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Effects of Stress on the Bioenergetics and Life-history of a Freshwater Predator

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a dissertation entitled "Effects of Stress on the Bioenergetics and Life-history of a Freshwater Predator" submitted by Deborah E.C. Smith in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

A bioenergetic model was used to describe the effects of natural stresses (food availability (high, medium and low), temperature (15°C and 20°C) and group size (1, 5, 10, 25, 50 and 100)) on energy acquisition and allocation patterns in the freshwater invertebrate predator *Nephelopsis obscura*.

In the high and medium food availability treatments, leeches were larger at 15 than at 20°C as a result of a lower proportion of energy allocated to total respiration. Leeches in the medium food treatments had higher absorption efficiency and allocated proportionally less energy to total respiration, maintaining growth and time to reproduction. In the low food treatments proportionally more energy was allocated to total respiration, as a result of higher activity, at the expense of growth and potential fecundity with no effect of temperature. The relative proportion of energy allocated to growth (lipid storage, somatic, reproductive) was the same in the high and medium food treatments at both temperatures. However, in the low food treatments at 15°C proportionally more energy was allocated to lipids at the expense of somatic growth, while the opposite occurred at 20°C.

In group sizes of either one or > 10 animals, *N. obscura* with *ad libitum* prey had slower growth and lower weekly food ingestion than groups of five. Size specific resting and active respiration decreased with increasing group size. In groups of 5 -50 the proportion of ingested energy allocated to total respiration was similar and higher than in group sizes > than 50 and single animals. Although the proportion of

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These results are discussed in terms of overwinter survival, feeding success and predation of N. obscura in the field. The bioenergetic responses of N. obscura are discussed in relation to responses during and after other natural and anthropogenic stresses. A stress resistant syndrome is described and compared to other aquatic species.

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CHAPTER ONE

GENERAL INTRODUCTION

STRESS

Attempts to define stress in physiological ecology have often led to semantic confusion. In some cases the term stress has been applied to properties of the environment which impose a force on a system, so that in this context the stress is a stimulus. However, stress has also been used to refer to the response of organisms to environmental change and when used in this sense, the causative factor is a stressor and the response a stress (Selye 1950). In recent years however, a consensus has been reached in physiological ecology to use stress when referring to stimuli which evoke a change in the system of interest (i.e., the cause), with the reaction (i.e., the effect) being termed the stress response (Bayne 1985; Rapport, Regier and Hutchinson 1985).

Stress can be either anthropogenic, or natural. There is increasing concern about the effects of anthropogenic stresses (e.g., heavy metals, pesticides, etc.) and ecologists are being required to make scientific predictions and analyses of responses at all ecological levels to anthropogenic stress (Grime 1989). Since organisms have evolved under the influence of natural stresses they have presumably developed effective responses to maximise fitness. Fitness refers to the relative contribution of genes to future generations (Townsend and Calow 1981), with fitness increasing as rate of gene transmission increases. The question remains as to whether or not organisms react in similar ways to natural stress as they do to anthropogenic stress. If animals have an adaptive response strategy and exhibit a common suite of traits in response to stress, then the response to novel environmental stresses will be the same as the response to natural stress, but it may not be adaptive.

There has been a tendency for the effects of anthropogenic stress to be studied in the laboratory with other environmental conditions optimal. However, in the field anthropogenic stresses are superimposed upon natural stresses, with potential additive, synergistic or antagonistic consequences. It is thus important that information is available on how organisms respond to both natural and anthropogenic stresses independently and in combination.

In northern temperate environments there are a number of different types of natural stress including changes in prey availability, oxygen concentration, temperature, pH, ionic concentration, etc. The aim of this study was to examine the responses of a freshwater predatory leech to different types of natural stress. Since all temperate aquatic organisms are exposed to such conditions the responses of this leech species are likely to be applicable to many other invertebrates. To isolate the effects of each stress examined, experiments were conducted in the laboratory to ensure that all other conditions were constant.

