, and on the duck's body under the feathers. P. papillifera were also conveyed on the body of the duck, but adult H. stagnalis and N. obscura were not. Adult leeches ingested by ducks were not recovered from the duck faeces, but viable N. obscura cocoons were recovered from the faeces of fed ducks. Passive dispersal by wind has never been recorded, but the transport of cocoons attached to macrophytes is possible. Davies (1979) showed that H. stagnalis, Helobdella triserialis (Blanchard 1849), Glossiphonia complanata (Linn. 1758), and Mooreobdella fervida (Verrill 1872) were dispersed to Anticosti Island by sea currents from the Quebec north shore of the Gulf of St. Lawrence.

In North America, predators of leeches include fish, birds, garter snakes, and salamanders (Pearse 1932; Bartonek and Murdy 1970; Bartonek and Trauger 1975; Able 1976; Arnold 1981; Davies *et al.* 1982; Kephart 1982; Kephart and Arnold 1982), as well as insects, gastropods, and amphipods (Hobbs and Figueroa 1958;

Pritchard 1964; Sawyer 1970; Anderson and Raasveldt 1974). Cywinska and Davies (1989) showed that four species of dytiscids, one species of odonate, two species of hemipterans, and two species of amphipods fed on one or more size classes of N. obscura. Cywinska and Davies (1989) also found that cocoons were consumed by amphipods and adult coleopterans. Predation on Erpobdella punctata (Leidy 1870) cocoons has also been recorded by gastropods (Sawyer 1970) and on Haemopsis sanguisuga (Linn. 1758) cocoons by mites (Bennike 1943). The only known vertebrate predators of leech cocoons are salamanders (Pearse 1932) and waterfowl (Davies et al. 1982).

On a geological time scale, only a very few freshwater ecosystems are long-lived and even on a shorter time scale of 1-20 years, many lentic ecosystems are ephemeral. In some very ephemeral ecosystems, leeches may not have sufficient time to reach carrying capacity (Davies 1991). However, evidence for interspecific competition between N. obscura and E. punctata has been presented by Davies *et al.* (1982) and between G. complanata and H. stagnalis by Wrona *et al.* (1981).

N. obscura (Erpobdellidae) is a predatory leech widely distributed in freshwater ecosystems in Canada and the northern U.S.A (Davies 1991). During the last 25 years extensive investigation has been conducted on its basic biology, ecology, physiology and life-history, showing that N. obscura is extremely phenotypically plastic. Over its geographic distribution N. obscura shows a wide variation in life-history traits. In prairie ponds of Alberta Davies and Everett (1977) found its mature body size rarely exceeds 700 mg with a minimum size at maturity of 170 mg. In

ponds in the Rocky Mountain foothills of Alberta and in Minnesota, N. obscura at maturity reaches 1,400 mg (Linton and Davies 1987) and 2,000 mg (Peterson 1983), respectively.

Davies and Everett (1977) found that N. obscura in southern Alberta produces two generations annually (Fig. 1.1). The first generation appears in spring (or early summer) and the second generation in late summer. The early summer generation is the progeny of the heavier individuals from the early summer generation of the previous year and from the late summer generation produced two years previously. The late summer generation is produced by portions of the previous year's early and late summer generations. Thus, in southern Alberta N. obscura produces cocoons after 12, 15 or 19 months. However, in Minnesota N. obscura appears to have a 24 month cycle (Peterson 1983).

In the field N. obscura invariably dies shortly after cocoon production, i.e. is semelparous (Davies and Everett 1977; Peterson 1983). However, under certain laboratory conditions N. obscura exhibits iteroparity. Thus, N. obscura is genetically potentially iteroparous but in the field usually exhibits phenotypic semelparity (Baird, Linton and Davies, 1986; Baird, Linton and Davies 1987; Davies and Singhal 1987).

N. obscura also shows high sensitivity to environmental changes. Water temperature regime affects the relative reproductive success of N. obscura and Wrona *et al.* (1987) demonstrated that the onset of cocoon production is temperature dependent. When N. obscura which were at or very close to cocoon production were

exposed to different temperature regimes, no cocoons were produced at 5°C, only 38% N. obscura produced cocoons at 10°C, but over 80% produced cocoons at 15° and 20°C. Wrona *et al.*(1987) also found that cocoon hatching success is influenced by temperature with successful hatching occurring at 15° and 20°C but not 5° or 10°C. Linton and Davies (1987) showed that between 10° and 20°C the reproductive output of N. obscura is positively correlated with temperature.

The growth of N. obscura is similarly sensitive to temperature changes. Growth rates are significantly positively correlated with increasing temperature with no growth occurring at 5°C and highest growth rates at 20°C (Wrona *et al.* 1987). In addition, the temperature regime acts as one of the primary components affecting depth distribution and seasonal movement (Gates and Davies 1987).

Food availability has direct impact on the growth and life history of N. obscura. Baird, Linton and Davies (1987) showed that the feeding rates declined significantly with a reduction in ration. A bioenergetics simulation model of the growth and life history of N. obscura (Linton and Davies 1987) showed that growth is more sensitive to differences in prey availability and abundance between years than to temperature variations. N. obscura displays different reproductive patterns when food rations are changed (Baird, Linton and Davies 1987). At 15°C, N. obscura behaved as an iteroparous species, decreasing energy allocation to reproduction with declining food ration. However, significant energy allocation to reproduction occurred even under starvation conditions indicating some 'recklessness', normally associated with semelparity (Calow 1978).

Because of the variation in dissolved oxygen concentrations encountered in different aquatic ecosystems, and temporally within a single habitat, N. obscura shows considerable respiratory plasticity. When subjected to different oxygen saturations (10%, 25%, 50% and 100%), both the resting and active respiration of N. obscura were positively correlated with oxygen concentrations (Wrona and Davies 1984). Dissolved oxygen also influences the feeding, survival, movement and distribution of N. obscura. Davies and Gates (1991) showed that dissolved oxygen concentration had a significant effect on prey capture rate with more prey consumed at higher oxygen saturation, irrespective of prey density. Davies *et al.* (1987) showed intraspecific differences in the survivorship of N. obscura to anoxic conditions with large individuals having longer survival times than small individuals at both 5°C and at 20°C. When exposed to hyperoxia (200%, 300%) survival of N. obscura increased with leech size and decreased with increased temperature (Davies and Gates 1991). Growth of N. obscura is also influenced by dissolved oxygen saturation (Davies *et al.* 1992). When subjected to five different dissolved oxygen regimes (three constant: 10%, 100%, 300%; and two diel cyclical: 10-100% and 100-300%) at 15°C, Davies *et al.*(1992) found that N. obscura showed highest growth rate in the diel 100-300% regime and the lowest in the constant 10% or diel 10-100% regimes.

Dratnal and Davies (1990) showed that prehistory plays an important role in changing energy allocation and life-history traits. Two groups of N. obscura were examined: one termed summer N. obscura were hatched in the laboratory from cocoons collected from the field; the second group, termed winter N. obscura were

collected from the field in the spring after surviving winter. Although both groups were maintained under identical conditions (20°C, ad libitum food, 100% oxygen saturation and 12:12 light:dark photoperiod regime), winter N. obscura had a longer developmental time (146 d for winter, 98 d for summer N. obscura) and larger size at maturity (385.7 mg for winter, 244.8 mg for summer N. obscura). Winter N. obscura ingested twice as much prey as the summer generation although their growth rates were similar. Winter N. obscura also shunted more energy into lipid storage than summer animals during their growth (from 6.13 to 15.04% for winter and 13.08 to 16.08% for summer N. obscura, respectively). With no evidence to suggest the occurrence of difference in genotype between winter and summer N .obscura Dratnal and Davies (1990) concluded that the observed differences were the result of indirect life history adaptations to stress experienced by winter N. obscura prior to collection (i.e. their prehistory).

No comparative study has been performed on these two generations of N. obscura produced each year, and it is not known whether they differ in terms of ecophysiology, growth and life-history traits. The environmental conditions prior to, and during the two major seasons of cocoon production (early and late summer) are different in terms of temperature, food availability, oxygen saturation and photoperiod regime. Water temperatures are higher and increasing during the early summer and about 4°C lower and decreasing in late summer (Fig. 1.2) (Gates 1984; Wrona, Linton and Davies 1987; Linton and Davies 1987). Food is abundant in early summer while in late summer strong intra-specific and inter-specific competition with E.

punctata occurs (Davies *et al.* 1981; Davies *et al.* 1984). As the environmental conditions are different between the two reproductive seasons (Davies *et al.* 1981; Davies *et al.* 1984; Gates 1984; Wrona, Linton and Davies 1987; Linton and Davies 1987), differences in life history and ecophysiology might occur between the two generations.

The overall aim of this study was to compare early summer (spring) and late summer generations of N. obscura to determine if they differ in terms of their ecophysiology, growth and / or life-history traits. The specific objectives were:

1. To determine whether differences in bioenergetics and lifehistory traits between these two generations can be attributed to differences in the prehistory of the parents.

2. To explain any differences between the two generations in terms of their ecological fitness.

3. To determine if genetic differences occur between the two generations of N. obscura from Stephenson's Pond, and offer an alternative explanation for any difference.

4. To determine if the Stephenson's Pond population is genetically different from those in the Rocky Mountain foothills of Alberta and from Utah (U.S.A.).

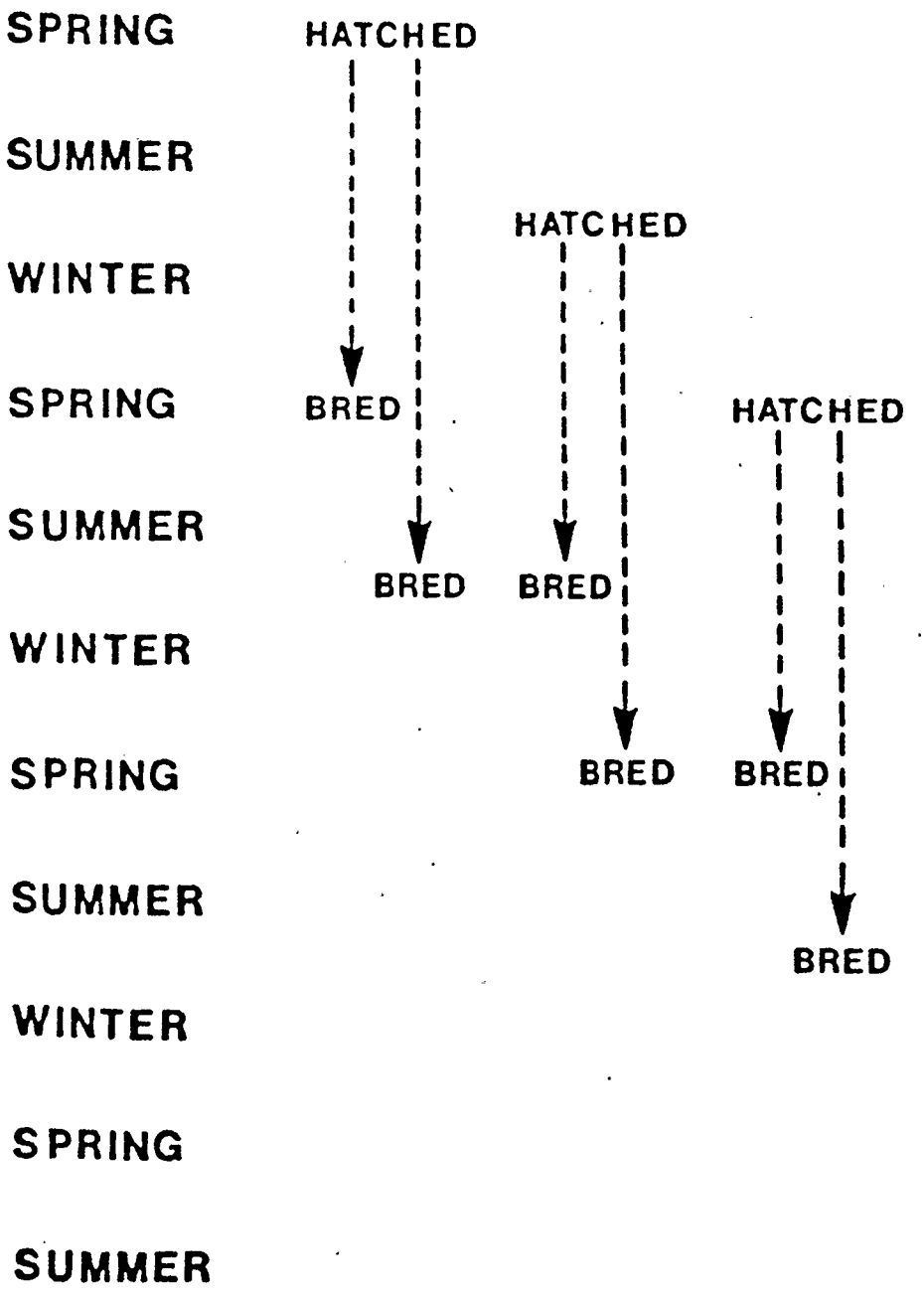


Figure 1.1 A schematic outline of the life history of the early and late summer generations of Nephelopsis obscura in southern Alberta (after Davies and Everett 1977)

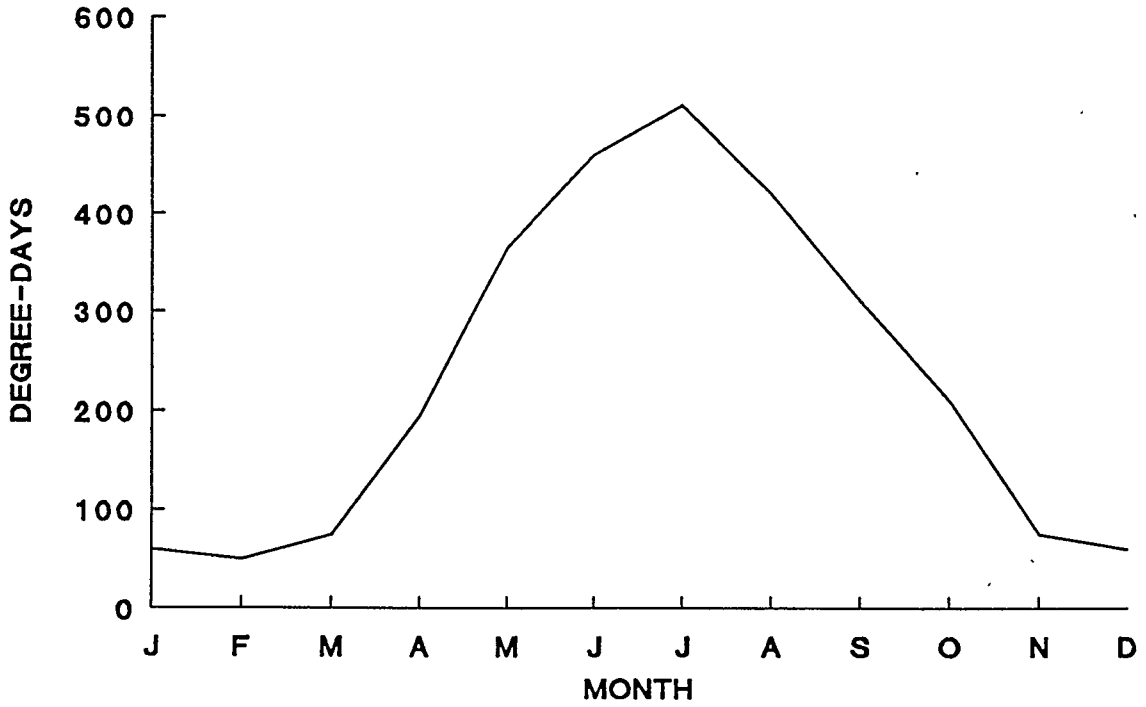


Figure 1.2 The annual water temperature regime in Stephenson Pond, Alberta (after Wrona *et al.* 1987)

1.1 Site Descriptions

Stephenson's Pond is located 51°9'N-114°16'W, in the knob and kettle topography of the prairie-foothills transition zone (Legget 1961), approximately 5 km northwest of Calgary, Alberta. The pond, enlarged by the placement of a earth-stone dike at the east end, has a small intermittent stream entering the western end. The surface area is about 2.2 hectares with a maximum depth of 2.5 m. It is highly eutrophic since the catchment basin is utilized by livestock, resulting in algal blooms in summer and dense aquatic microphyte stands of Potamogeton richardsoni (Benn.) and Myriophyllum exalbescens (Fern.) Populus balsamifera (Linn.) and Salix sp. form the dominant riparian vegetation on the southern portion of the catchment basin with grasses, sedges and shrubs constituting the remainder. The bottom substrate of Stephenson's Pond consists of mud and allochthonous debris, and a limited amount of rock in the dike area. N. obscura is the numerically dominant erpobdellid with E. punctata subdominant (Davies 1973; Davies, Reynoldson and Everett 1977; Linton, Davies and Wrona 1982; Davies and Gates 1991). T. rude, G. complanata, H. stagnalis and Placobdella papillifera (Moore) also occur.

1.2 Statistical Methods

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 Ho : $\mu_1 = \mu_2 = \mu_3 = \ldots\mu_K$, where, K = the number of experimental groups. Depending on the number (n) of treatment variables (factors) examined, a one-way ANOVA was performed following Zar (1984) using the Statistical Analysis System (SAS) package (SAS Institute, Inc. 1979). When the null hypothesis of equality of population means was rejected, a Student-Newman-Keuls (SNK) a posteriori multiple range test was used to determine between which population means the differences occurred (Zar 1984).

1.2.2 Student's t-test

A two-tailed Student's t-test was used to compare the means of the two generations of N. obscura with the hypothesis Ho: $\mu_1 = \mu_2$ (where μ_1 = the mean of the early summer generation, and μ_2 = the mean of the late summer generation).

1.2.3 Regression and Covariance Analysis

Least-squares linear regression analysis (Zar 1984) was used to examine the relationship between two variables 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 Student's t-test with equality of slopes and intercepts among regressions tested using analysis of covariance (Zar 1984).

1.2.4 Goodness-of-Fit

Contingency Chi-Square table analysis was used to test for homogeneity of genotypic frequencies between early summer and late summer N. obscura from Stephenson's pond.

All the hypotheses were performed at the $P = 0.05$ level of probability.