Three types of natural stress were examined: prey availability, temperature and group size. Changes in prey availability can result from several different processes and was examined in this study by manipulating frequency of feeding while prey quality and quantity per meal were constant. This scenario is representative of field conditions for many aquatic predators where prey occur in patches, resulting in variable meal frequencies with the predators attaining satiation before the prey is depleted. Despite the potential importance of the effects of changes in meal frequency in influencing feeding behaviour and prey consumption of a predator this has not been previously addressed. In the field organisms experience temperatures ranging from 0.5 - 25°C, but in this study experiments were conducted at 15° and 20°C representing the mean minimum and maximum summer temperatures in the field spanning the range of temperatures over which cocoon production is most successful. The majority of animals in the presence of other conspecifics live in groups of different sizes although the effects of aggregation on the ecology of organisms has not been extensively investigated. This study examines the effects of various group sizes with the number of animals in each group representing realistic group sizes previously recorded for other leech species either in the laboratory or in the field.

Stresses vary in frequency, duration and intensity so that individuals may experience different environments at different times and offspring may encounter a different environment from their parents. In the face of environmental variability the ability of an organism to express different phenotypes will provide a selective advantage. Changes in the phenotype induced by environmental cues is termed phenotypic plasticity (reviewed by Bradshaw 1965; Schlichting 1986; Stearns 1989b). Although phenotypic plasticity is presumably adaptive to natural stresses it may be non-adaptive to anthropogenic changes.

For organisms exhibiting phenotypic plasticity, the adaptive value of a particular phenotype depends on the net increment in relative fitness (Via 1987). Since fitness cannot usually be measured directly, correlates of fitness (e.g. growth, survival,

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fecundity) are frequently used. At the individual level, anything that impairs survival chances and/or reproductive output and/or extends time between reproductive bouts will lead to a reduction in fitness (see Sibly and Calow 1986). Each correlate of fitness is optimised to maximise overall fitness and consequently a reduction or increase in a single correlate does not necessarily indicate a change in fitness. For example, reduced survival does not necessarily indicate reduced fitness since it might be compensated for by enhanced growth.

If an organism can compensate for stress as a result of phenotypic plasticity, it is possible there are common suites of traits (e.g., time to maturity, growth rate, respiration rate) that characterise this evolutionary process. For example, many plants in high stress environments share a common suite of traits including high root:shoot ratios and concentrations of secondary metabolites and low rates of growth, photosynthesis, and transpiration. Based on these general patterns, Grime (1977) suggested that this suite of traits represents an adaptive strategy enabling plants to survive in stressful environments. These modifications have subsequently been termed Stress Resistant Syndrome (SRS) by Chapin, Autumn and Pugnaire (1993).

Only traits showing phenotypic plasticity are included in the SRS. However, some traits remain unchanged over a wide range of environmental conditions. For example, when exposed to different concentrations of zinc solution or changes in acidity respiration costs of *Gammarus pulex* (L.) remain unchanged (Maltby, Naylor and Calow 1990). Phenotypic plasticity however, may lead to compensation and flexible traits are good indictors of stress (Bayne 1985).

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Adaptive plasticity evolves under natural selection (Schlichting 1986; Stearns 1989b) but there are conflicting views on the mechanisms involved (Via 1993). It has been suggested that phenotypic plasticity is itself the target for selection, and that genes for plasticity exist separate from the genes that affect individual traits (Bradshaw 1965; Schlichting and Levin 1984, 1986). However, current models (Via and Lande 1985, 1987; Via 1987) illustrate how phenotypic plasticity can evolve by selection toward different optimum phenotypes in different environments.

The responses of organisms to stress can be considered at different levels: cellular, individual, population and community (Cappuzzo 1985). However, if a reduction in fitness does not occur, the environmental change cannot be classified as a stress (Calow 1989; Kohen and Bayne 1989) and in such cases acclimation or compensation (Bayne 1985) occurs.

A major disadvantage of considering reactions to stress at the cellular level is that it is very difficult to make the link between biochemical processes and fitness. For example, a number of authors have used nucleic acids as growth indices and as indicators of environmental stress (Barron and Adelman 1984; Mohapatra and Noble 1992; Peterson and Brown-Peterson 1992; Wang *et al.* 1993). However, the use of nucleic acids as growth indices is based on a number of assumptions which may not be fulfilled under all circumstances (Miglavs and Jobling 1989) i.e., that measurement of DNA gives an indication of cell numbers and that measurement of RNA is an index of protein synthetic potential so that changes in RNA:DNA ratio can be used as an indicator of change in rate of tissue protein synthesis and hence growth rate, if protein synthesis per ribosome proceeds at a constant rate. However, there is evidence that changes in nutritional status can lead to changes in both ribosomal numbers and activity (Smith 1981; Loughna and Goldspink 1984; Miglavs and Jobling 1989) and growth is the result of both tissue synthesis (anabolic) and degradation (catabolic) processes, whereas measurements of RNA:DNA provide only estimates of synthetic activity. Since growth rate is the only variable considered, conclusions about changes in fitness are very difficult. Assessing stress responses at the molecular or cellular level are thus not generally holistic.