2.0 ENERGETICS AND LIFE HISTORY OF EARLY AND LATE SUMMER GENERATION NEPHELOPSIS OBSCURA

2.1 Introduction

While the study of the ecological energetics of populations is not new, the use of physiological constraints on energy allocation to explain variability in life histories is a relatively recent development (Calow 1978; Sterns 1980; Linton and Davies 1987; Dratnal and Davies 1990). Since energy is usually limited, there is often conflict within an organism for its use, resulting in physiological trade-offs (Sibly and Calow 1986). Partitioning of energy between metabolic demands has profound effects on the ecological success of animals. In an effort to increase their fitness organisms may exhibit flexibility in the allocation of energy in response to environmental conditions. Physiological adaptations of resource acquisition and use are not only the basis of the way organisms function physiologically, but also the basis of their form (allocation of resources between different structures) and behaviour (allocation of resources between different activities) (Sibly and Calow 1986) which are subsequently reflected in life history traits. For example, if an organism allocates more energy into somatic growth rather than reproductive growth, it will probably take a longer time to reach maturity, have a larger size at maturity and may exhibit iteroparity. Variability in life history traits is demonstrated by the energetic partitioning within an organism. In comparative study of life history, the advantage of using an energetics model is that variables can be quantified and correlations between quantified variables and life

history traits established. For example, total production (P) can be partitioned into energy available to support growth and reproduction. The proportion of production partitioned to reproduction (Re), i.e. Re/P, provides one meaningful measure of reproductive effort, and the values of Re/P ranged from 6 to 69% for a variety of molluscs (Browne and Russell-Hunter 1978). Browne and Russell-Hunter concluded (1978) that viviparous species with the young develop within the uterus, and nourished until they are born by nutrients received from the mother's blood through a placenta have lower Re/P (mean Re/P = 6.25%) than oviparous species (laying eggs that hatch outside the mother's body) (mean Re/P = 24.24%). Based upon the study of 16 species of freshwater and marine gastropods and bivalves, Browne and Russell-Hunter (1978) also found that semelparous species diverted a greater proportion of their non-respired assimilation (=production) to reproduction than iteroparous species (mean Re/P = 29.9% versus 18.2%, respectively). Calow (1978) also noted that iteroparous species of freshwater molluscs generally had lower Re/P (mean = 28.3%) than semelparous species (mean = 61.0%). Thus, reproductive effort can be used as a criterion to distinguish between semelparity and iteroparity or viviparity and oviparity. Zaika (1972) studied 25 species of bivalves and found a relationship between P:B ratios (P = total production and B = mean standing crop biomass (Burky *et al.* 1985)) and life span. Banse and Mosher (1980) discussed many of the variables which influence P:B ratios and report that short life span invertebrate species have a higher P:B ratio and *vice versa*. Sebens (1979) developed a model by which some life history traits can be predicted with respect to the maximum body size

and reproductive size. The basis of this model is the dynamic balance between energy absorbed by an organism and energy used for metabolism. In addition, Reddy, Dratnal and Davies (1992) showed that analyses of changes in energy reserves with age, size and developmental stage provides additional information on the adaptiveness of the life history.

Because N.obscura has phenotypic plasticity in its response to environmental changes (Peterson 1983; Wrona and Davies 1984; Baird, Linton and Davies 1986; Davies *et al.* 1987; Linton and Davies 1987; Gates and Davies 1987; Baird, Linton and Davies 1987; Wrona *et al.* 1987; Davies and Gates 1991 a; Davies and Gates 1991 b), the pre-history can alter its life-history traits (Dratnal and Davies 1990). Because the parents of the early summer and late summer N. obscura experience different environments (Davies *et al.* 1981; 1984; Gates 1984; Wrona and Davies 1987; Linton and Davies 1987), it is hypothesized that different energy acquisition and allocation patterns may occur between the early summer and late summer generations, which might subsequently induce differences in life-history traits between the two generations.

2.2 Objective

The objective of this study was to quantitatively determine the differences between early summer and late summer generations of N. obscura from Stephenson's Pond in terms of energy acquisition, energy allocation, growth and reproduction.

2.3 Materials and Method

2.3.1 Bioenergetic Model

The bioenergetic model used is based on the energy balance equation of Sibly and Calow (1986) :

$$I - Fe = A = R + G + U \qquad (1)$$

Where I = the total energy content of the food ingested, Fe = the energy content of faeces, A = the energy absorbed across the gut wall, R = the energy expended as metabolism, G = energy production including somatic growth and reproductive growth, U = the energy value of excretory and secretory products. However, because of the difficulties in separating leech faeces from mucus (Dratnal and Davies 1990) energy absorption by N. obscura cannot be directly assessed. In addition as energy utilized in excretion is negligible (Calow *et al.* 1982; Vandenbos unpublished data), equation (1) can be modified as:

$$I - (Fe + Mu) = E = R + G \qquad (2)$$

where E = energy available for growth and metabolism other than mucus production.

This model assumes that the sole source of energy is food consumption and that all energy consumed (I) is metabolized through respiration (R), lost through waste production faeces (Fe) + mucus (Mu), or accumulated as growth (somatic and reproductive).

2.3.2 Experimental Design

2.3.2.1 Collection

Nephelopsis obscura cocoons were collected immediately after deposition from Stephenson's Pond in late July and early September, 1990 and maintained at 20°C, 100% oxygen saturation with a 12 h:12 h light:dark photoperiod regime until they hatched, after approximately 4 weeks. The hatchlings for the July cocoons (early summer generation) and the September cocoons (late summer generation) were maintained under identical laboratory conditions and provided with ad libitum food (Tubifex tubifex Müller) until they reached about 10 - 12 mg.

2.3.2.2 Feeding and Growth Experiment

Twenty leeches were randomly selected from the early summer (mean size 11.0 $\pm$ 0.1 mg) and late summer (mean size 10.7 $\pm$ 0.3 mg) stocks of hatchlings and maintained individually in plastic containers (30 cm^2) with aerated, filtered (50μm) pond water.

Since some variable determinations e.g. energy content and total lipid are destructive, parallel sets of early summer and late summer N. obscura were maintained under identical conditions to the experimental leeches.

Hatchlings from both generations were maintained under identical conditions

(20°C, 12 h:12 h light:dark photoperiod regime, and 100% oxygen saturation). Each leech was provided with about 100 mg T. tubifex three times a week for 3 h. To correct for errors due to biomass changes of T. tubifex during the feeding periods, similar weights of T. tubifex were added to five containers without leeches (controls) and left for 3 h. The difference between the initial wet weight and final wet weight of T. tubifex from the experimental containers containing leeches gives the apparent food ingested by N. obscura. The difference between the initial and final wet weight of T. tubifex from the controls without leeches gives the prey biomass change during the feeding period. The apparent food ingested by N. obscura was corrected by subtracting the mean weight changes of T. tubifex in the controls to give the actual biomass of prey ingested by N. obscura. To reduce errors due to food remaining in the gut or increased water intake sometimes observed in N. obscura immediately after feeding (Dratnal and Davies 1990), leech growth (G) was measured weekly 2 d after feeding. The difference between two successive weights of N. obscura represented leech growth in terms of mg wet weight (WWT).

To convert feeding and leech growth into energy equivalents, ten leeches were randomly selected each month from both generations, freeze-dried to a constant weight and the calorific value (converted to joules) determined using a Philipson microbomb calorimeter. The energy equivalent of the prey T. tubifex was similarly determined each month.

The experiments were continued until the leeches reached sexual maturity, as indicated by the development of a visible clitellum (Davies and Everett, 1977), with

the size at maturity and time to maturity recorded.

2.3.2.3 Energy Losses in Faeces Plus Mucus

To determine the energy available for growth and metabolism, energy losses in faeces plus mucus were calculated. Faeces plus mucus were collected each day for 4 d on pre-weighed Millipore filters (0.45 μm) which were subsequently dried to a constant weight at 40°C. The difference between the initial and final weights represented the dry weight of faeces plus mucus.

To calculate the energy content of faeces plus mucus, water from the experimental containers was collected each day for 6 d to provide sufficient material to measure the calorific value. The water samples were centrifuged at 12,000 rpm using a continuous flow-through system (0.15 $L \cdot min^{-1}$). The faeces plus mucus was freeze-dried to a constant weight and the energy content determined using a Philipson microbomb calorimeter.

Errors associated with these methods (mainly bacterial decomposition and bacteria increments) are assumed to be a constant proportion of depositions (Dratnal and Davies, 1990), and therefore should not affect the relative estimations for both generations.

2.3.2.4 Energy Absorption Efficiency

After the determination of energy consumed from the prey (I) by N. obscura and energy loss in faeces plus mucus (Fe + Mu), the energy absorption efficiency (AE) was calculated from the equation (Tedengren *et al.* 1990):

$$AE = \frac{I - (Fe + Mu)}{I} \quad (3)$$

2.3.2.5 Growth Efficiency

Growth efficiency was determined as gross growth efficiency (GE_g) and as net growth efficiency (GE_n) from the equations (Garton 1984):

$$GE_g = \frac{G}{I} \quad (4)$$

$$GE_n = \frac{G}{A} \quad (5)$$

2.3.2.6 Lipid Storage

Energy storage by both generations of N. obscura was calculated as percentage total lipid concentration measured following the method of Folch *et al* (1957). For each assessment five leeches were individually freeze-dried to a constant weight and homogenized in 16 ml 1:1 chloroform:methanol mixture. The homogenate was transferred to a 25 ml test tube, heated to boiling (61°C), cooled, and 9 ml chloroform added before being filtered into a separate funnel. A volume of 10 ml normal saline (0.9 %) was added to the contents, shaken vigorously, with the lower layer collected into a preweighed beaker and subsequently dried at 70°C to a constant weight. The difference between the two beaker weights gave the amount of lipid present in the sample.

2.4 Results

2.4.1 Leech Growth

2.4.1.1 Growth Pattern

Some mortality occurred in the experiments and thus the results are based on fourteen early summer and fifteen late summer specimens of N. obscura.

The growth patterns of the early summer leeches and late summer leeches were similar, both showing sigmoid growth (Figs. 2.1 and 2.2). Initially leeches grew with an accelerating rate until the inflection point after which growth continued at a decelerating rate. The asymptote appeared after 182 d for early summer N. obscura and 145 d for late summer N. obscura, respectively. The growth of early summer N. obscura is described quantitatively by regressing age against biomass:

$$\dot{W}_E = \frac{772.5}{1+\exp^{(4.06-0.039T_E)}} \qquad (6)$$

$r^2 = 0.976$

Similarly the growth of late summer N. obscura can be described by:

$$\dot{W}_L = \frac{907.2}{1+\exp^{(3.60-0.041T_L)}} \qquad (7)$$

$r^2 = 0.970$

where T_E and W_E represent the age (d) and biomass (mg WWT) for early summer N. obscura and T_L and W_L represent the age (d) biomass (mg WWT) for late summer N. obscura.

2.4.1.2 Growth Rate

The inflection points calculated from equations 3 and 4 occur after 104 d at a biomass of 324.5 mg for early summer and after 88 d at a biomass of 446.2 mg for late summer leeches. Thus late summer leeches reach the inflection point earlier and with a larger body size than early summer leeches.

To compare the growth between the two generations the growth curves were divided into two phases by the inflection point. The accelerating phase and the decelerating phase for both generations fitted an exponential function with a linear relationship between the natural log of wet weight (WWT) and time (T) described by the equation:

$$Log(W) = a + b(T) \qquad (8)$$

where a and b are constants estimated by least squares regression. The regression coefficient (b) was used as an index for leech growth rate (Table 2.1). Analysis of covariance showed that the values of the slopes are significantly different between early summer and late summer leeches; indicating that late summer N. obscura grow

faster than early summer leeches during the accelerating phase. During the decelerating growth phase there was no significant difference in slopes of the early summer and late summer generations; indicating similar growth rates for both generations. Within a generation, however, significant differences between slopes was found in the two growth phases for both the early summer and late summer N.obscura, with leeches growing much faster during the early growth phase than during the adult growth phase. To make a comparison of size-specific growth, energy acquisition and allocation, four different sizes (Stage I, II, III, and IV) for each generation were chosen. The first two stages are before the inflection point and the last two stages after the inflection point. Individual sizes at a particular stage are similar between the two generations (Table 2.2).

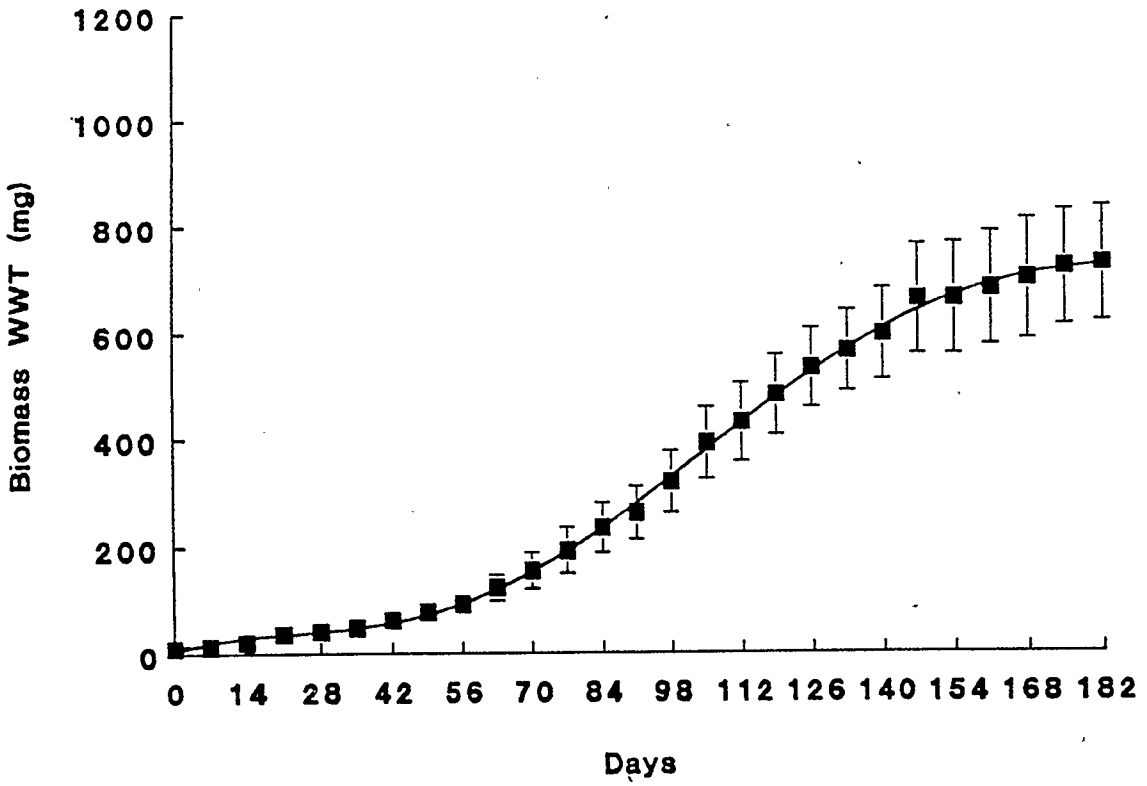


Figure 2.1 Growth curve of the early summer generation Nephelopsis obscura

Vertical bars represent standard errors.

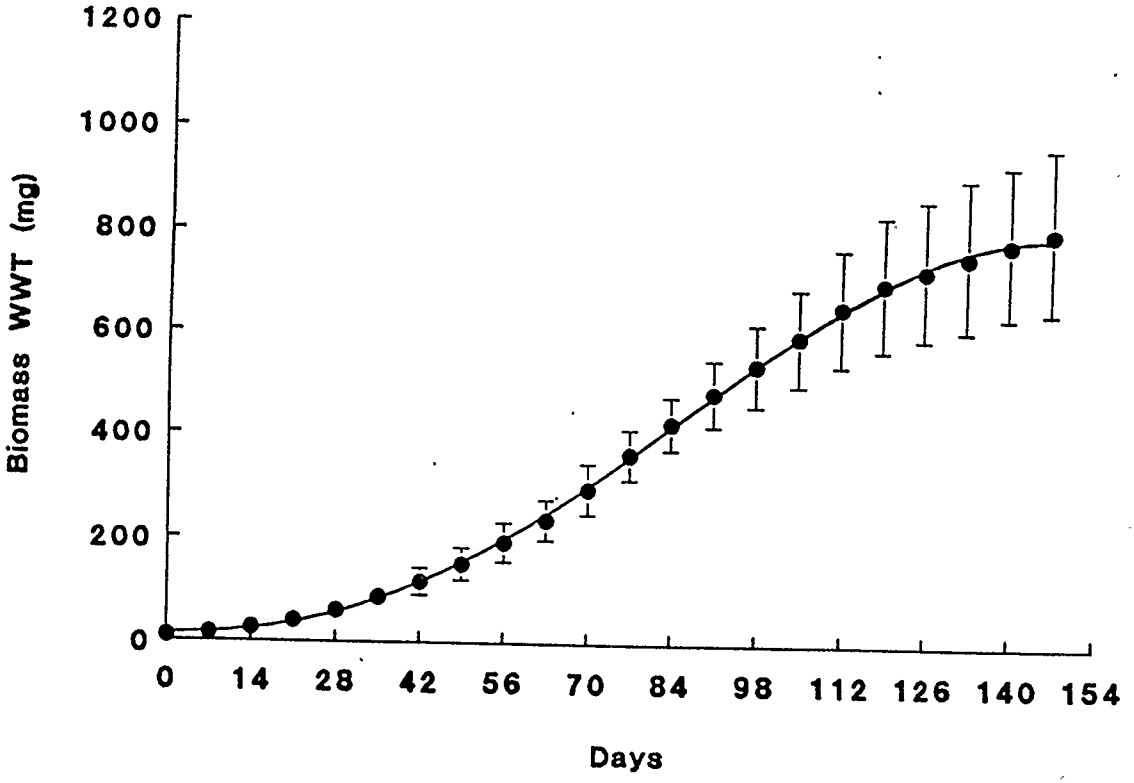


Figure 2.2 Growth curve of the late summer generation Nephelopsis obscura

Vertical bars represent standard errors.

TABLE 2.1

Mean values of intercept (a) and slope (b) (the exponential growth rate) for early summer and late summer Nephelopsis obscura. Values are given as mean ± standard error. * = significant difference at $P < 0.05$. *** = significant difference at $P < 0.001$. NS = no significant difference. n = sample size.