In this study the physiological responses of individuals to environmental stress will be examined using a bioenergetic model. Information obtained from changes in energy acquisition and allocation (i.e., short-term physiological changes) will be used to assess fitness correlates and to predict potential long-term demographic changes.

BIOENERGETIC APPROACH

Physiological adaptations of resource use are important because they are the basis of both the form (allocation of resources between different structures) and behaviour (allocation of resources between different activities) of organisms (Sibly and Calow 1986). The resource considered in this study is energy which is necessary for all life processes and is generally considered to be a good currency that can be used for any organism (Kleiber 1961; Calow 1985). There are a number of advantages of choosing energy as the currency for this study rather than carbon or nitrogen. Energy is likely to be the best general currency when prey and predator are of similar biochemical composition (i.e., for carnivores), although less appropriate for herbivores and detritivores which may encounter shortages of protein, nitrogen, vitamins and trace elements (Sibly and Calow 1986). Energy can also be used to quantify activities and processes (e.g., feeding, respiration, excretory rates) as well as states and structures (e.g., size, gonads, lipids) and moreover, is relatively easy to measure. In addition, results from the majority of other studies in the literature are expressed in energy units or in units which can easily be converted to energy allowing comparisons to be made.

The rate of energy intake by foraging animals is limited (Karasov 1986; Wieser 1991; Kozlowski 1991) and primarily constrained by prey availability. To be consumed, prey items must first be detected, approached, selected, manipulated and ingested (i.e., must be available and accessible given the constraints of the morphology and sensory capacities of the predator). Predator characteristics (e.g.,

sensory modality, mouth size, foraging mode) and prey characteristics (e.g., size, activity, conspicuousness) will partially determine rates of prey ingestion. If prey are readily available to a predator, the number of prey eaten increases up to an asymptote level as prey density increases. This is known as the functional response curve (Holling 1959; Ringler and Brodowski 1983). The asymptote may result either from satiation of the predator or from limitations of food processing capabilities.

Since the rate of energy acquisition is limited, energy allocated to one compartment of the energy budget model (Fig. 1) (e.g., respiration) is unavailable to others (e.g., growth). Consequently, trade-offs occur between conflicting energetic demands (Stearns 1976, 1977, 1989a; Bell 1980; Shine 1980; Reznick 1985). Allocation to growth, maintenance or reproduction need not be immediate, as energy may be stored (usually as lipid) when abundant and then used later when energy demands are high or resource availability is low (Larson 1991; Reznick and Braun 1987; Meffe and Snelson 1993).

Energy utilization patterns that maximise fitness will be favoured by natural selection with the evolution of an optimal allocation pattern. The optimal allocation pattern depends on the environmental conditions experienced and fitness cost-benefits incurred when resources are allocated in different ways. Energy allocation patterns however, are not static and the optimal allocation of resources will likely change during the life-cycle (Perrin and Sibly 1993).

Figure 1. Energy budget model used for Nephelopsis obscura.

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Changes in the pattern of energy allocation of organisms in different environmental conditions, (e.g., food levels and temperature), have been documented (e.g., Cui and Wootton 1988; McCauley, Murdoch and Nisbet 1990). However, these studies have not examined the energy budget model as a whole and thus important questions regarding trade-offs and their consequences for life-history could not be addressed. In this study several components in the energy budget (Fig 1) are examined over the entire life of the animal.