Growth phase	Accelerating	Decelerating	n	Test of Covariance
Intercept	a			
Generation				
Early summer	1.11 ± 0.08	2.26 ± 0.12	14	***
	NS	*		
Late summer	1.20 ± 0.09	2.41 ± 0.17	15	***
Slope	b			
Generation				
Early summer	0.015 ± 0.001	0.003 ± 0.001	14	***
	***	NS		
Late summer	0.018 ± 0.001	0.003 ± 0.001	15	***

TABLE 2.2

Four different stages of early summer and late summer Nephelopsis obscura used in the study of size-specific growth, energy acquisition and allocation. Size of N. obscura is given as mean ± standard error in mg wet weight. n = 10. NS = no significant difference.

Growth phase	Accelerating		Decelerating	
Stage	I	II	III	IV
Generation				
Early summer	23.7 ± 1.0	301.6 ± 5.3	447.6 ± 8.9	690.3 ± 10.9
	NS	NS	NS	NS
Late summer	23.5 ± 1.0	299.6 ± 11.3	455.5 ± 12.2	691.2 ± 16.6

2.4.2 Energy Content of Nephelopsis obscura and Tubifex tubifex

One-way ANOVA shows no significant difference in energy content of N. obscura amongst the different sizes for both early summer and late summer generations (Table 2.3). Student's t-test indicates no significant difference in energy content of N. obscura between the two generations (Table 2.3). One-way ANOVA shows that the energy content of T. tubifex during the experiments showed no significant differences (Table 2.4). Therefore the values of the energy content of N. obscura were pooled, as were the energy values of T. tubifex. The mean value of 21.9 $KJ \cdot g^{-1}$ (dry weight) for N. obscura and 23.1 $KJ \cdot g^{-1}$(dry weight) for T. tubifex used for the calculation of the energy budget for N. obscura.

TABLE 2.3

Energy content of early summer and late summer Nephelopsis obscura. Energy value is given as mean ± standard error in $KJ \cdot g^{-1}$(dry weight of N. obscura). NS = no significant difference between generations or amongst different stages within generation. n = 10.

Growth phase	Accelerating		Decelerating		ANOVA
Stage	I	II	III	IV	
Generation					
Early summer	21.6 ± 1.3	21.8 ± 1.4	22.7 ± 1.3	22.3 ± 1.5	NS
	NS	NS	NS	NS	
Late summer	21.8 ± 2.9	21.9 ± 2.1	22.1 ± 0.8	21.8 ± 1.3	NS

TABLE 2.4

Energy content of Tubifex tubifex consumed by the early summer and late summer Nephelopsis obscura during the experiments as mean ± standard error in $KJ \cdot g^{-1}$(dry weight of T. tubifex). NS = no significant difference. n = 10.

Growth phase	Accelerating		Decelerating		ANOVA
Stage	I	II	III	IV	
Generation					
Early summer	22.7 ± 0.5	23.2 ± 1.0	23.1 ± 0.9	23.4 ± 1.1	NS
	NS	NS	NS	NS	
Late summer	23.1 ± 0.9	23.4 ± 1.1	23.3 ± 1.1	22.9 ± 0.8	NS

2.4.3 Food Intake

Table 2.5 shows the feeding rates ($KJ \cdot d^{-1} \cdot ind^{-1}$) by the early summer and late summer generations of N. obscura from hatchling to sexual maturity. Student's t- tests indicate that at each stage there is no significant difference in feeding rate between the two generations. However, within a generation there is a significant difference in feeding rate amongst stages in both generations with smaller leeches consumed more prey per unit leech DWT than large leeches (Table 2.5). Feeding rates for both generations gradually increased until the growth curve inflection point was reached when feeding rates decreased in both generations (Figs. 2.3 and 2.4).

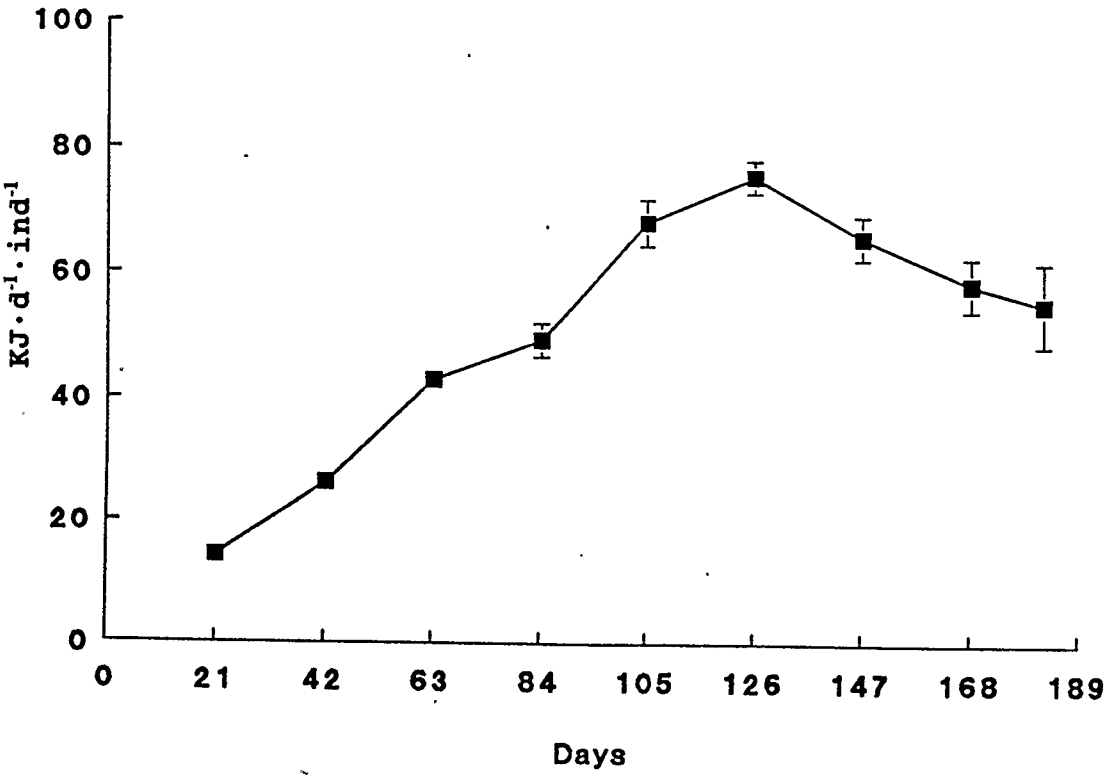


Figure 2.3 Feeding rate of early summer Nephelopsis obscura

Vertical bars represent standard errors.

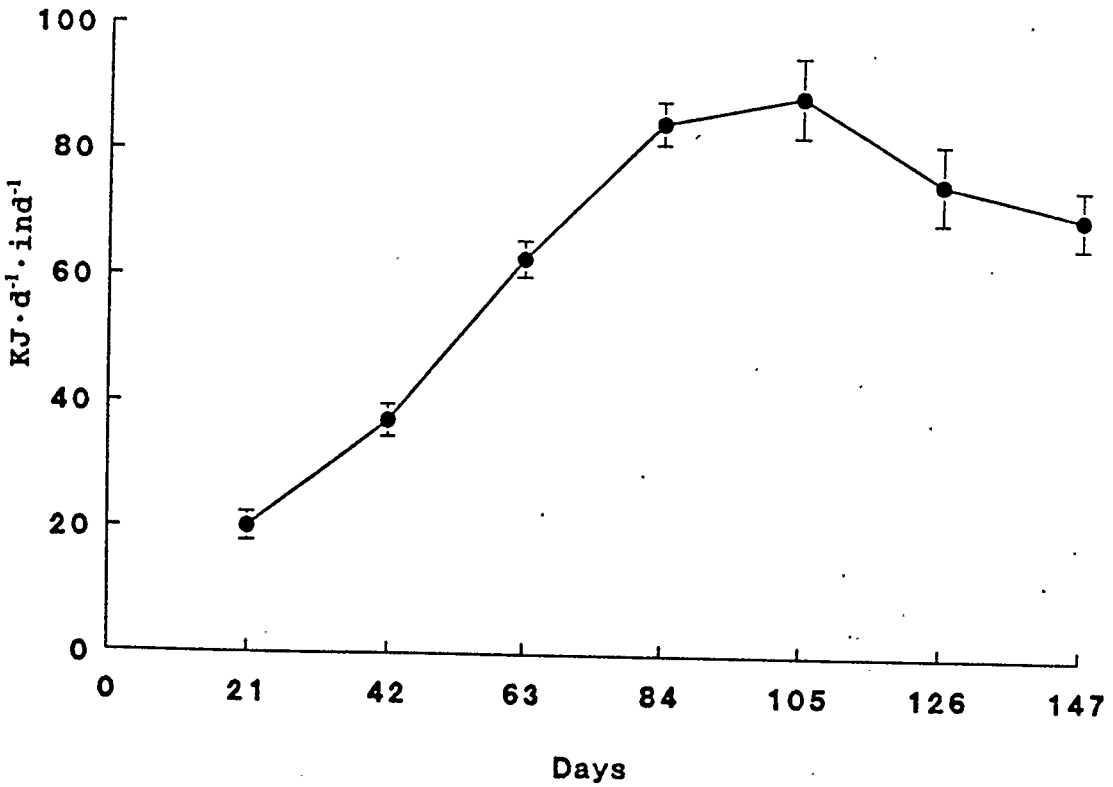


Figure 2.4 Feeding rate of late summer Nephelopsis obscura

Vertical bars represent standard errors.

2.4.4 Energy Losses in Faeces Plus Mucus

During the accelerating growth phase, late summer N. obscura lost less energy in faeces plus mucus than did the early summer animals, i.e. a higher proportion of energy was available in late summer leeches for growth and metabolism. However, during the decelerating growth phase the energy losses in faeces plus mucus were not significantly different between generations (Table 2.6). One-way ANOVA test shows that there is significant difference in energy loss in faeces plus mucus amongst stages within generation for both early and late summer N. obscura. With the stage increase, energy loss in faeces plus mucus significantly decreased.

2.4.5 Energy Absorption

Energy absorption for both generations were calculated from Equation 2 by subtracting energy lost in faeces plus mucus from the energy of the prey consumed.

The calculated values of energy absorption (Table 2.7) show that during the accelerating phase energy absorbed by late summer leeches was significantly higher than in early summer leeches, whereas during the decelerating phase energy absorption was similar in both generations. One-way ANOVA shows that within a generation different stages are significantly different from each other with respect to energy absorption for both early summer and late summer N. obscura, with a smaller size having a higher energy absorption.

2.4.6 Energy Absorption Efficiency

Calculated energy absorption efficiencies are given in Table 2.8. Student's t-test shows that during the accelerating growth phase late summer N. obscura had significantly higher energy absorption efficiency than early summer leeches. Within a generation, however, both generations showed similar trend with only stage I having lower energy absorption efficiency compared to other stages.

TABLE 2.5

Feeding rates (mean ± standard error KJ (Tubifex tubifex) • day^{-1} • g^{-1} (dry weight of Nephelopsis obscura)) of early summer and late summer N. obscura. *** = significant difference at $P < 0.001$. NS = no significant difference. n = 10.

Growth phase	Accelerating		Decelerating		ANOVA
Stage	I	II	III	IV	
Generation					
Early summer	3.79 ± 0.16	1.38 ± 0.02	1.11 ± 0.07	0.56 ± 0.03	***
	NS	NS	NS	NS	
Late summer	3.90 ± 0.13	1.40 ± 0.03	1.12 ± 0.03	0.61 ± 0.06	***

2.4.7 Energy Allocated to Growth

The data for energy allocated to growth (Table 2.9) are consistent with the growth patterns for the two generations. There is a significant difference with respect to energy allocated to growth between the two generations in the accelerating phase with late summer leeches allocating more energy into growth than early summer leeches. This results in a higher growth rate for late summer leeches and therefore the late summer leeches reach the inflection point earlier at a larger size than early summer leeches. During the decelerating phase, however, the amount of energy allocated to growth by the two generations is similar (Table 2.9), resulting in similar growth rates. There is a significant difference amongst different stages in energy allocation to growth within a generation. Both early summer and late summer N. obscura exhibit the same trends with the smallest stage having highest energy allocation to growth and the largest stage having the lowest energy allocation to growth.

2.4.8 Growth Efficiency

The patterns of gross growth efficiency were similar to the patterns of net growth efficiency in both generations (Table 2.11 and 2.12). Significant difference existed between the two generations at stage I and stage II in terms of gross and net growth efficiency. One-way ANOVA shows that growth efficiency differed significantly amongst stages within generations with higher efficiency at stage II and stage III.

2.4.9 Energy Allocated to Respiration

Since the energy absorbed equals the energy available for growth and metabolism the energy allocated to metabolism can be calculated from Equation 2 by subtracting the amount of energy allocated to growth from the total amount of energy absorbed. Student's t-test shows that there is no significant difference in energy allocated to respiration at each stage between the two generations (Table 2.9). One-way ANOVA shows that within a generation the energy utilized in respiration is significantly different amongst stages for both early summer and late summer N. obscura with a significant decrease in energy allocated to metabolism from stage I to stage IV.

TABLE 2.6

Size-specific energy lost in faeces plus mucus for early summer and late summer Nephelopsis obscura. Energy value is given as $KJ \cdot day^{-1} \cdot g^{-1}$(dry weight of N. obscura) (mean $\pm$ standard error). * = significant difference at $P < 0.05$. *** = significant difference at $P < 0.001$. NS = no significant difference. n = 10.

Growth phase	Accelerating		Decelerating		ANOVA
Stage	I	II	III	IV	
Generation					
Early summer	1.46 ± 0.10	0.34 ± 0.02	0.22 ± 0.03	0.13 ± 0.01	***
	*	*	NS	NS	
Late summer	1.12 ± 0.07	0.27 ± 0.03	0.21 ± 0.02	0.14 ± 0.02	***

TABLE 2.7

Size-specific energy absorption for early summer and late summer Nephelopsis obscura. Energy value is given as mean ± standard error in KJ (Tubifex tubifex) · day^{-1} · g^{-1}(dry weight of N. obscura). * = significant difference at $P < 0.05$. ** = significant difference at $P < 0.01$. *** = significant difference at $P < 0.001$. NS. = no significant difference. n = 10.

Growth phase	Accelerating		Decelerating		ANOVA
Stage	I	II	III	IV	
Generation					
Early summer	2.33 ± 0.10	1.03 ± 0.02	0.89 ± 0.05	0.37 ± 0.03	***
	*	**	NS	NS	
Late summer	2.79 ± 0.08	1.13 ± 0.02	0.86 ± 0.04	0.42 ± 0.04	***

TABLE 2.8

Energy absorption efficiency for early summer and late summer Nephelopsis obscura as mean ± standard error in percentage. * = significant difference at $P < 0.05$. *** = significant difference at $P < 0.001$. NS = no significant difference. n = 10.

Growth phase	Accelerating		Decelerating		ANOVA
Stage	I	II	III	IV	
Generation					
Early summer	0.62 ± 0.02	0.75 ± 0.04	0.81 ± 0.02	0.73 ± 0.05	***
	***	*	NS	NS	
Late summer	0.71 ± 0.01	0.81 ± 0.02	0.80 ± 0.02	0.75 ± 0.04	***

TABLE 2.9

Size-specific energy allocated to growth for early summer and late summer Nephelopsis obscura. Energy value is given as mean ± standard error in KJ (N. obscura) · day^{-1} · g^{-1} (dry weight of N. obscura). * = significant difference at $P < 0.05$; *** = significant difference at $P < 0.001$. NS = no significant difference. n = 10.

Growth phase	Accelerating		Decelerating		ANOVA
Stage	I	II	III	IV	
Generation					
Early summer	0.95 ± 0.08	0.56 ± 0.02	0.47 ± 0.02	0.05 ± 0.06	***
	***	*	NS	NS	
Late summer	1.44 ± 0.06	0.66 ± 0.01	0.48 ± 0.03	0.14 ± 0.04	***

TABLE 2.10

Size-specific energy utilized in respiration for early summer and late summer Nephelopsis obscura. Energy value is given as mean ± standard error in $KJ \cdot day^{-1} \cdot g^{-1}$(dry weight of N. obscura). *** = significant difference at $p < 0.001$. NS. = no significant difference. n = 10.

Growth phase	Accelerating		Decelerating		ANOVA
Stage	I	II	III	IV	
Generation					
Early summer	1.38 ± 0.08	0.47 ± 0.03	0.42 ± 0.03	0.32 ± 0.06	***
	NS	NS	NS	NS	
Late summer	1.35 ± 0.06	0.47 ± 0.02	0.38 ± 0.05	0.28 ± 0.02	***

TABLE 2.11

Gross growth efficiency for early summer and late summer Nephelopsis obscura as mean ± standard error in percentage. *** = significant difference at $P < 0.001$. * = significant difference at $P < 0.05$. NS = no significant difference. n = 10.

Growth phase	Accelerating		Decelerating		ANOVA
Stage	I	II	III	IV	
Generation					
Early summer	0.25 ± 0.02	0.41 ± 0.01	0.43 ± 0.01	0.09 ± 0.14	***
	***	**	NS	NS	
Late summer	0.37 ± 0.01	0.47 ± 0.01	0.45 ± 0.03	0.21 ± 0.08	***

TABLE 2.12

Net growth efficiency for early summer and late summer Nephelopsis obscura as mean ± standard error in percentage. *** = significant difference at $P < 0.001$. * = significant difference at $P < 0.05$. NS = no significant difference. n = 10.

Growth phase	Accelerating		Decelerating		ANOVA
Stage	I	II	III	IV	
Generation					
Early summer	0.41 ± 0.03	0.55 ± 0.02	0.53 ± 0.02	0.13 ± 0.19	***
	***	*	NS	NS	
Late summer	0.52 ± 0.02	0.58 ± 0.01	0.57 ± 0.04	0.25 ± 0.12	***

2.4.10 Lipid Storage

The patterns for lipid storage for early summer and late summer generations of N. obscura were very different (Fig. 2.5). The hatchlings of late summer leeches had a much higher lipid content and this was maintained throughout the life history until sexual maturity (approximately 700 mg) after which total lipid concentration decreased. Early summer hatchlings had a lower total lipid content which increased with size and reached a maximum value at maturity similar to that in late summer N. obscura after which total lipid content subsequently decreased. Since a significant difference in lipid content exists between hatchlings of the two generations and the calorific value of lipid is twice as much as protein and carbohydrate, a significant difference in energy content of leeches between hatchlings of the two generations was expected. The data in Table 2.3, however, indicates that the energy contents of leeches at stage I are not significantly different between the two generations. Based on the calorific value of lipid, protein and carbohydrate, the calculated energy content of N. obscura would be expected higher in late summer than in early summer by 0.05%. As the variance in calorific bomb assesments is larger than 0.05%, an explanation for the contradictory results is provided.

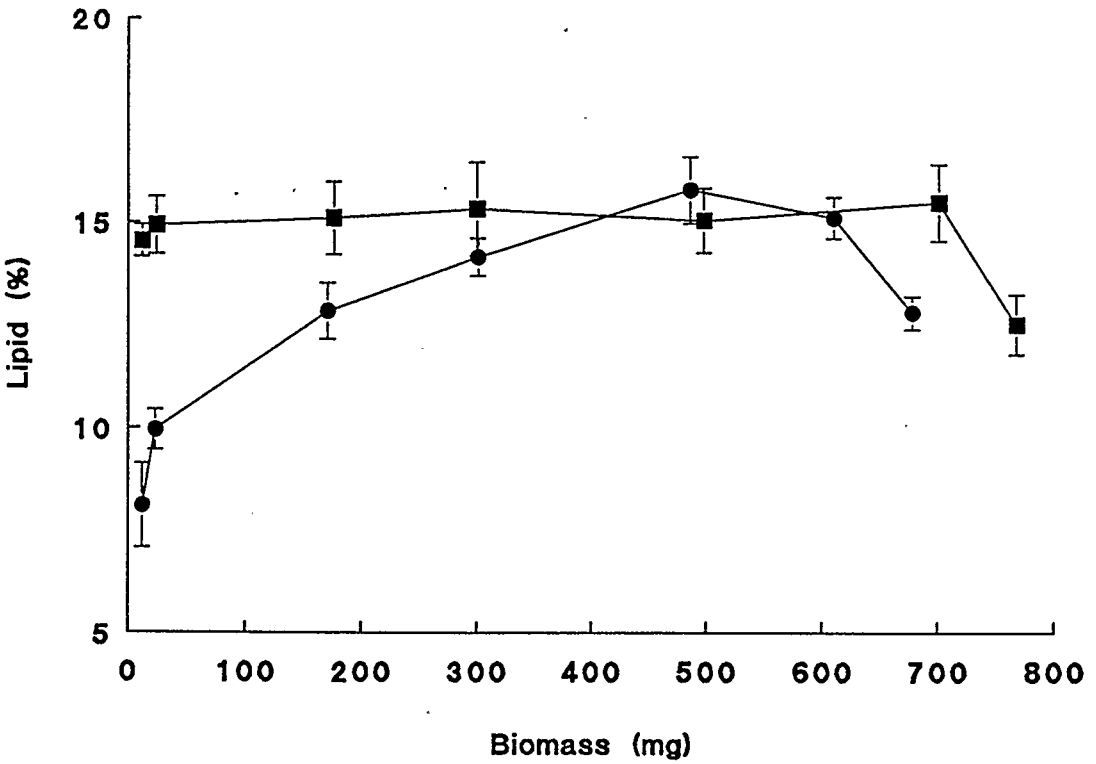


Figure 2.5 Lipid content of early summer and late summer

<u>Nephelopsis obscura</u> ● early summer ■ late summer

Vertical bars represent standard errors.

2.4.11 Leech Development

2.4.11.1 Size at Energy Shifting and Maturity

Based upon the principle of allocation and assuming the energy allocated to growth is limited, a trade-off must occur between somatic and reproductive growth. Most organisms go through some period during which all the energy is allocated to growth, thus maximizing growth rate at the expense of the early reproduction (Sebens 1987). Organisms cannot allocate all absorbed energy into somatic growth throughout their life history, or they would become very large but produce no offspring, a strategy which has zero neo-Darwinian fitness (Calow 1981). Sebens (1979) considered size at which the energy available for production should switch growth of somatic tissues to building gonads and gametes. Sebens (1979) developed a model which predicts the size at which reproduction should occur, is based upon the energy balance between intake and expenditure which can be described by two allometric equations:

$$E_A = a_1 W^{b_1} \quad (9)$$

$$E_C = a_2 W^{b_2} \quad (10)$$

When the difference between energy absorption (E_A) and energy cost (E_C) is at a maximum the organism can allocate more energy into reproduction. This size

(W_{OPT}) is given by:

$$W_{OPT} = \left(\frac{a_2 b_2}{a_1 b_1}\right)^{\frac{1}{a_1 - a_2}} \quad (11)$$

Sebens' model can also predict maximum body size. When energy expenditure (E_C) equals energy intake (E_A) the organism has no energy available to allocate to growth and maximum size is attained (equation 12):

$$W_{MAX} = \left(\frac{a_1}{a_2}\right)^{\frac{1}{b_1 - b_2}} \quad (12)$$

Sebens' model has been proved to be valid for a variety of marine invertebrates (Sebens 1979; Wilson 1988), as well as some fish (Weatherley 1972). However, N. obscura did not always increase energy absorption with increase in body size, an energy absorption decreased when it reaches the size around the inflection point on the growth curve. Thus, energy intake in N. obscura does not fit Sebens' model, i.e. cannot be described by equation 9. In addition, when N. obscura reaches W_{OPT} it does not completely stop somatic growth and allocate all available energy to reproduction. Based on the observed data in this study Sebens' model has been modified for N. obscura:

$$E_{EA} = a_A + b_A W_E - c_A W_E^2 \quad (13)$$

$$E_{EC} = a_C + b_C W_E - c_C W_E^2 \quad (14)$$

$$E_{LA} = a_A + b_B W_L - c_A W_L^2 \quad (15)$$

$$E_{LC} = a_C + b_C W_L - c_C W_L^2 \quad (16)$$

$$W_{OPT} = -\frac{b_A - b_C}{2(c_A - c_C)} \quad (17)$$

$$W_{MAX} = \frac{-(b_A - b_C) - ((b_A - b_C)^2 - 4(c_A - c_C)(a_A - a_C))^{\frac{1}{2}}}{2(c_A - c_C)} \quad (18)$$

where E_{EA} and E_{EC} are energy absorption and energy cost for the early summer generation N. obscura; E_{LA} and E_{LC} are energy absorption and energy cost for the late summer generation N. obscura; W_E and W_L are wet weight for the early summer and late summer generation N. obscura; W_{OPT} is optimal size for energy shift to reproduction, W_{MAX} is maximum size or size at maturity for N. obscura; a_A, a_C, b_A, b_C, c_A and c_C are constants estimated by least squares regression. The following equations describe the relationship between size and energy absorption and energy cost in early summer and late summer N. obscura:

$$E_{EA} = 2.90983 + 0.29018 W_E - 0.00033 W_E^2 \quad (19)$$

$r^2 = 0.9903$

$$E_{EC}=4.34585+0.09280W_E-0.00006W_E^2 \quad (20)$$

$r^2 = 0.9915$

$$E_{LA}=6.93072+0.27906W_L-0.000297W_L^2 \quad (21)$$

$r^2 = 0.9901$

$$E_{LC}=3.2639+0.11022W_L-0.00009W_L^2 \quad (22)$$

$r^2 = 0.9658$

Figs. 2.6 and 2.7 schematically outline the relationship between energy absorption and expenditure, the optimal size for N. obscura to shunt maximum energy to reproductive growth (defined as the energy shifting stage), and maximum body size (the size at maturity as this size coincides with the complete development of both male and female gonads).

Using equations 17 and 18, the optimal size at the energy shifting and size at maturity for both the early summer and late summer N. obscura were determined. The predicted and observed value for size at energy shifting and size at maturity are

given in Tables 2.13 and 2.14. Student's t-test shows a significant difference in observed value for sizes at the energy shifting stage and size at maturity between the two generations. Late summer N. obscura has a larger W_{OPT} size and larger mature size than early summer leeches.

2.4.11.2 Age at Energy Shifting Stage and Maturity

The modified Sebens' model only predicts the size at the energy shifting stage and at maturity. Since a quantitative relationship between biomass and age has been established for both early summer and late summer generation N. obscura (see equations 3 and 4), the corresponding age at energy shifting stage and maturity were thus calculated. Tables 2.15 and 2.16 shows that late summer leeches start building gonads and reach maturity at a significantly younger age than early summer leeches. With the exception for the observed age for the energy shifting stage in early summer N. obscura, all the observed values were very similar to the model's predictions (Table 2.15).

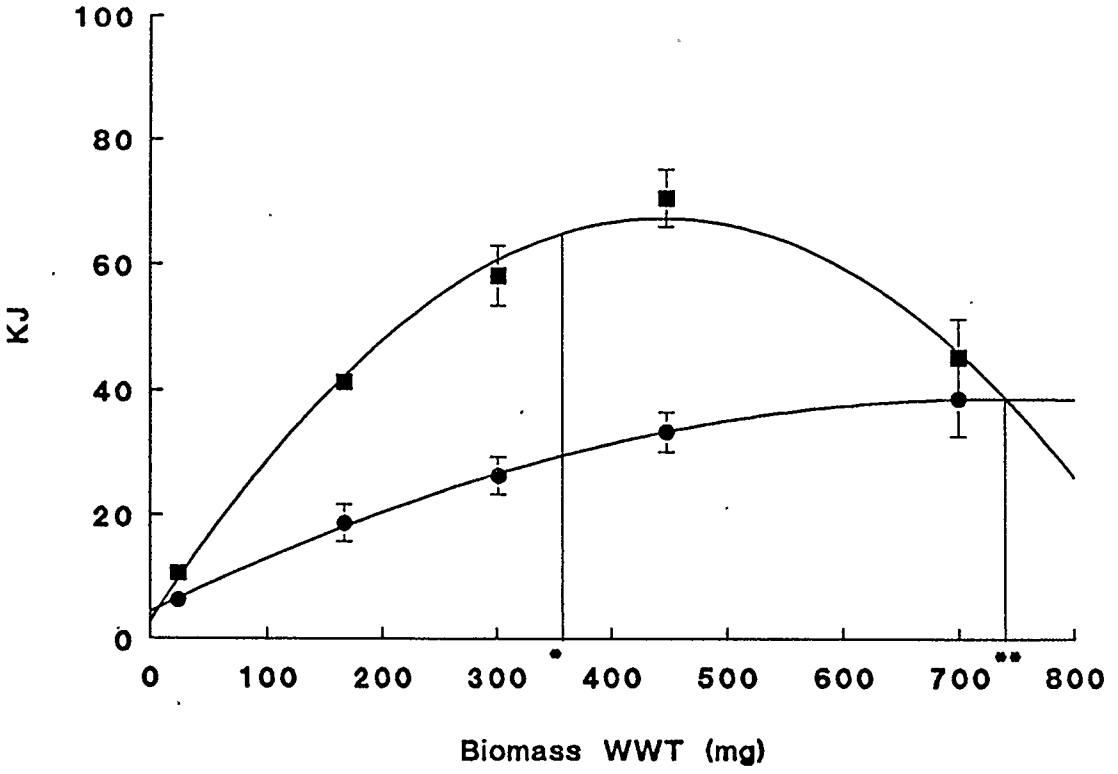


Figure 2.6 Energetics model for early summer Nephelopsis obscura

● cost ■ absorption

*** model predicted size at energy shifting stage**

**** model predicted size at maturity**

Vertical bars represent standard errors.

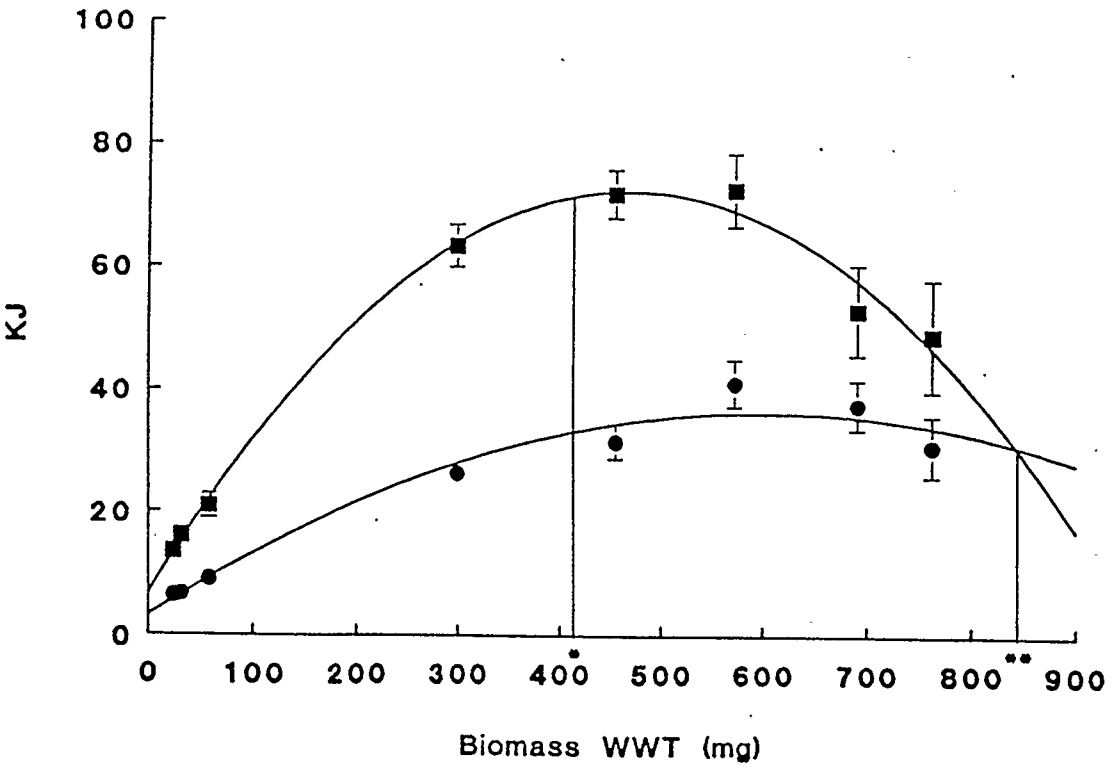


Figure 2.7 Energetics model for late summer Nephelopsis obscura

● cost ■ absorption

* model predicted size at energy shifting stage

** model predicted size at maturity

Vertical bars represent standard errors.

TABLE 2.13

Size at the energy shifting stage (W_{OPT}) for early summer and late summer Nephelopsis obscura. Size is given as mean ± standard error wet weight of N. obscura. * = significant difference at $P < 0.05$. NS = no significant difference. Sample size = n.

W_{OPT}	Predicted	Observed	n
Generation			
Early summer	373.8	368.7 ± 16.9 *	14
Late summer	411.8	406.5 ± 12.0	15

TABLE 2.14

Size at maturity for early summer and late summer Nephelopsis obscura. Size is given as mean ± standard error in wet weight of N obscura. * = significant difference at $P < 0.05$. NS = no significant difference. Sample size = n.

Size at maturity	Predicted	Observed	n
Generation			
Early summer	740.3	747.8 ± 29.4 *	14
Late summer	841.2	860.9 ± 42.6	15

TABLE 2.15

Age at the energy shifting stage (A_{OPT}) for early summer and late summer Nephelopsis obscura. Age is given as mean ± standard error in d. *** = significant difference at $P < 0.001$. NS = not significant. Sample size = n.

A_{opt}	Predicted	Observed	n
Generation			
Early summer	103	97 ± 1	14
		***	
Late summer	83	84 ± 3	15

TABLE 2.16

Age at maturity for early summer and late summer Nephelopsis obscura. Age is given as mean ± standard error in d. ** = significant difference at $P < 0.01$. NS = not significant. Sample size = n.

Age at maturity	Predicted	Observed	n
Generation			
Early summer	184	176 ± 11 **	14
Late summer	149	145 ± 4	15

2.5 Discussion

2.5.1 Growth

Growth patterns are categorized into two major types: determinate and indeterminate (Sebens 1987). Determinate (or constrained) growth refers to growth trajectories and / or final sizes that are primarily under genetic control. Environmental influences usually act early in the life history to set the growth pattern, and major deviations, especially once maturity has been reached, are not possible. Indeterminate (or plastic) growth, however, refers to the potential for continuous size change throughout the life-history as environmental conditions change. Organisms belonging to this category often have asymptotic size which can increase or decrease over an order of magnitude or more. The intrapopulation maximum mature size of N. obscura can vary from 170 to 2000 mg in the field (Davies and Everett 1977; Peterson 1983; Linton and Davies 1987) and from 700 mg to more than 3000 mg under different conditions of food ration and temperature (Linton and Davies 1987). Substantial degrowth occurs when food availability decreases and regrowth can occur if food availability increases (Gates 1984). Winter stress can change the asymptotic size at maturity (Dratnal and Davies 1990), overwintered leeches reaching a mean asymptotic size at maturity of 385.7 mg compared to 244.8 mg for leeches which had not overwintered. The results of this study shows that N. obscura from different generations that hatch at different seasons differ in asymptotic sizes at maturity. Late

summer generation N. obscura reached maturity earlier with a significantly larger size (860.9 ± 46.2 mg) than early summer generations leeches (747.8 ± 29.4 mg). Clearly N. obscura shows indeterminate growth. Therefore, the difference in growth of N. obscura both between and within populations is due either to phenotypic plasticity or genotypic differences, with the former more probable.