Individual patterns of resource allocation can be used to predict consequences of stress to demographic parameters at the population level using mathematical models (Nisbet et al. 1989; Kooijman, van der Hoeven and van der Werf 1989; Calow and Sibly 1990). These models are based on the premise that within certain limits metabolism varies, thus influencing growth, reproduction and consequently abundance. Warren and Davies (1967) defined scope for growth (SfG) as the difference between energy intake and total metabolic losses, specifying the energy available for tissue production. This is frequently used as a population-relevant index of stress, because reductions in it indicate a longer time between birth and reproduction and between reproductive events (from a smaller amount of energy allocated to somatic growth) and reduced fecundity (from a smaller amount of energy allocated to production of offspring). Some models base predictions about population dynamics solely on SfG. However a reduction in SfG will not necessarily result in changes in abundance at the population level. For example, if reduced SfG is a consequence of increased maintenance respiration, due to the deployment of stressresisting mechanisms, the consequent enhanced survival of individuals might compensate for reduced recruitment. The precise outcome will depend upon the exact form of all the functional relationships involved and is addressed by the model of Sibly and Calow (1990).

Scope for activity (SfA) is the difference between basal (that level of metabolism below which the animal cannot survive) and maximum metabolic rate and specifies the energy available for other aerobic activities (Fry 1947, 1971). As SfA decreases less energy is available for stress resisting processes and thus survival is presumed to decrease. Since SfA and SfG can be used to estimate values of survival, time to and between reproductive bouts and fecundity, predictions about changes in fitness and population dynamics can be made. Since quantification of basal metabolism is not possible in practise, SfA is often calculated as the difference between active respiration (i.e., the metabolic costs incurred while active), and resting respiration (i.e., the metabolic costs incurred while stationary including costs of maintenance, growth and repair) (Chapter 2).

The overall aim of this study is to assess how patterns of energy acquisition and its subsequent allocation to life processes are affected by natural stress. Four general questions are examined:

- (1) Which aspects of energy acquisition and allocation are altered as a result of stress?
- (2) Does the extent of alterations in the energy balance change throughout the life of the animal?

- (3) Can an animal exposed to stress show compensation and maintain fitness?
- (4) Are the physiological traits affected by one type of stress the same as those for other types of natural stress?

TEST SPECIES

Nephelopsis obscura Verrill (Erpobdellidae), is a widely distributed species of predatory freshwater leech. It occurs throughout Canada and the northern U.S.A. (Davies 1991). In many small ponds and lakes, leeches are top of the trophic pyramid and are ideal for studying intra- and inter- specific resource competition, niche overlap, and predator-prey interactions (Davies 1991). In lentic ecosystems of Alberta and Western Canada *N. obscura* is usually the numerically dominant erpobdellid (Davies 1973, Davies and Everett 1977). In larger lakes and rivers it is often an important component of the diet of fish and is grown commercially for fish bait in some regions (Peterson 1982, 1983). Like other Erpobdellidae, *N. obscura* primarily feeds on macroinvertebrates and zooplankton which are usually swallowed whole or their body fluids sucked out (Davies and Everett 1975). Predators of leeches in North America include fish, birds, garter snakes and salamanders, as well as insects, gastropods and amphipods (Cywinska and Davies 1989).

Extensive investigations have been conducted on the basic biology, ecology, physiology and life history of *N. obscura*. It is phenotypically plastic in its responses to environmental changes including temperature (Baird, Linton and Davies 1986, 1987; Wrona, Linton and Davies 1987), oxygen regime (Davies, Yang and Wrona 1987; Davies and Gates 1991a, b; Davies, Monita and Linton 1992; Reddy and Davies 1993), and winter stresses (Dratnal and Davies 1990; Reddy, Dratnal and Davies 1992; Davies and Kalarani 1993; Dratnal *et al.* 1993).

Studies on the reproductive biology of N. obscura in different environments have

shown plasticity in life-history traits such as time to first reproduction, size at maturity and number of breeding bouts. In the Rocky Mountains of Western Alberta and in Minnesota, *N. obscura* reproduces after a minimum body size of 1,200 mg is reached (Peterson 1982, 1983; Linton and Davies 1987). However, in the prairie-foothills ponds of southern Alberta, reproduction can be initiated at 150-200 mg (Davies and Everett 1977; Singhal, Davies and Baird 1985; Davies and Singhal 1988).

In southern Alberta *N. obscura* annually produces two primarily semelparous generations both overwintering prior to reaching maturity. The first generation is produced in early summer and the second generation in late summer with cocoons produced either 12, 15 or 19 months after hatching (Davies and Everett 1977; Singhal, Davies and Baird 1985). In Minnesota *N. obscura* reproduces after 24 months (Peterson 1982, 1983).