Late summer generation N. obscura grew significantly faster than early summer leeches during the accelerating growth phase, but both generations had similar growth rates during the decelerating growth phase. The difference in growth rates between the two generations during the accelerating phase is likely adaptive to the different environmental conditions experienced by the leeches in each generation. Late summer generation leeches encounter winter about two months earlier and at a smaller size than the early summer generation. Based on laboratory data, late summer generation N. obscura first encounter winter at a mean size of 116.3 mg, while the early summer generation N. obscura first encounter winter at a mean size of 430.7 mg. Davies *et al.*(1987) demonstrated that N. obscura subjected to winter conditions (5°C with anoxia) show intra-specific size differences in survivorship. Small individuals (10-15 mg) had much higher mortality than medium size individuals (50-140 mg), and large size individuals (150-250 mg) had the lowest mortality. Three primary components contribute to fitness: survival, fecundity and generation time. To maximize fitness, survival and fecundity are maximized and generation time minimized (Sibly and Calow 1986). Since body size is positively related to survival and fecundity a faster growth rate resulting in a larger body size before winter

enhances the probability of survival and thus the fitness of the late summer generation N. obscura. However, why do early summer generation N. obscura not grow as fast as the late summer generation? This appears to be contrary to the principle of allocation, which assumes that an organism should operate at maximal rates and efficiencies of resource utilization (Rollo 1986). If there were no fitness cost involved, selection would maximize growth rates within these constraints because this would minimize development time and increase survival (Sibly and Calow 1986). Rollo (1986) proposed that organisms do not always operate at maximum rates, but rather are tuned to the mean and variance of resource supply. Organisms with submaximal rates have greater compensatory scope and therefore have a better chance to survive in an unpredictable and / or variable environment than organisms with maximum rates. Comparison of the growth rates between the two generations of N. obscura during the accelerating growth phase shows that early summer generation leeches operate at a submaximal rate.

Predation rates on N. obscura are inversely related to size (Cywinska and Davies 1989) with 4-5 mg individuals consumed by eight predatory species. As body size increases, the number of predatory species decreases so that N. obscura > 30 mg are consumed by only three species. Comparison of the total predation mortality rates suggests a negative relationship between mortality and size of N. obscura, with the highest mortality 446.6 leech $\cdot$ week^{-1} on 4-5 mg N. obscura, 275 leech $\cdot$ week^{-1} on 6-10 mg N. obscura , 70.3 leech $\cdot$ week^{-1} on 30-50 mg N. obscura and only 44.1 leech $\cdot$ week^{-1} on 100-150 mg N. obscura. The faster growth of the late summer

generation N. obscura resulting in a large overwintering size would also thus reduce predation mortality.

Why do late summer generation N. obscura not increase their feeding rate instead of increasing absorption efficiency to have a faster growth rates? Gut capacity probably constrains food intake and thus to increase available energy they increase food absorption efficiency. Facing competition for food in the fall (Davies *et al.* 1981) processing less food with higher efficiency would appear to be a better strategy. The possible mechanisms for late summer N. obscura increasing energy absorption efficiency are probably increased gut retention time and/or increased enzyme secretion.

2.5.2 Lipid Storage

Lipid plays an important energetic role in the life histories of aquatic invertebrates (Giese 1966; Hadley 1985). Because lipid represents up to twice the potential metabolic energy per unit weight, compared to proteins or carbohydrates, lipid reserves offer the most efficient means of storing energy to support metabolic needs during low food availability or to meet reproductive needs (Quigley et al. 1989; Norrbin et al. 1990). N. obscura which have overwintered initially primarily allocate energy surplus to lipid deposition rather than maximizing the somatic growth (Dratnal and Davies 1990). Dratnal and Davies (1990) concluded that winter stresses tune energy allocation to lipid storage which enhances survivorship in an unpredictable environment.

Late summer generation N. obscura hatchlings have a significantly higher lipid content (14.6 $\pm$ 0.2%) compared to early summer generation hatchlings (8.3 $\pm$ 0.5%). The tactics by which animals apportion their energy to growth, reproduction, storage and activity at different life history stages directly affects ecological success and fitness (Calow 1977). An increased proportion of assimilated food is allocated to reserves rather than to immediate use as a means of ameliorating temporal mismatches between food supply and energy demand (Larson 1991). High lipid content is essential for the late summer generation leeches because they encounter winter condition sooner and at a smaller size than early summer generation leeches. Reddy *et al.* (1992) demonstrated that in the laboratory small N. obscura, after experiencing

winter conditions (5°C, 10% oxygen saturation) start to utilize lipid as energy reserves after 20 d. Attaining a large size as rapidly as possible is usually considered to increase fitness (Sibly and Calow 1986) by improving survivorship of juveniles. This might explain why early summer generation hatchlings have a relatively low lipid concentration and do not increase their lipid accumulations rapidly. With limited energy resources, trade-offs must occur between competing physiological demands. Allocation of energy primarily to somatic structure and / or activity rather than to energy storage increases the size at maturity, which, in turn, increases the probability of survival and fecundity. There are also additional costs to energy storage (Pond 1981): space must be found for it within the organism's body and it must be supported and / or carried around. This requires additional energy expenditure, biochemical machinery and structural tissue. Late summer generation leeches presumably offset those costs through enhanced winter survivorship.

Lipid storage decreased when N. obscura of both generations reached the size just prior to maturation (610.7 $\pm$ 8.5 mg for early summer and 701.5 $\pm$ 22.1 mg for late summer generation leeches) suggesting that lipid is used for the final stages of gonad maturation and / or clitellum production. The similarity of the lipid levels (15.1 $\pm$ 0.5% for early summer and 15.5 $\pm$ 0.5% for late summer generation leeches) suggest that before full sexual maturation can be attained this threshold lipid level must be reached to initiate clitellum production and / or cocoon production.

2.5.3 The Sebens' Model

In Sebens' energetic model, the definition for W_{OPT} is the size at which an organism terminates growth with all energy allocated to gonad production. Calow (1981) suggested that the switch from growth to reproduction in animals may occur gradually and not sharply as in the case for plants (Cohen 1971; Amir 1979) where the diversion of resources from soma to reproduction causes a reduction in the growth of photosynthetic tissue (Bazzaz *et al.* 1979) and hance in resource input per unit weight of tissue, the optimal theoretical solution does seem to be a sharp switch (Cohen 1971). N. obscura continues somatic growth after reaching W_{OPT} and proportionally allocates energy to both somatic and reproductive growth (Figs. 2.1 and 2.2). Thus, Sebens' model is useful to estimate the size at reproduction and maximum body size for a variety of species his narrow definition of W_{OPT} restricts the applicability of the model. If W_{OPT} is redefined as the size at energy shifting stage, the model can be applied to both organisms that have a sharp switch from growth to reproduction and to those organisms that allocate proportionally more energy to reproductive growth when W_{OPT} is attained.

Using the modified Sebens' model for N. obscura there is a good match between the observed data and the model's predictions. The inflection point in the growth curve occurs when the instantaneous rate change of energy is maximized and therefore represents the maximum growth rate. Since W_{OPT} is the size at which maximum energy is available (for both somatic and reproductive growth), it is not surprising that it coincides with the inflection point. In N. obscura energy absorption

(E) is not the total energy absorption but excludes the energy in mucus, which for practical purpose cannot be separated from faeces produced. The model's predictions would be underestimated if N. obscura produced large amounts of mucus with high calorific value as in other invertebrates (Calow 1974; Richman, Loya and Slobodkin 1975). A good match between the model's predictions and observations indicate that N. obscura does not produce a large amount of mucus under the experimental conditions. At the growth inflection point both early summer and late summer generation N. obscura show a morphological narrowing at the anterior of the body. Davies and Singhal (1988) showed that N. obscura reaches sexual maturity (both testisacs and ovisacs with fully developed gametes) at a size of about 250 mg, while at a size of 126 to 150 mg, coinciding with the inflection point in the growth curve, N. obscura initiates the production of male and female gametes. The morphological narrowing can thus be used as an index for the inflection point and the energy shifting stage.

The energy balance model can also be used to gain insight into some of the effects of resource availability and energy expenditure under different environmental conditions. Comparison between the early summer generation of N. obscura used in this study and the early summer generation used by Dratnal and Davies (1990) shows that the only difference in experimental condition was the presence or absence of mud as a substratum. This difference resulted in significant differences in the size at sexual maturity (Fig. 2.8). Leeches maintained in a mud substratum either used more energy to capture the same amount of prey, or alternatively used the same amount of energy

but captured less prey than leeches in containers without mud. Given either scenario, the effects are shown in Fig. 2.9. Comparison between the two groups of leeches shows the inflection point and size at sexual maturity for leeches with a mud substrate are both moved to the left of Fig. 2.9. This indicates that leeches using more energy or capturing less food will be of a smaller size at the inflection point and at sexual maturity. Based on the feeding rate data, the food intake is similar for both groups ($0.016\dot{3} \pm 0.0013$ mg WWT T. tubifex $\cdot d^{-1} \cdot$ mg WWT N. obscura^{-1} for the group with a mud substrate (Dratnal and Davies 1990) and 0.0177 ± 0.0012 mg WWT T. tubifex $\cdot d^{-1} \cdot$ mg WWT N. obscura^{-1} for the group without a mud substrate). Therefore leeches with mud must spend more energy to capture a similar amount of prey compared to leeches without mud.

2.5.4 The Phenotypic Hypothesis

The early summer and late summer generation N. obscura differ in terms of their bioenergetics and life-history traits. There are two possible reasons for these differences. The two generations are either genetically different or both show phenotypic plasticity. Davies and Everett (1977) demonstrated (Fig. 1.1) that the parents of the early summer generation N. obscura hatch in either the early summer or the late summer of the previous year. Since there are no obvious spatial isolation mechanism between the early summer and late summer generation N. obscura in Stephenson's Pond genetic differences between the two generations appears unlikely. An individual's prehistory can influence its ecophysiology and Stearns (1980) showed that regardless of the origin of the parents, mosquito fish (Gambusia affinis Linn.) that spend the first week of their life in fresh water produce offspring 8-15% heavier than the offspring of females that had spent the first week in brackish water. Kelsch and Neill (1990) demonstrated that bluegills (Lepomis macrochirus Linn.) have different preferred temperatures after being subjected to different acclimation temperatures. Similarly, Dratnal and Davies (1990) showed that N. obscura with different prehistories differed ecophysiologically. Since both the early summer and late summer generation N. obscura studied in these experiments were hatched from cocoons in the laboratory, and the offspring maintained under identical conditions, the observed differences in ecophysiology and life history traits can only be accounted for by differences in the prehistory of their parents, or by genetic differences. There is

evidence to support the argument that differences in prehistory can change the reproductive output which may consequently alter the ecophysiology of the offspring. Lack (1954) hypothesized that for organisms which produce gametes from limited resources, there is a trade-off between fecundity (n) and survival of offspring (S_j). With more offspring produced, their size will decrease as well their survival (z). Kolding and Fenchel (1981) established a graphic model assuming that poorer trophic conditions lead to a reduction in survival for eggs of a particular size, because they contain less energy resources (Fig. 2.10). Maximum $S_j n$ then shifts to the right of Fig. 2.10, and the predicted optimum egg size increases. Hence, bigger but fewer eggs should be produced in poor trophic conditions. In support, Kolding and Fenchel (1981) found that winter breeding amphipods had fewer and larger eggs than summer breeding amphipods. Skdsheim (1984) reported that individual female amphipods produced small broods of large eggs in winter, and larger broods of smaller eggs in spring with a reverse trend observed from summer to fall. These changes were coupled with increased survival probability of newly hatched juveniles in spring and summer. Kusano (1988) showed that female amphipods had nearly constant clutch volume during the reproductive season, but produced a large clutch of small eggs in mid-winter and a small clutch of large eggs in late spring. Linton and Davies (1987) demonstrated that for N. obscura egg number was positively correlated with temperature while the energy content of eggs was negatively correlated with temperature. During late summer the environmental conditions for N. obscura in Stephenson's Pond are poorer than in early summer with respect to lower, decreasing

water temperatures and dissolved oxygen, as well as the occurrence of severe inter- and intra-specific competition for food (Davies *et al.* 1981; 1984; Gates 1984; Wrona and Davies 1987; Linton and Davies 1987). Thus, in late summer bigger, or higher energy content eggs might be expected to be produced by N. obscura. In contrast, early summer breeding N. obscura might be expected to produce more, but smaller or lower energy content eggs, because there are no food shortages and temperature and dissolved oxygen concentrations are higher and increasing. In this situation hatchlings from the smaller or lower energy content eggs will, however, still have a good probability of surviving to maturity. Difference in quality between the eggs produced by the two generations of N. obscura could induce different ecophysiology, growth and life-history traits. In addition, different prehistories of the parents may also tune the physiological mechanism of the offspring with the same genes expressing different phenotypes.

In the field, differences between generations may be even larger because of the larger differences in environment experienced by the leeches of different generations.

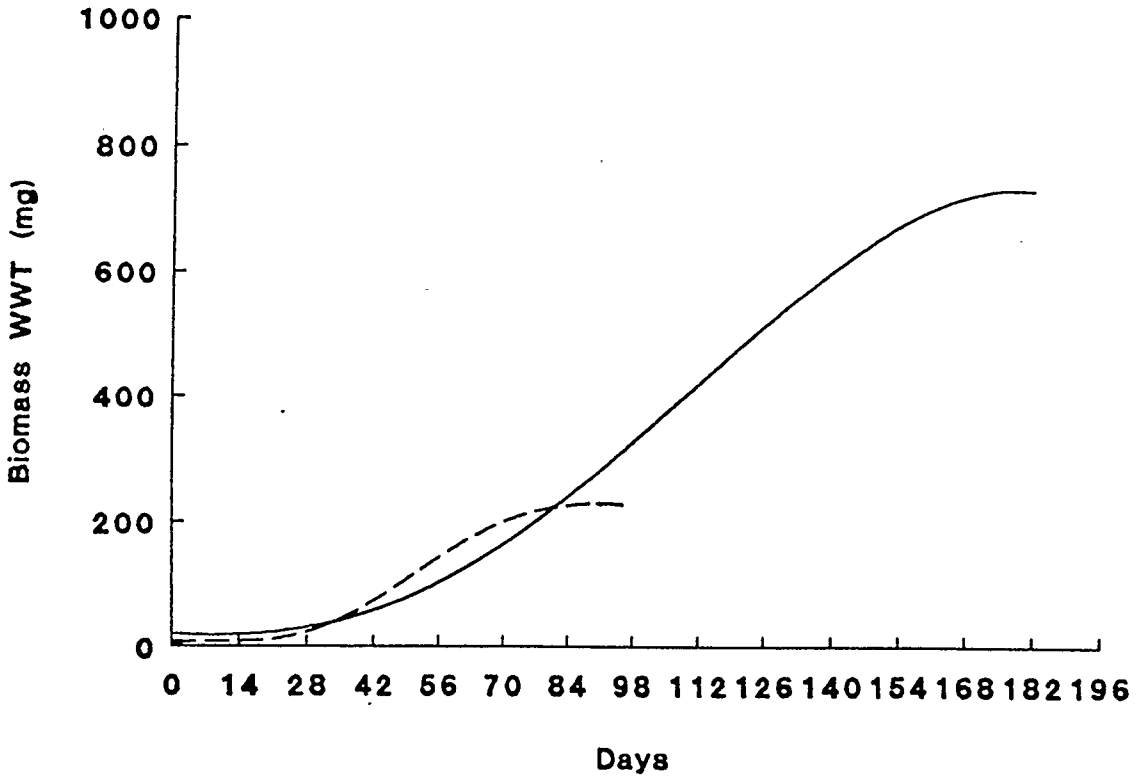


Figure 2.8 The growth patterns of late summer Nephelopsis obscura which fed on ad libitum Tubifex tubifex either with (- - - - -) or without (———) mud substrate

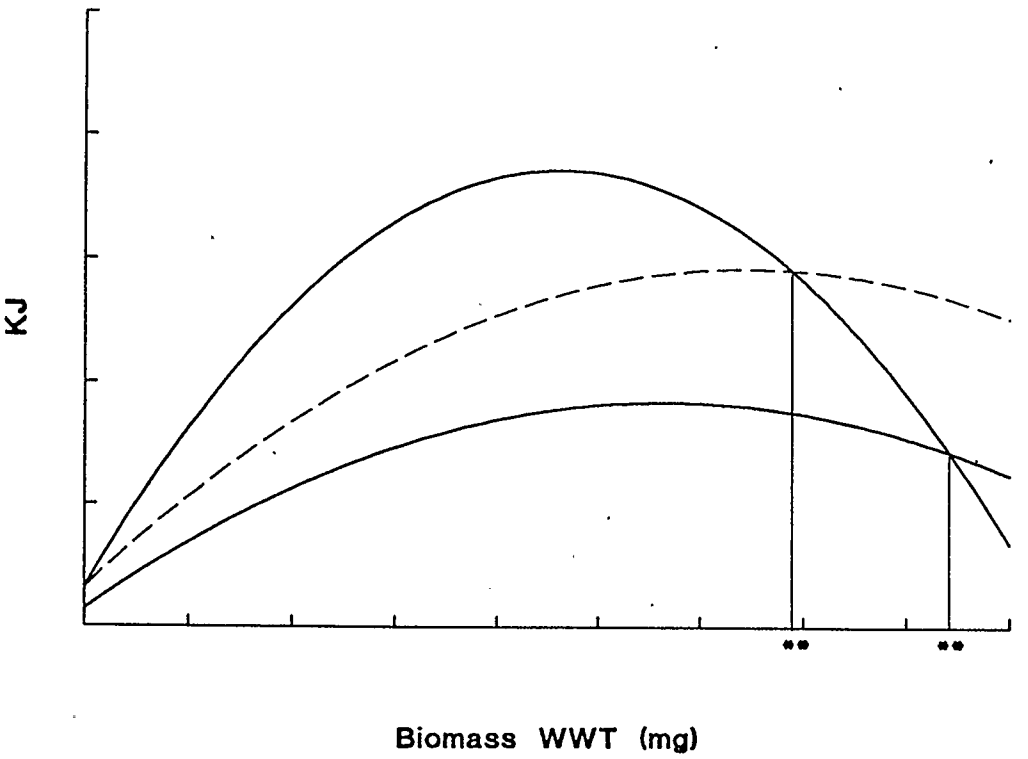


Figure 2.9 Effect of the presence (- - - - -) or absence (———) of a mud substrate on the size at maturity of <u>Nephelopsis obscura</u>

**** size at maturity**

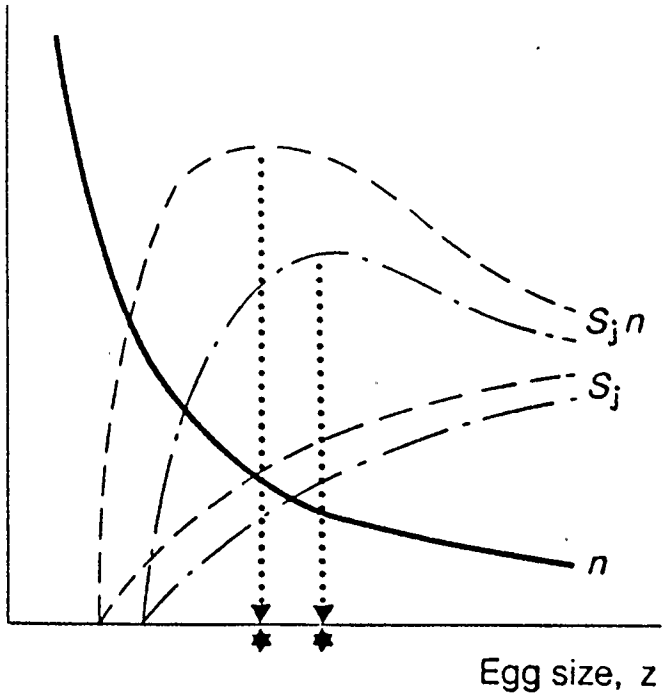


Figure 2.10 Effect of egg size on fecundity and juvenile survival. Fitness is proportional to $S_j n$. The star shows the optimum size. If S_j is reduced (as might happen under poor trophic conditions) natural selection should favour bigger eggs. Redrawn from Kolding & Fenchel (1981).

2.6 Conclusion

A comparison of early summer and late summer generations N. obscura shows that certain ecophysiological and life history traits differ between generations. This is probably due to seasonal differences in environmental factors which directly affect the physiological processes of the parents of these two generations which subsequently results in ecophysiological differences between the two generations.

Although the growth patterns of both generations are similar, with a sigmoid growth curve, late summer N. obscura grow faster during the accelerating growth phase resulting in a larger size at the inflection point, the size of which N. obscura starts shunting larger amount of energy to reproduction, and a larger maximum body size.

Both generations of N. obscura consumed similar amount of food per unit dry weight and allocated similar amount energy to metabolism. The late summer generation N. obscura, however, had higher energy absorption because of lower energy losses in faeces plus mucus during the early life stages and therefore had more energy to allocate to somatic growth compared to the early summer generation. This accounts for the observed faster growth rate of late summer generation N. obscura during their early life history.

Energy storage patterns are also different between the two generations. The late summer generation N. obscura had much higher lipid content at hatching, and

maintained this level until just before maturity. In comparison, the early summer generation N. obscura hatched with a lower lipid content but gradually increased the lipid content until they reached the same level as the late summer generation just prior to maturation.

Differences between generations of N. obscura might be adaptive to differences in the environments experienced by each generation. Considering size specific mortality (Davies *et al.* 1988), cost of fast growth (Sibly and Calow 1986) and food availability in the field (Davies *et al.* 1981; Davies *et al.* 1984), the faster growth rate of juvenile late summer N. obscura and the comparatively slow growth rate of early summer N. obscura are both strategies which maximize the number of individuals surviving to maturity and reproduction.

The good match between the observed data and the predictions of the modified Sebens' models support the validity of the energetics model.

3.0 GENETIC EXAMINATION

3.1 Introduction

With increasing evidence (Davies and Everett 1977; Peterson 1983; Baird, Linton and Davies 1986; Baird, Linton and Davies 1987; Linton and Davies 1987; Dratnal and Davies 1990) of variability in the life histories both among and within populations of N. obscura, the question remains whether or not this variability is entirely due to phenotypic plasticity or is, at least in some part, due to genetic differences. Baird, Linton and Davies (1986) investigated post-reproductive mortality and showed that under different environmental conditions, N. obscura from a single population showed a range of post-reproductive mortality patterns. Their results supported the phenotypic plasticity hypothesis rather than genotypic variability which would be expected to give a narrow range of responses.

Using energy allocation to reproduction as a criterion distinguishing iteroparity from semelparity, Baird *et al.*(1987) provided additional evidence to support the phenotypic plasticity hypothesis. They concluded that the observed temperature-related shifts of energy allocation to reproduction by N. obscura could be explained in terms of the relative mortality risks associated with breeding at different temperatures resulting in a flexible life-history strategy.

Linton and Davies (1987) developed an energetics model of the growth and life history of N. obscura. Although constructed using data from the Stephenson's

Pond population, presumed to be toward one extreme of the observed spectrum of life history traits and growth patterns, the model accurately simulated the growth of N. obscura throughout its geographic range. Thus, Linton and Davies (1987) concluded that the variability in size and life-history of N. obscura observed in the field could be explained as the result of a plastic phenotype responding to different environmental conditions.

Further support for the phenotypic plasticity hypothesis came from the study of Dratnal and Davies (1990) who showed that the prehistory of individuals played an important role in changing energy allocation and life-history traits. Overwintered N. obscura differed significantly from summer N. obscura which had not overwintered with respect to food intake, energy storage, size at maturity and age at maturity. Dratnal and Davies (1990) concluded that the observed differences between winter and summer N. obscura were indirect life history adaptations to the stresses experienced by winter N. obscura prior to collection. They found no evidence to suggest genetic differences between winter and summer N. obscura. However, direct genetic studies have not been conducted on different generations or populations of N. obscura, and thus the possibility exists that some of the intraspecific differences observed in N. obscura are genetically determined.

Leary *et al.* (1989) reported that detectable genetic differences in rainbow trout (Oncorhynchus mykiss Linn.) are associated with differences in spawning time, with the progeny from the earliest and latest spawning dates having lower levels of heterozygosity than progeny from the middle spawning dates. Kautsky *et al.* (1990)

showed that in blue mussel (Mytilus edulis Linn.) variation in growth rates and maximum sizes over its geographic distribution can largely be explained by physiological differences due to environmental conditions. However, some differences in morphology and fecundity were partly genetically determined.

The objective of this study was to determine if there were genetic differences within and between populations of N. obscura utilizing an electrophoretic examination of protein (isozymes).

There are several alternative approaches to determine if genetic differences occur between two populations. Reciprocal transplantations is one commonly used method to determine if ecophysiological differences between populations from different geographic localities are due to phenotypical plasticity or to genetic differences (Kautsky *et al.* 1990). The advantage of using this method is that it is direct and easy to distinguish the environmentally induced differences from inherited differences if the differences between populations disappear when one population is transplanted to the ecosystem naturally occupied by the other population. This method, however, is time consuming if the life cycle of the test organism is relative long. This method also ignores the effect of the prehistory of the organisms on their growth and / or life history traits. Because the transplanted population differs from the local population in terms of ecophysiology, differences can be either due to genetic difference between the populations or to different prehistories of the populations (Sterns 1980; Dratnal and Davies 1990; Kelsch and Neill 1990).

To determine if variations in life-history parameters among three neighbouring

populations of the freshwater pulmonate, Lymnaea peregra (Müller) could be ascribed to differences in environmental factors or to a genetic basis, Lam and Calow (1989) conducted a series of controlled-breeding experiments and genetically or environmentally induced differences were easily distinguished. This method may reduce the effect of prehistory as the hatchlings of first generation (F_1) and second generation (F_2) were compared in terms of life history traits. If the variations observed in the F_1 generation disappear in the F_2 generation, this variations could potentially be the remnants of the phenotypic differences exhibited by the parent under field conditions. This method is, therefore, superior to the reciprocal transplantations method. However, results cannot be obtained until the completion of the life cycle of the F_2 generation and thus the duration of Lam and Calow (1989) type experiment are even longer than reciprocal transplantation experiments.

Originating in the 1930's (Tiselius 1937), electrophoresis, coupled with the zymogram technique (Hunter and Markert 1957), has been the tool of choice for studies of heritable variation by geneticists, systematists, and population biologists (Gottlieb 1977; Brown 1979; Hamrick *et al.* 1979; Ellstrrand 1984; Crawford 1985). In comparison with other methods, electrophoretic isozymes analysis is widely used for its relative efficiency and cost effectiveness, particularly in studies of intraspecific variability (Kephart 1990). Once the method is standardized, results can be obtained rapidly. The capacity to visualize enzymes directly is another advantage in using this approach which allows the detection of multiple gene products catalyzing the same reaction. It also allows the detection of genetic variation irrespective of the effects of

mutations on quantitative activity. In addition, the commercial availability of high quality substrates allows many of the isozyme methods considerable specificity. There are, however, several disadvantages to this method. With thousands of enzymes it is almost impossible to screen all enzymes present in a species.If enzymes showing differences between populations are not screened it might incorrectly be concluded that there is no genetic difference between the populations. Even if genetic differences are found, it cannot be said that the differences between populations in life history traits are genetically determined until correlations between genotype and life history traits are established.

A variety of methods can be used for the separation of isozymes and / or allozymes, including: polyacrylamide gel electrophoresis, agarose gel electrophoresis, paper electrophoresis, thin-layer electrophoresis, cellulose acetate electrophoresis, affinity electrophoresis and starch gel electrophoresis (Andrews 1990). In comparison with other methods, the advantages of using starch gel electrophoresis are its cost effectiveness, its simplicity and the degree of resolution which is at present time exceeded only by polyaceylamide gel electrophoresis (Andrews 1990). If the genetics of a species has not previously been studied the electrophoretic techniques must be standardized. Because standardization is time-consuming and costly, starch gel electrophoresis is usually recommended for preparative studies (Andrews.1990). The disadvantage of using starch gel electrophoresis is that quantitative measurement of enzymes by densitometry is difficult and rather inaccurate.

3.2 Materials and Methods

3.2.1 Sample Preparation

Specimens from three populations of N. obscura were sampled and tested electrophoretically. N. obscura cocoons from Stephenson's Pond were collected in both early summer and late summer and hatched in the laboratory at 20°C, with 100% oxygen saturation, and a 12:12 L:D photoperiod regime. The hatchlings were maintained under identical conditions and provided with ad libitum prey (Tubifex tubifex) three times a week. Individuals from the other two populations were collected as adults from the Rocky Mountain foothills of Alberta, and the from Utah, U. S. A., with a mean size of 550 mg and 1200 mg, respectively. These specimens were acclimated to the same laboratory conditions for one month. Leeches from all three sites were starved for 3 d, to ensure complete digestion of the last meal, prior to being prepared for electrophoresis. Leeches were individually homogenized in distilled water and preserved at -80°C.

3.2.2 Starch Gel Electrophoresis

Horizontal starch gel electrophoresis was used followed the procedures of Packer and Owen (1989). In general, the principle of electrophoretic separation of proteins depends upon passing an electric current through an electrophoretic media (starch), and the possession of varying electrical charge by the protein (isozymes) to be separated. To transmit electricity, the electrophoretic media must contain an ionized solution or buffer. Therefore, the electrophoretic apparatus consists of electrode buffer trays, a starch gel tray and a power supply (Figs. 3.1 and 3.2). Electrical current was supplied by a Model LKB 2197 Power Supply.

To prepare standard starch gel, 45 g hydrolysed potato starch powder was dissolved in 460 ml gel buffer in a 1 L Pyrex side-arm flask. While being mixed vigorously the flask was heated for about 5 min. and then a size 8 rubber stopper placed in the top of the flask, with the side arm connected to an aspirator to degas the solution. When bubbles stopped forming the starch solution was poured into the gel tray, maintained at room temperature (20°C) for 1 h and then refrigerated at 4°-5°C for approximately 2 h.

Up to thirty wicks (4 by 14 mm pieces of Whatman No. 3 filter paper) were impregnated with each sample of leech homogenate and placed in slices on the starch gels.

Standard horizontal electrophoresis was performed at 55 Ma at 5°C for 4-7 h, the duration varying with different enzymes. At completion of a run, the gels were

cut into three slices and each stained for a specific enzyme (Table 3.1). The buffer systems and staining techniques followed Packer and Owen (1989) (Tables 3.1, 3.2). A buffer system consists of a gel buffer used in preparing the gel and an electrode buffer, which is an ionized solution that conducts current through the gel during a run. Proteins, which are zwitterions, carry positive charges as a result of ionized amino groups and negative charges contributed by ionized carboxyl groups. Their net charge, and thus their migration in the electrical field of the gel, depends on the pH of the buffer system. Because different enzymes are ionized under different pH conditions, different buffer systems are chosen to produce the best separation of isozymes on the gel. In this study three different buffer systems were used (Table 3.2). The procedural steps of starch gel electrophoresis are summarised in Fig. 3.3.

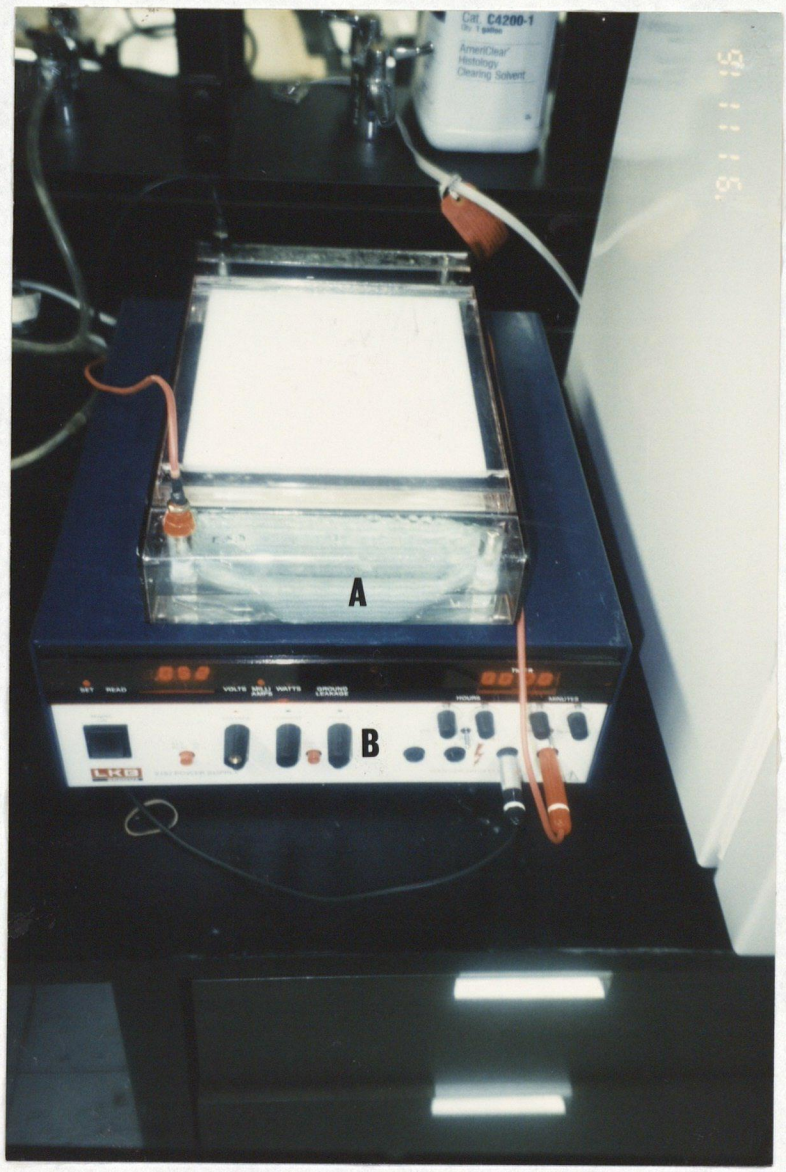


Figure 3.1 Electrophoresis Apparatus

A - Gel and electrode buffer trays

B - Power supply

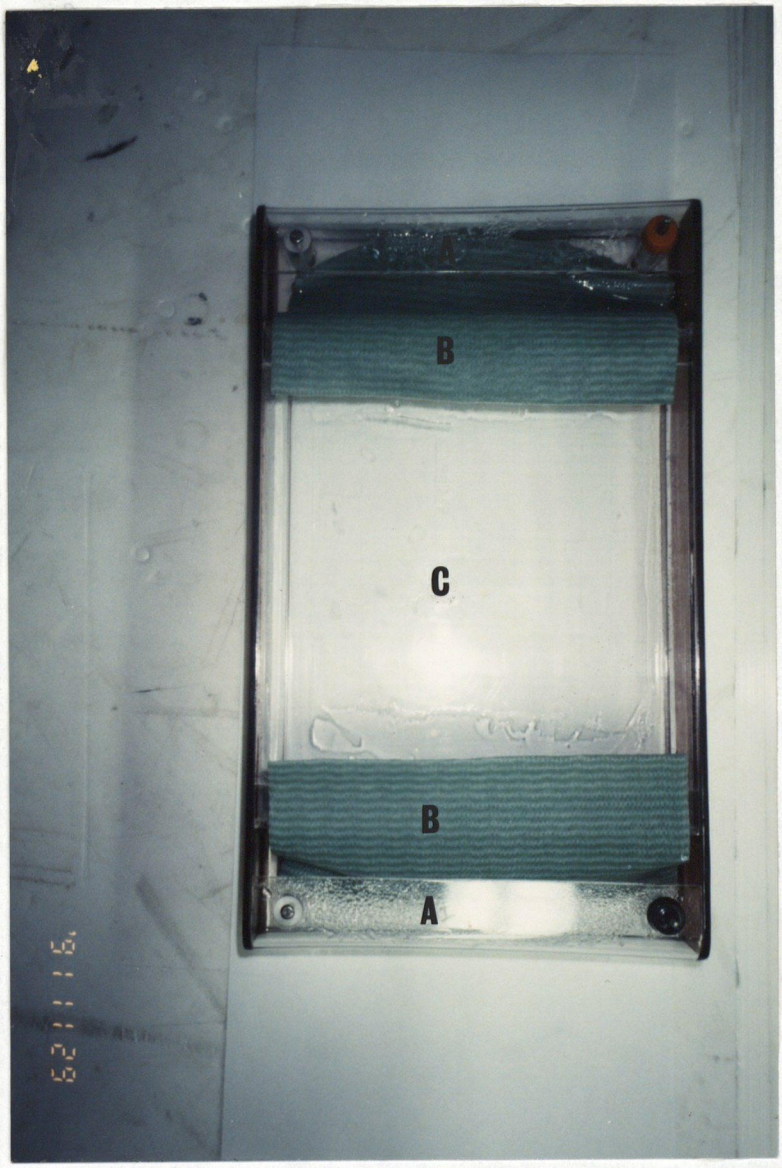


Figure 3.2 Schematic representation of the buffer and gel trays

A - Buffer trays B - Cloth wicks C - Gel tray

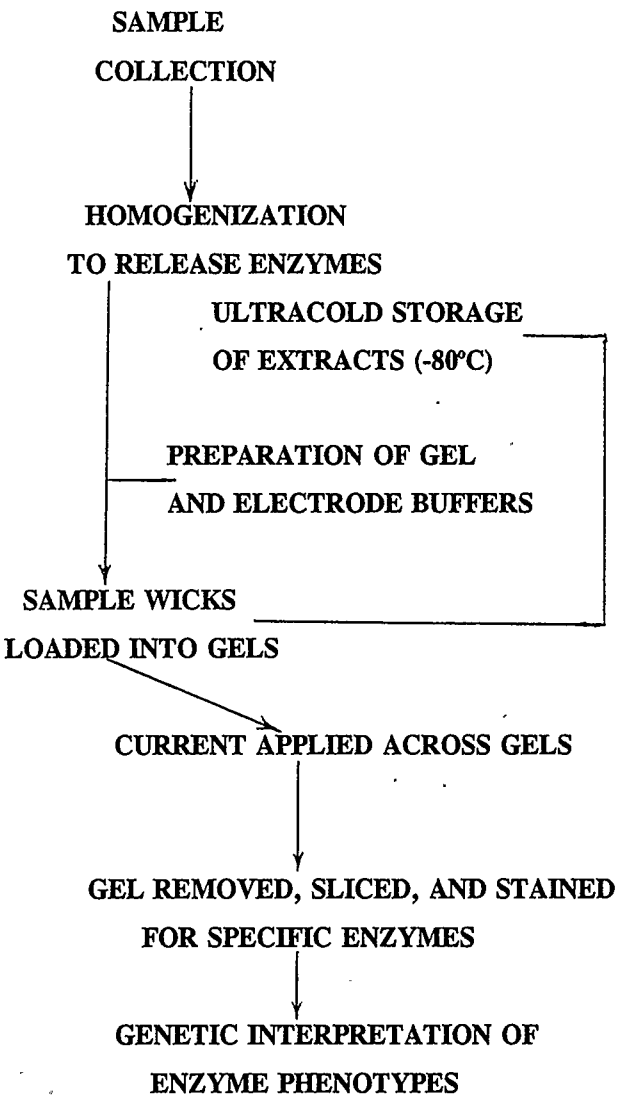


Figure 3.3 Flow chart of procedural steps for enzyme electrophoresis

TABLE 3.1

List of enzymes screened and stain recipes.