In the field *N. obscura* usually dies shortly after cocoon production (Davies and Everett 1977; Peterson 1983; Baird, Linton and Davies 1986, 1987; Davies and Singhal 1988). However, under certain conditions in the laboratory *N. obscura* have been demonstrated to be iteroparous (Baird, Linton and Davies 1986) and there is evidence that a small proportion of the population are also iteroparous in the field (Davies and Everett 1977; Davies and Singhal 1988).

SITE DESCRIPTION

Stephenson's Pond is a permanent water body located 51°C 9'N-114° 16'W, in the prairie foothills zone approximately 5 km northwest of the city limits of Calgary, Alberta, Canada. The surface area is about 2.2 hectares with a maximum depth of 2.5 m. There is an earth-stone dike at the east end and a small intermittent steam enters the pond from the western end. The catchment basin is occupied by livestock and the water is hyper-eutrophic. As a result, in the summer large algal blooms occur with dense stands of emergent macrophytes including Potamogeton richardsoni (Benn.) and Myriophyllum exalbescens (Fern.). The dominant riparian vegetation on the southern shore consists of Populus balsamifera (L.) and Salix sp. with grasses, sedges and small shrubs constituting the remainder. The bottom substrate consists of mud and allochthonous material, with some rocks and boulders in the area of the dike at the east end. Nephelopsis obscura is the dominant leech species with Erpobdella punctata (Leidy) subdominant (Davies 1973; Davies, Reynoldson and Everett 1977; Linton, Davies and Wrona 1982; Davies and Gates 1991a). Other leeches present in lower abundances include Glossiphonia complanata (L.), Helobdella stagnalis (L.), Theromyzon trizonare (Davies and Oosthuizen) and Placobdella papillifera (Verrill).

CHAPTER TWO

MATERIALS AND METHODS

BIOENERGETIC MODEL

For all organisms allocation of energy to growth can be described by:

Growth = Income - Losses

Winberg (1956) was the first to develop a balanced energy equation:

$$I - Fe = A = RT + U + G$$

Where: I = food ingested

Fe = egestion A = energy absorbed RT = total respiration U = excretion G = growth

This equation (reviewed by Calow 1981), integrates the major physiological processes considered to be the important components of growth and has formed the basis of growth models for fish (Kitchell, Stewart and Weininger 1977; Stewart *et al.* 1983; Hewett and Johnson 1987; Beauchamp, Stewart and Thomas 1989) and invertebrates (Bayne, Widdows and Thompson 1976; Paloheimo, Crabtree and Taylor 1982).

The bioenergetic model used here is based on the modified energy balance equation of Sibly and Calow (1986) where for an individual, an energy budget for a defined period of time is:

$$I - Fe - U = A = RT + Gs + Gr + Gf$$

Where: I = food ingested

Fe = faeces
U = excretory products
A = energy assimilated
RT = total respiration
Gs = somatic growth
Gr = reproductive growth

Gf = storage

RT includes hourly resting (Rm) and active respiration costs and time spent resting (Tm) and active (Ta):

RT = (Rm X Tm) + (Ra X Ta)

Clarke (1993) noted that in traditional energy budgets respiration is usually considered as a single discrete process, when in reality it is a summation of many processes. In this study, Rm includes respiratory costs of all physiological processes when the animal is inactive and incorporates costs of maintenance and repair as well as somatic, gonad, faeces and mucus production. Thus Rm is not equal to basal metabolism (Chapter 1). Ra is the cost of respiration while the animal is active and thus includes all processes incorporated in Rm plus the additional costs of activity.

Because of the difficulties in separating leech faeces from mucus (Mu) (Dratnal and Davies 1990) energy assimilation by *N. obscura* cannot be directly assessed, and since energy lost in excretion is negligible (Brafield 1985; Kalarani and Davies 1994), the equation can be modified as:

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$$I - (Fe + Mu) = A = RT + Gs + Gr + Gf$$

QUANTIFICATION OF MODEL COMPONENTS

In this section the methods used to measure the separate components of the model (i.e., feeding, growth, respiration, etc.) are described. Further details regarding experimental design, number of animals, number of replicates and sampling dates are provided for each experiment in Chapters 3 and 4.