Gel buffer systems defined in Table 3.2

Enzyme	Gel buffer	Stain recipe
Aspartate aminotransferase (AAT)	I	40 mg L-asparrtic acid, 20 mg α-ketoglutaric acid, 0.2 mg Pyrodixal-5'-phosphate, 60 mg Fast Blue BB salt, 10 ml Tris (pH 8.0), 0.5 ml MTT, 50 μl PMS, 15 ml Agar
Aconitase (ACON)	I	1 ml Aconitic acid stock (0.07 g), 10 ml Tris (pH 8.0), 0.5 ml NADP, 50 μl IDH, 0.5 ml $MgCl_2$, 0.5 ml MTT, 50 μl PMS, 15 ml Agar
Adenylate Kinase (AK)	I	0.15 g Glucose, 0.08 g ADP, 10 ml Tris (pH 8.0), 0.5 ml NADP, 50 μl G6PDH, 2 spatulas Hexokinase, 0.5 ml $MgCl_2$, 0.5 ml MTT, 50 μl PMS, 15 ml Agar

Table 3.1. Cont.

Enzyme	Gel buffer	Stain recipe
Aldolase (ALD)	III	0.2 g Fructose 1,6 diphosphate, 10 ml Tris (pH 7.5), 0.5 ml Arsenic stock, 20 μl TPI, 0.5 ml NAD, 0.5 ml MTT, 50 μl PMS, 15 ml Agar
Diaphorase (DIA)	II	20 mg Menadione, 15 mg NADH, 10 ml Tris (pH 8.0), 0.5 ml MTT, 50 μl PMS, 15 ml Agar
Esterase (EST)	I	60 mg α-Napthyl acetate and 60 mg β-Napthyl acetate dissolve in 1 ml acetate, 10 ml Phosphate buffer (pH 6.0), 0.15 g Fast Blue BB dissolve in 15 ml H_2O
Fumarate hydratase (FUM)	I	0.1 mg Fumaric acid, 10 ml Tris (8.0), 0.5 ml NAD, 50 μl MDH, 0.5 ml MTT, 50 μl PMS, 15 ml Agar

Table 3.1. Cont.

Enzyme	Gel buffer	Stain recipe
Glycerol-3-phosphate dehydrogenase (G3PDH)	I	0.1 g α-glycerophosphate, 25 mg EDTA, 10 ml Tris (pH 8.5), 0.5 ml MTT, 50 μl PMS, 15 ml Agar
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	I	0.25 g Fructose 1,6 diphosphate, 50 μl Aldolase, 10 ml Tris (pH 8.0), 0.5 ml arsenic stock, 0.5 ml NAD, 0.5 ml MTT, 50 μl PMS, 15 ml Agar
Glucose-6-phosphate dehydrogenase (G6PDH)	I	0.15 g Glucose-6-phosphate, 10 ml Tris (pH 7.5), 0.5 ml $MgCl_2$, 0.5 ml NADP, 0.5 ml MTT, 50 μl PMS, 15 ml Agar
β-Hydroxybutyate dehydrogenase (HBDH)	I+NAD	0.15 g Hydroxy butyric acid, 10 ml Tris (pH 8.5), 0.5 ml NAD, 0.5 ml MTT, 50 μl PMS, 15 ml Agar

Table 3.1. Cont.

Enzyme	Gel buffer	Stain recipe
Hexokinase (HK)	I	0.15 g Glucose, 20 mg ATP, 10 ml Tris (pH 8.0), 0.5 ml $MgCl_2$, 0.5 ml NADP, 50 μl G6PDH, 0.5 ml MTT, 50 μl PMS, 15 ml Agar
Isocitrate dehydrogenase (IDH 1)	I	0.1 g Isocitric acid, 25 mg $MnCl_2$, 10 ml Tris (pH 8.0), 0.5 ml NAD, 0.5 ml MTT, 50 μl PMS, 15 ml Agar
Isocitrate dehydrogenase (IDH 2)	I	0.1 g Isocitric acid, 25 mg $MnCl_2$, 10 ml Tris (pH 8.0), 0.5 ml NADP, 0.5 ml MTT, 50 μl PMS, 15 ml Agar
Leucine aminopeptidase (LAP)	I	20 mg L-leucine β nepthylainde dissolve in 5 ml Formamide, 20 ml Phosphate buffer (pH 6.0), 20 mg Fast Black K salt
Lactate dehydrogenase (LDH)	I+NAD	2.5 ml Lactic acid concentrate, 10 ml Tris (pH 8.5), 0.5 ml NAD, 0.5 ml MTT, 50 μl PMS, 15 ml Agar

Table 3.1. Cont.

Enzyme	Gel buffer	Stain recipe
Malate dehydrogenase (MDH)	III	0.15 g Malic acid, 50 mg EDTA, 10 ml Tris (pH 9.0), 0.5 ml NAD, 0.5 ml MTT, 50 μl PMS, 15 ml Agar
Malic enzyme (ME)	I	0.125 g Malic acid, 10 ml Tris (pH 8.5), 0.5 ml $MgCl_2$, 0.5 ml NADP, 0.5 ml MTT, 50 μl PMS, 15 ml Agar
Octanol dehydrogenase (ODH)	I	1 ml 2-octanol/ethanol, 10 ml Tris (pH 8.0), 0.5 ml NAD, 0.5 ml MTT, 50 μl PMS, 15 ml Agar
6-phosphogluconate dehydrogenase (6PGD)	III	50 mg 6-phosphoglycerate, 10 ml Tris (pH 8.0), 0.5 ml NADP, 0.5 ml $MgCl_2$, 0.5 ml MTT, 50 μl PMS, 15 ml Agar
Phosphoglucose isomerase (PGI)	I	0.06 g 3-phosphoglycerate, 0.05 g ATP, 0.02 g NADH, 10 ml Tris (pH 8.0), 0.5 ml $MgCl_2$, 50 μl G3PDH, 15 ml Agar

Table 3.1. Cont.

Enzyme	Gel buffer	Stain recipe
Phosphoglucomutase (PGM)	I	0.15 g Glucose-1-phosphate, 10 ml Tris (pH 8.5), 0.5 ml NADP, 0.5 ml $MgCl_2$, 50 μl G6PDH, 0.5 ml MTT, 50 μl PMS, 15 ml Agar
Pyruvate kinase (PK)	I	0.15 g Glucose, 15 mg ADP, 15 mg PPEP, 15 mg Fructose 1,6 diphosphate, 10 ml Tris (pH 8.5), 0.5 ml NADP, 0.5 ml $MgCl_2$, 0.5 ml KCl, 0.5 μl G6PDH, 0.5 ml MTT, 50 μl PMS, 15 ml Agar
Sorbitol dehydrogenase (SDH)	I	0.05 g sorbitol, 10 ml Tris (pH 8.0), 0.5 ml NAD, 0.5 ml MTT, 50 μl PMS, 15 ml Agar
Superoxide dismutase (SOD)		0.5 ml MTT, 50 μl PMS, 15 ml Agar

Table 3.1. Cont.

Enzyme	Gel buffer	Stain recipe
Succinate dehydrogenase (SUCDH)	I	2.5 mg EDTA, 0.125 g Succinate, 8 mg ATP, 2.5 mg Pyruvate, 10 ml Phosphate buffer (pH 7.0), 0.5 ml NAD, 0.5 ml MTT, 50 μl PMS, 15 ml Agar
Triose phosphate isomerase (TPI)	I	1 mg EDTA, 15 drops TPI substrate, 10 ml Tris (pH 8.0), 0.5 ml arsenic stock, 0.5 ml NAD, 0.5 ml MTT, 50 μl PMS, 15 ml Agar
Xanthine dehydrogenase (XDH)	I+NAD	0.04 g Hypoxanthine, 10 ml Tris (pH 7.5), 0.5 ml NAD, 0.5 ml KCl, 0.5 ml MTT, 50 μl PMS, 15 ml Agar

TABLE 3.2

Buffer systems used. Analytical grade reagents per litre; pH at 20°C

Buffer system	Electrode	Gel
I	18.8 g Tris, 8.3 g Citric acid, pH 8.5	Dilute 66.7 ml electrode buffer to 1 L distilled water,pH 8.5
II	83.2 g Tris, 30.2 g Citric acid, pH 8.0	Dilute 100 ml electrode buffer to 3 L distilled water, pH 8.0
III	7.7 g Tris, 10 ml N-3-asminopropylmorpholine, pH 6.1	Dilute 100 ml electrode buffer to 1 L distilled water, pH 6.1

3.3 Results

3.3.1 Comparison amongst Populations

Of the twenty-eight enzymes screened twelve showed no activity (no band on the gels), eight showed poor resolution (bands on the gel not clear enough to be photographically recorded) and eight showed good resolution (bands clearly recorded photographically). The active enzymes as well as the number of loci for each enzyme are given in Table 3.3.

In general, all three populations showed very low levels of genetic variation with fifteen of sixteen active enzymes are monomorphic. Fixed genetic differences do, however, occur amongst populations with four enzymes showing differences in mobilities between or amongst populations (Figs. 3.4, 3.5, 3.6 and 3.7). Hexokinase (HK) is represented by a single invariant band for each population; the mobility, however, differs amongst populations, with the Stephenson's Pond population (SP) having a faster band compared to the populations from the Rocky mountain foothills (RMP) and Utah, (UTAH) (Fig. 3.4). Diaphorase (DIA) is represented by a single band with the same mobility in the RMP and UTAH populations but by two invariant bands in the SP population (Fig. 3.5). Pyruvate kinase (PK) is represented by three bands in all three populations; however, the fastest band shows different mobilities amongst populations with the SP population having a faster band than the RMP and UTAH populations (Fig. 3.6). Phosphoglucomutase (PGM) is represented by three

invariant bands for the SP and RMP populations, but also differs in mobility; the UTAH population, in comparison, shows two bands (Fig. 3.7).

Malate dehydrogenase (MDH) showed high resolution on gels (Fig. 3.8), however, this enzyme is very sensitive to temperature. Different electrophoretic patterns appeared when fresh and frozen samples were used. Presumably due to protein degradation, the pattern from frozen samples is irregular and impossible to interpret. Thus frozen samples were not used to screen MDH. Since all samples of RMP and UTAH populations were frozen, comparison amongst populations for MDH were not possible.

TABLE 3.3

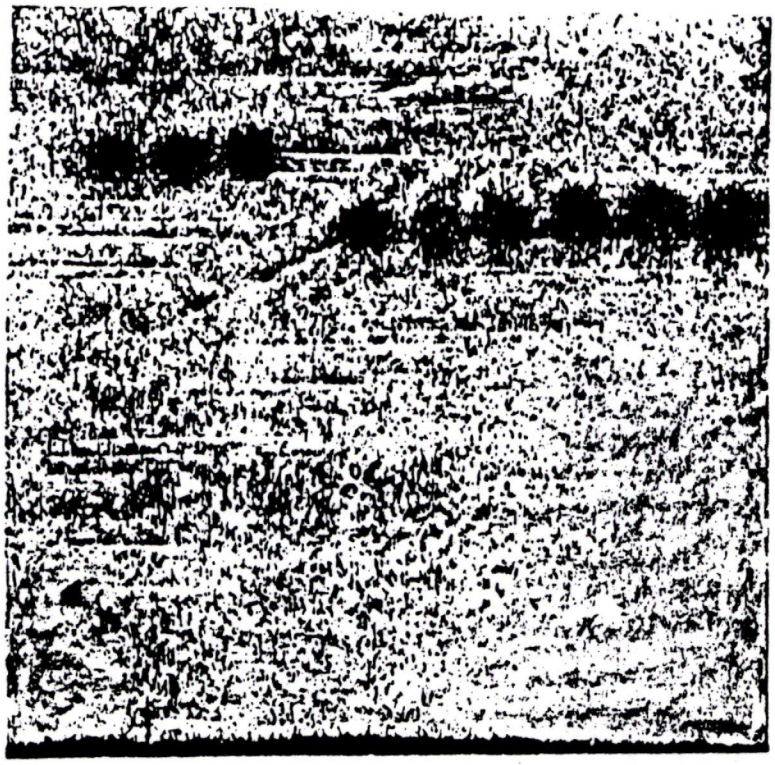
List of active enzymes (abbreviation given in Table 3.1)

P - poor resolution; G - good resolution

Enzyme	Population	No. of loci	Resolution
ALD	SP	1	P
	RMP	1	P
	UTAH	1	P
AK	SP	1	G
	RMP	1	G
	UTAH	1	G
HK	SP	1	G
	RMP	1	G
	UTAH	1	G
DIA	SP	2	G
	RMP	1	G
	UTAH	1	G
PGM	SP	3	G
	RMP	3	G
	UTAH	2	G
6PGD	SP	1	G
	RMP	1	G
	UTAH	1	G
LAP	SP	1	P
	RMP	1	P
	UTAH	1	P
PK	SP	3	G
	RMP	3	G
	UTAH	3	G

Table 3.3. Cont.

Enzyme	Population	No. of loci	Resolution
GPI	SP	1	P
	RMP	1	P
	UTAH	1	P
EST	SP	1	P
	RMP	1	P
	UTAH	1	P
TPI	SP	1	P
	RMP	1	P
	UTAH	1	P
ME	SP	1	P
	RMP	1	P
	UTAH	1	P
G6PDH	SP	1	P
	RMP	1	P
	UTAH	1	P
HBDH	SP	1	P
	RMP	1	P
	UTAH	1	P
SOD	SP	1	G
	RMP	1	G
	UTAH	1	G
MDH	SP	Polymorphic	G
	RMP	-	-
	UTAH	-	-



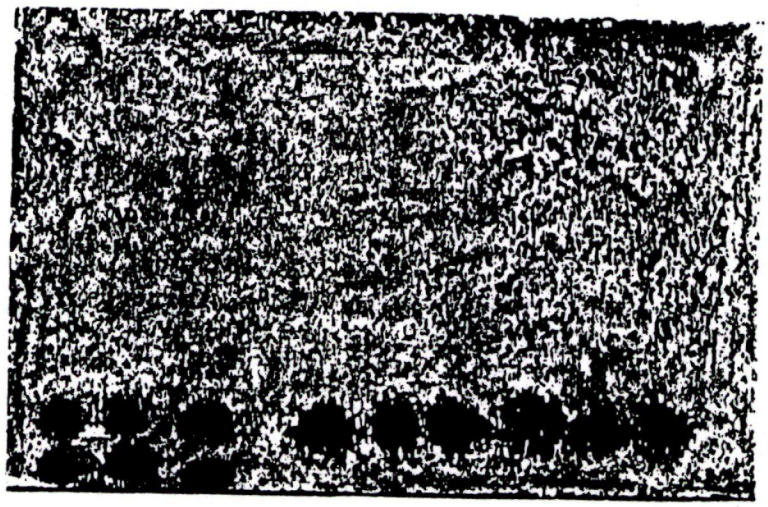
SP RMP UTAH

Figure 3.4 Electrophoretic pattern of HK

Each point represents one individual leech.

SP=Stephenson's Pond; RMP=Rocky mountain pond;

UTAH=Utah pond



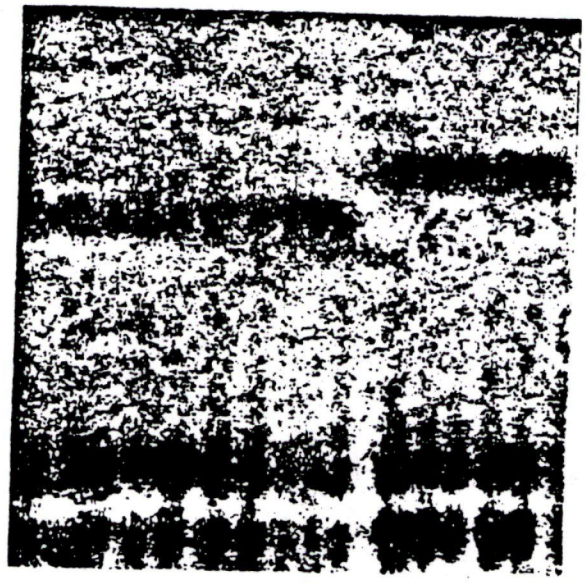
SP RMP UTAH

Figure 3.5 Electrophoretic pattern of DIA

Each point represents one individual leech.