FEEDING

As gut analyses (Davies and Everett 1975) and serological analyses (Davies, Wrona and Everett 1978) show that oligochaetes form a major part of the diet of *N. obscura* in the field, a diet of *Tubifex tubifex* (Müller) was provided. The diet of other leech species also consists mainly of oligochaetes and chironomid larvae (Young and Ironmonger 1979; Dall 1983; Barton and Metcalfe 1985; Young and Procter 1985; Young and Spelling 1989).

Tubifex tubifex are mud-dwelling oligochaetes but experiments were conducted without a mud substratum to allow easy and precise measurement of prey consumption by *N. obscura*. Each leech was provided with approximately 100 mg *T*. tubifex three times a week for 3 h. The difference between the initial and final wet weight of *T. tubifex* from the experimental containers containing leeches gave the apparent food ingested by *N. obscura*. Similar weights of *T. tubifex* handled in the same manner were added to five containers without leeches (controls) and left for 3 h. 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.

Food ingestion was converted to energy values using a conversion factor of 23.1 J.mg DWT⁻¹ (Qian 1992). This value is similar to the average energy value for five undetermined Tubificidae worm species (22.5 \pm 4.6 J mg DWT⁻¹) reported by Cummins and Wuycheck (1971).

GROWTH

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 biomass was measured each week 48 h after feeding. Leeches were blotted to remove excess water and wet weight (WWT) measured to the nearest \pm 0.1 mg. Although energy content of *N. obscura* increased with increasing body size (Qian 1992), presumably because of differences in the proportion of total lipid, these differences were not significant and leech biomass was thus converted to energetic value (J) using the mean value of 21.9 J.mg DWT⁻¹ (Qian 1992), a similar value (22.4 J.mg DWT⁻¹) to that reported by Driver (1981) for *N. obscura*.

FAECES PLUS MUCUS

Faeces plus mucus were collected daily from animals maintained in individual plastic containers over a 4 d period on pre-weighed Millipore filters (0.45 μ m) which were subsequently dried to a constant weight at 40°C. The difference between the initial and final weights of filters represented the DWT of the faeces plus mucus.

To calculate the energy content of the faeces plus mucus, water from the stock tanks was collected each d for 3 d to provide sufficient material. Decomposition of the faecal material and bacterial growth are assumed to be a constant proportion of deposition (Dratnal and Davies 1990). The water samples were centrifuged at 12,000 rpm using a continuous flow-through system (0.15 L.min⁻¹). The collected samples of faeces plus mucus were freeze-dried and the energy content determined using a Philipson (1964) microbomb calorimeter.

LIPID STORAGE

Total lipid was measured following the methods of Folch, Lees and Stanley (1957). For each assessment five leeches were individually freeze-dried to a constant weight and homogenized in a 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. Lipids were converted to energy units using a conversion factor of 39.3 J.mg DWT⁻¹ (Pond 1981).

RESPIRATION

Since energy absorbed equals the energy available for growth and respiration,
energy allocated to total respiration (RT) can be calculated as the difference between energy absorbed and energy utilized for growth (RT = A - G) (Qian and Davies 1994). Since calculation of RT by difference does not provide potentially important information on how the individual components of respiration change in response to stress, hourly Rm and Ra respiration costs and time of activity (Tm and Ta) were measured.

Metabolic heat losses which represent the costs of aerobic respiration are usually estimated from the rate of oxygen consumption by the animal in a respirometer (Wootton 1990). Numerous respirometer systems have been developed, broadly classified as static or flow through systems each with their respective advantages and disadvantages (reviewed in Lampert 1984; Wrona and Davies 1984). In this study an activity-specific computerised flow through respirometer (Davies, Wrona and Kalarani 1992) was utilized. The unique design of this respirometer makes it particularly useful in bioenergetic studies since it is capable of correlating oxygen uptake with defined activity patterns. Measurements were taken 48 h after feeding to exclude the energetic costs of specific dynamic action (Kalarani and Davies 1994) and 1 h after introduction into the respirometer to allow acclimation (Davies, Wrona and Kalarani 1992). The respirometer system consists of four major components:

(1) flow-through respiration chambers

(2) an activity monitoring system

(3) a temperature controlled water circulation and filtration system

(4) a microcomputer data acquisition and control system.