SP=Stephenson's Pond; RMP=Rocky mountain pond;

UTAH=Utah pond



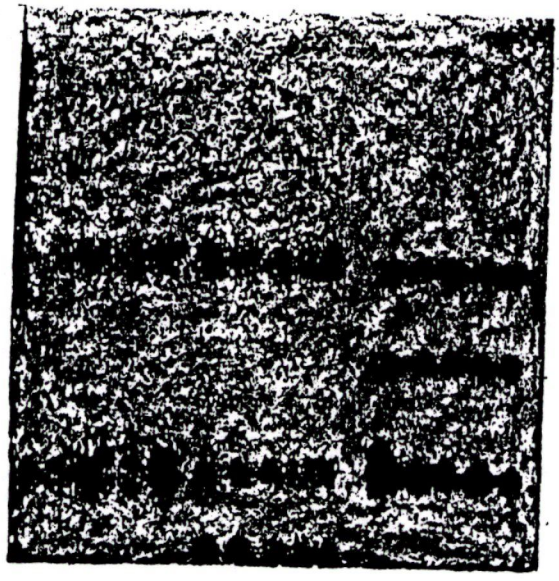
UTAH RMP SP

Figure 3.6 Electrophoretic pattern of PK

Each point represents one individual leech.

SP=Stephenson's Pond; RMP=Rocky mountain pond;

UTAH=Utah pond



UTAH RMP SP

Figure 3.7 Electrophoretic pattern of PGM

Each point represents one individual leech.

SP=Stephenson's Pond; RMP=Rocky mountain pond;

UTAH=Utah pond

3.3.2 Comparison between Early and Late Summer Generation *Nephelopsis obscura* from Stephenson's Pond

From the electrophoretic analysis of the two generations of N. obscura from Stephenson's Pond, the only enzyme showing polymorphism with high resolution (Fig. 3.8) was malate dehydrogenase (MDH). Three patterns were shown on the gels for both early summer and late summer N. obscura. Some individuals showed a fast single band, some a slow single band, and others showed three bands. These electrophoretic patterns are typical of genes coding enzymes made of two polypeptide chains (Pasteur *et al.* 1988). Each band contains molecules consisting of two polypeptide chains that bind at random. In homozygous individuals, the two peptide chains are identical and the gels show a single band. In heterozygous individuals, the random binding of the two kinds of peptide chains produces three kinds of molecules and individuals show three bands. If A and B refer to the alleles coding the two peptide chains, the genotype of individuals showing a fast single band is represented as AA; individuals showing a slow single band as BB; and individuals showing three bands as AB. The genotypic frequencies of the MDH locus in early summer and late summer N. obscura are given in Table 3.4. Contingency table chi-square analysis shows a significant difference between the two generations in terms of the genotypic frequency of MDH. There are more heterozygotes in the late summer generation N. obscura than in the early summer generation. Homozygous individuals with a single

fast band (AA) are dominant in early summer generation leeches. In both generations the frequency of homozygotes with a slow single band (BB) is very low (0.1 for early summer leeches and 0.03 for late summer leeches).

To check for significant variations from the Hardy-Weinberg Law in terms of allelic frequency at the MDH locus, a Contingency Chi-Square table analysis was performed with the results in Table 3.5. The allelic frequency of the early summer N. obscura deviated from the Hardy-Weinberg Law in MDH locus suggesting that selection for a certain genotype occurs.

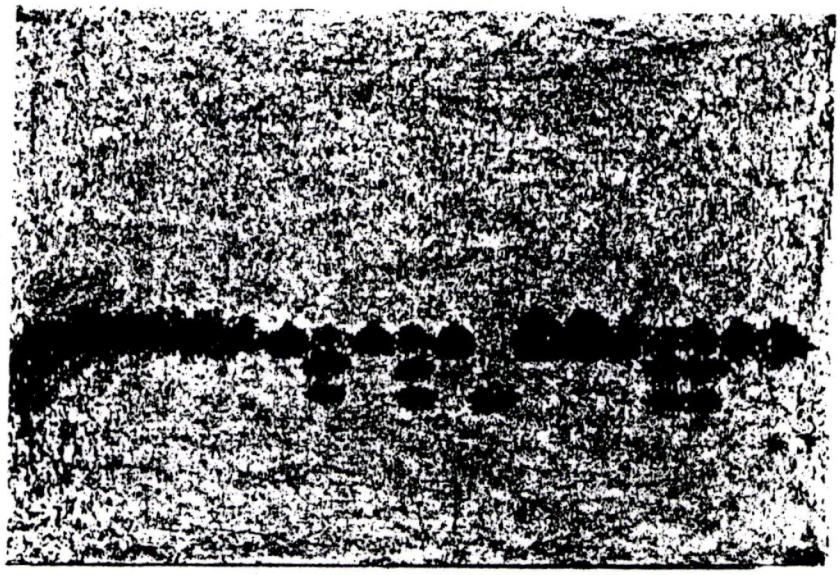


Figure 3.8 Electrophoretic pattern of MDH of the population from Stephenson's Pond (SP)

TABLE 3.4

Genotypic frequencies of malate dehydrogenase (MDH) in the early summer and late summer Nephelopsis obscura. Sample size = 60.

Genotype	Early summer	Late summer	X^2	P
AA	0.667	0.517		
BB	0.100	0.033	7.26	0.026
AB	0.233	0.450		

TABLE 3.5

Test of the Hardy-Weinberg Law of allelic frequency at the MDH locus for the early summer and late summer Nephelopsis obscura

Generation	Genotype	Observed frequency	Expected frequency	X^2	P
Early summer	AA	40	36.78		
	BB	6	2.82	4.84	<0.05
	AB	14	20.40		
Late summer	AA	31	33.06		
	BB	2	4.02	1.85	> 0.05
	AB	27	22.98		

3.4 Discussion

3.4.1 Genetic Variation and Phenotypic Plasticity in Heterogenous Environments

Levins (1968), in a theoretical presentation, suggested that genetic variability would be higher in an unstable, heterogenous compared to stable, homogenous environment. The hypothesis was that genetic variability would be required to accommodate variations in the environment. Recently, support for this hypothesis has been presented as data on the genetics of populations has accumulated (Felsenstein 1976; Hedrick *et al.* 1976; McLeod *et al.* 1981; Dimichele and Powers 1982; Chappell *et al.* 1984; Burky *et al.* 1985; Barnes *et al.* 1986; Hedrick 1986; LaBerge and Hann 1990). McLeod *et al.*(1981) studied two populations of freshwater clam, Nusculium partumeium (Say), one collected from an ephemeral pond and the other from a permanent pond. McLeod *et al.* (1981) found that five loci were polymorphic out of total twenty-two loci examined for the population from ephemeral pond. The population from the permanent pond, however, was completely monomorphic at every locus. McLeod *et al.* (1981) thus concluded that the genetic polymorphism of the population from ephemeral pond is an adaptation to its habitat with genetic polymorphism presumably allowing the population to adapt to the changeable condition. LaBerge and Hann (1990) reported that Daphnia pulex (Leydig) and Simocephalus vetulus (O.F.Müller) (Cladocera: Daphniidae) inhabiting an ephemeral

pond had high genetic diversity. They also found physiological difference within species in terms of temperature tolerance between genotypes and concluded that the rapid changes of conditions in the pond played a large role in determining the species and genotypes able to exist in such an unpredictable and changeable environment.

In this study, however, even in a variable ecosystem, e.g. Stephenson's Pond, N. obscura has a very low genetic variation with only one polymorphic enzyme out of sixteen active enzymes screened. How can N. obscura survive in such a variable environment with low genetic variation? Phenotypic plasticity may be the answer. Bradshaw and Hardwick (1989) proposed that there are two types of adaptations, constitutive and facultative. The former relates to genetic variability and the later to phenotypic plasticity. Bradshaw and Hardwick (1989) suggested that in the situation where stress operates in a temporary or fluctuating manner, facultative adaptations, produced within a single genotype through phenotypic plasticity, will be more appropriate. This is contrary to the evidence provided by McLeod *et al.* (1981) and Berge and Hann (1990). Difference in the type of adaptation can thus be considered species-specific rather than environmentally determined. Evidence indicates that organism living in a variable environment must have specific adaptations, either constitutive or facultative (McLeod *et al.* 1981; Bradshaw and Hardwick 1989; Berge and Hann 1990). The fact that N. obscura has low genetic variation thus strongly supports the phenotypic plasticity hypothesis.

3.4.2 Genetic Differences amongst Populations

Fixed differences in four enzymes are demonstrated amongst populations, showing that the populations from Stephenson's Pond (SP), Rocky Mountain foothill (RMP) and Utah (UTAH) are genetically different. While the RMP population differs from the UTAH population in only one enzyme (PGM), the SP population differs from both the RMP and the UTAH populations in four enzymes. All four enzymes showing differences amongst populations are involved in, or related to, anaerobic glycolysis pathway (Fig. 3.8). Diaphorase or dihydrolipoyl dehydrogenase (DIA) is one of the three enzymes in the pyruvate dehydrogenase complex involved in the following reaction:

$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \longrightarrow \text{acetyl CoA} + \text{CO}_2 + \text{NADH}$$

Diaphorase is thus a key enzyme determining whether aerobic or anaerobic pathways are used. The genetic differences amongst population of N. obscura suggest that the oxygen regime in SP is different from that in RMP and UTAH, which are similar to each other. Since the duration of ice coverage in RMP and UTAH are similar and much longer than at SP, it is highly probable that anoxic conditions last longer in RMP and UTAH than in SP. Genetic similarities correlating with environmental types shows that environmental factors such as oxygen saturation and temperature, act as selective force with different genotype favoured in different oxygen regimes. Thus, it is hypothesized that the tolerance to changes in dissolved oxygen saturation will be

different amongst populations from SP, RMP and UTAH but the populations from RMP and Utah will be most similar.

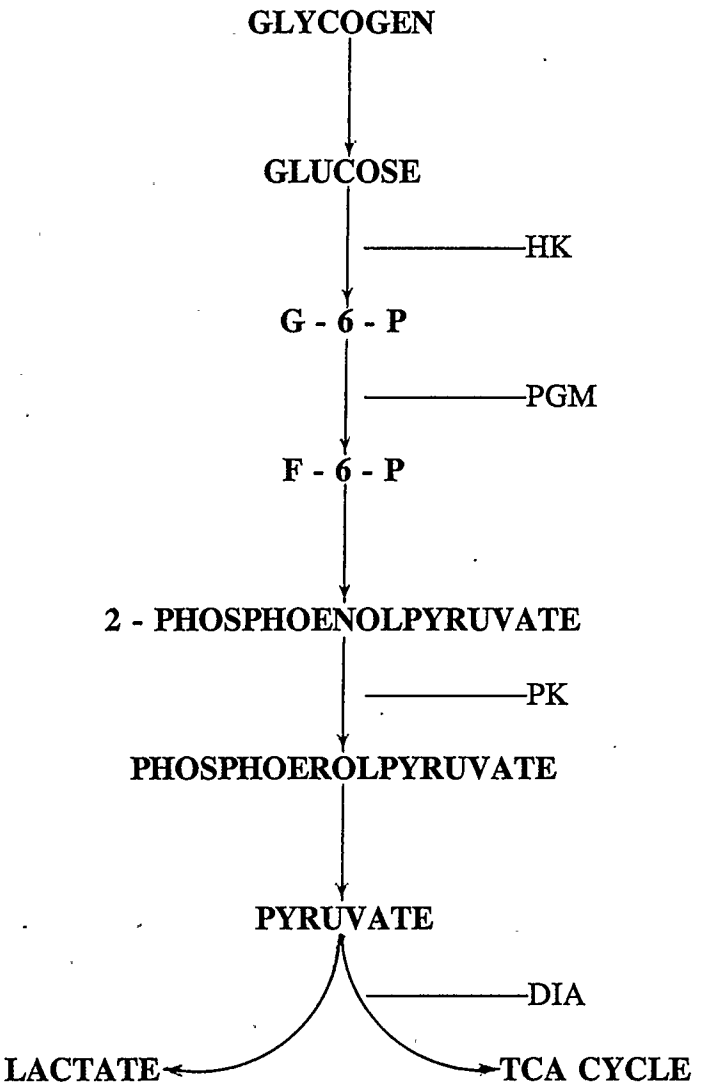


Figure 3.9 Anaerobic glycolysis

3.4.3 Early Summer and Late Summer Generations of Nephelopsis obscura in Stephenson's Pond

A significant difference occurs between the early summer and late summer generations of N. obscura with respect to the genotypic frequency of malate dehydrogenase (MDH). There is no fixed genetic difference between generations and there is only one locus (MDH) showing a difference in frequency. The fact that the population did not remain in Hardy-Weinberg equilibrium at the MDH locus can result from selection or genetic drift. Because of the very large population size of N. obscura in Stepheson's Pond, the occurence of genetic drift is unlikely. Thus, the deviation from the Hardy-Weinberg equilibrium suggests that selection for heterozygotes occurs in the field. In the laboratory Nevo *et al.* (1986) tested three pairs of species belonging to three genera and families of marine gastropods for resistance to diverse inorganic and organic pollutants. Their results suggest that fitness is positively correlated with heterozygosity. Lavie and Nevo (1987) reported that when the marine gastropods Littorina punctat (Philippi) and Littorina neritoides (Philippi) were exposed to cadmium and mercury pollution, different genotypes had different resistances. Similarly, Baird *et al.* (1991) conducted a comparative study of genotype sensitivity to pollutants using clones of Daphnia magna (Straus), and showed that different genotypes had different mortality responses to pollutants. For N. obscura temperature and oxygen concentrations act as selective forces. In the late summer with decreasing temperature and lower oxygen concentrations if homozygotes

show higher mortality the proportion of heterozygotes in the population would increase. This assumption can be tested by weekly sampling of N. obscura from the early summer through late summer. If the proportion of heterozygotes gradually increases, the selection hypothesis is supported.

Several methods can be used to further test the phenotypic plasticity hypothesis. One method would be to collect cocoons of both generations of N. obscura from Stephenson's Pond and hatchlings of both generations maintained under identical conditions and record variables in life history traits. After the completion of the life cycle individual genotypes could be determined using electrophoresis and life history trait data for individuals with the same genotype pooled. Another method is to collect pre-reproductive adults of both early summer and late summer generations of N. obscura with two individuals maintained together. When cocoons are produced, individuals genotypes could be examined and the genotypes of the offsprings determined. If their parents are homozygotes AA or BB the offsprings must also be homozygotes AA or BB. If one of the parents has the AA genotype and the other the BB genotype, the offsprings must be heterozygotes AB. Using either of the above methods, leeches could be exposed to identical laboratory conditions. If the individuals with the same genotype but hatched in different seasons differ in terms of growth and ecophysiology, the phenotypic hypothesis would be supported.

In the literature there is a growing body of evidence suggesting that fitness is positively correlated with heterozygosity (Garton 1984; Koehn and Gaffney 1984; Mitton and Grant 1984; Nevo *et al.* 1986; Lavie and Nevo 1987). It can be

hypothesized that the heterozygous individuals of N. obscura grow faster, allocating energy more efficiently to lipid storage, ultimately increase the probability of their surviving winter. This hypothesis can be tested in the laboratory by maintaining late summer N. obscura under ambient field conditions and recording the variability in life history traits. If differences occur between different genotypes in terms of life history traits the heterozygous-increased fitness hypothesis is supported.

3.5 Conclusion

This study provides the first data on the genetic structure of Nephelopsis obscura. Fixed genetic differences are detected amongst populations of N. obscura from three different geographic localities. Genetic differences amongst populations appears to be a reflection of the oxygen regimes experienced by the populations.

Since there is no fixed genetic difference between early summer and late summer generations leeches and since there is only one locus (MDH) showing difference in terms of frequency of heterozygotes, the phenotypic hypothesis is supported. In addition, the very low genetic variation shown by N. obscura indicates facultative adaptation which strongly supports the phenotypic plasticity hypothesis.

4.0 CONCLUSIONS

The objectives of this study were to: test the hypothesis that if the early summer and late summer generations differ ecophysiologically this is due to differences in the prehistory of their parents; explain any differences between the two generations in terms of their ecological success; determine if genetic differences occur between the two generations of N. obscura from Stephenson's Pond and to determine if the population from Stephenson's Pond is genetically different from those in the Rocky mountain foothills of Alberta and Utah.

Significant differences were found between the early summer and late summer N. obscura in the following:

1. Growth rate

Late summer N. obscura grew faster than early summer leeches during the accelerating growth phase resulting in reaching inflection point and maturity with a larger size and shorter time.

2. Energy acquisition and allocation

During the accelerating growth phase late summer N. obscura absorbed more energy from prey resulting from less energy loss in faeces and mucus than early summer N. obscura. Therefore, late summer N. obscura allocated more energy to growth, even though both generations allocated similar amount of energy to metabolism. This may account for the higher growth rate and growth efficiency.

3. Life history traits

Late summer N. obscura had a larger mature size and shorter developmental time in comparison to the early summer generation.

In terms of energy reserves, late summer N. obscura hatchlings had significantly higher lipid content compared to the early summer generation.

4. Genetics

The genetic variation in both generations of N. obscura was very low, only MDH locus being polymorphic. The genotypic frequency of the MDH locus was different between the two generations with more heterozygotes in late summer N. obscura than in early summer leeches.

The differences in growth and life history traits between the two generations are likely adaptive to different environmental conditions experienced by the two generations.

The observed ecophysiological differences could be either due to phenotypic plasticity, i.e. due to the different prehistory their parents experienced or to genetic difference between the two generations. The genetic examination showed no fixed genetic difference and only the MDH locus showed difference in genotypic frequency. Therefore, the phenotypic plasticity hypothesis is supported. The low genetic variation exhibited by N. obscura indicated its facultative adaptation which also strongly supports the phenotypic plasticity hypothesis.

Fixed genetic difference amongst populations from three localities were detected in this study at four enzymes indicated that the three populations are genetically distinct. All four enzymes are involved in or related to anaerobic

glycolysis pathway presumably suggesting that the dissolved oxygen saturation is a selective force with different genotype favoured by different environments. The genetic similarity and dissimilarity amongst populations was likely a reflection of the environmental conditions of their ecosystems.

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