Three experimental and one control chamber constitute the respirometer system. Each chamber is a modified gas-tight glass syringe and by moving the position of the plunger, the internal dimensions can be adjusted to accommodate different sizes of animals. Readings from the control and each experimental chamber were taken every second for 30 min for each animal.

The activity of the animals in a respiration chamber is monitored using an impedance converter connected to two electrodes, one placed at each end of the chamber. Any movement of the animal results in a change in electrical impedance between the two electrodes, which is detected by the impedance converter whose signal is transmitted to an amplifier. The output from the amplifier is connected to the computer and also displayed on an oscilloscope.

Water of known temperature and oxygen concentration from a water bath is continuously drawn through each respiration chamber by four peristaltic pumps, each connected independently to one of four solenoid valves. A fifth oxygen electrode pump sucks water through the oxygen electrode during the measurement of respiration, by a Strathkelvin oxygen meter. Water flow through all the peristaltic pumps eventually returns to the water bath for reoxygenation, filtration and recirculation.

Automation and control of the operation of the respirometer is achieved using the software package Labtech Notebook, the ADLAB analog/digital data acquisition and control board, and an exterminator interface board. The microcomputer processes and stores information on the water bath temperature, the activity signal from the

impedance converter and amplifier as well as the oxygen tension from the oxygen meter. (For further details of the respirometer system including a schematic diagram of the flow-through respirometer see Davies, Wrona and Kalarani 1992).

The rate of oxygen consumption of the animal is converted to J using an oxygen coefficient of 0.0202 J. μ l O₂.h⁻¹ (Elliott and Davison 1975).

Simultaneous to the respiration measurements, activity of the animals was recorded for the first 10 min of every h over a 24 h period (12:12 h light:dark regime) using an infra-red sensitive video camera. The time spent resting (no movement) and time of total activity including locomotory movement (defined as any movement resulting in linear displacement of the whole body (e.g., swimming and crawling) (Davies and Kasserra 1989) and random movement (defined as movement resulting in non-linear body displacement e.g. dorsal-ventral undulations of the body with the posterior sucker remaining attatched, probing with the anterior portion of the body (Wrona and Davies 1984) was measured.

REPRODUCTION

To assess somatic and reproductive growth animals were randomly selected every 2 weeks and dissected. The male and/or female gonads were removed to determine the biomass of reproductive and somatic tissues. Gonads were freeze dried and a conversion factor of 24.0 J.mg DWT⁻¹ (Biernacka 1994) used to convert gonad biomass into energy units. Slides and smears of gonads were made to determine gametic development throughout the life-history using histological techniques.

Spermatogenic and ovarian cells were classified into six spermatic and three oocytic stages of development based on the number, size, shape and distribution of individual cells and cell clusters following Singhal, Davies and Baird (1985).

STATISTICAL METHODS

Prior to conducting statistical analysis the assumptions of normality and homogeneity of variances were checked with Lilliefors test and F-max test respectively. When the assumptions were violated, data were transformed or nonparametric analyses used, as appropriate. Unless otherwise stated all tests were conducted following Zar (1984) using a microcomputer with SYSTAT (version 5.02) (Wilkinson 1990). The significance of all tests was determined at p < 0.05 with differences stated as being highly significant when p < 0.01. Variation is reported as mean \pm standard deviation unless otherwise stated.

CHAPTER THREE

INFLUENCE OF PREY AVAILABILITY AND TEMPERATURE ON THE ECOLOGICAL ENERGETICS OF NEPHELOPSIS OBSCURA.

INTRODUCTION

The objective of this chapter was to investigate acquisition and allocation of energy by *Nephelopsis obscura* at different levels of prey availability and at different temperatures. Prey availability and the amount of energy consumed are primary factors determining the amount of energy allocated to growth, energy storage, gonad development, and metabolism. (Adams, McLean and Parrotta 1982). Temperature has a secondary influence on energy acquisition and energy partitioning in many organisms (Adams, McLean and Parrotta 1982) including *N. obscura* (Linton and Davies 1987).

As is common for most carnivores, the feeding process of *N. obscura* consists of a series of discrete events where meals of a particular size are eaten over a prescribed time, separated by between-meal interfeeding intervals (Calow 1980; Wrona and Calow 1988). Many invertebrates experience variable inter-feeding intervals due to the heterogenous nature of benthic prey distribution in freshwater ecosystems (Hildrew and Townsend 1982). This study contrasts the optimal pattern of energy acquisition and allocation when prey patches are of the same type but with differences in meal periodicity. To ensure a consistent food supply *ad libitum* prey (*T. tubifex*) was provided throughout each feeding period.

One objective of this study was to determine if there is a relationship between

number of meals available per week and the size of the meals ingested. The marginal value theorem (Charnov 1976, Parker and Stuart 1976) predicts that organisms in environments yielding a high overall rate of energy intake should exploit individual patches less than those in environments where there is a lower overall rate of energy intake. With increased time between patches (i.e., as feeding frequency decreases), the optimal foraging theory predicts that animals should remain feeding longer and that meal sizes should increase due to the decrease in the average net rate of energy gain from the environment.

Once energy has been acquired, it can be allocated to different components of the energy budget. It is hypothesized that with different levels of prey availability (based on meal frequency) and different temperatures, animals will adjust energy acquisition and differentially allocate energy to components of the bioenergy budget.

Some aspects of the relationship between life history and food acquisition by *N*. *obscura* have been examined previously. Linton and Davies (1987) showed that growth is more sensitive to differences in prey availability and abundance between years than to changes in temperature, and that reproductive output is related to body size. Post-reproductive mortality is influenced by weight loss during reproduction, post-reproductive feeding and body size (Baird, Linton and Davies 1986, 1987) so that while primarily semelparous in the field *N. obscura* is genetically potentially iteroparous. Food availability modulated by changes in behaviour of prey *T. tubifex* have also been shown to affect growth, time to reproduction and size at maturity of *N. obscura* (Dratnal, Dratnal and Davies 1992).

Sexual maturity in *N. obscura* is also at least partially controlled by temperature (Baird, Linton and Davies 1986, 1987; Linton and Davies 1987; Wrona, Linton and Davies 1987; Biernacka 1994). Water temperature regime affects the reproductive success of *N. obscura* and Wrona, Linton and Davies (1987) demonstrated that the onset of cocoon production is temperature dependent. When *N. obscura* at, or very close to, cocoon production were exposed to different temperature regimes, no cocoons were produced at 5°C, only 38% produced cocoons at 10°C, but over 80% produced cocoons at 15°C and 20°C. Wrona, Linton and Davies (1987) also found that cocoon hatching success is influenced by temperature with successful hatching occurring only between 15°C and 20°C. Linton and Davies (1987) showed that between 10°C and 20°C reproductive output of *N. obscura* is positively correlated with temperature.

In this study, the following questions have been examined:

- (i) Which aspects of energy acquisition and allocation are altered as a result of changes in food availability?
- (ii) Which aspects of energy acquisition and allocation are altered as a result of changes in temperature?
- (iii) What is the relationship between number of meals per week and food ingestion per meal?
- (iv) Do changes in the energy balance vary through the life cycle?
- (v) Can an animal exposed to reduced food availability show compensation and maintain fitness?

(vi) Which of the two temperatures (representing upper and lower range of summer conditions in the field) is optimal for growth and reproduction of *N. obscura*?

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METHODS

COLLECTION AND CULTURE

To ensure that all animals had the same pre-history, cocoons recently deposited on macrophytes by *N. obscura* were collected from Stephenson's Pond. In the laboratory the cocoons were maintained in aerated (100% oxygen saturation) pond water at either 15°C or 20°C with a 12:12 h light:dark regime (simulating summer field conditions) until they hatched after approximately 4 weeks. The hatchlings were transferred to large holding tanks under identical conditions but provided with an *ad libitum* supply of prey (*T. tubifex*).

FEEDING AND GROWTH EXPERIMENTS

Three treatments based on the number of feedings per week: three, two or one (high, medium and low food availability respectively) were established. At each feeding the leeches were provided with *ad libitum* prey for 3 h after which the prey was removed from the tanks. To ensure a super-abundant food supply at each meal, the quantity of prey provided was more than twice that of the asymptotic level of prey that could be ingested per individual per meal (Dratnal and Davies 1990).

For each treatment 450 animals were maintained in each of two large stock tanks $(area=880 \text{ cm}^2, \text{ volume}=15000 \text{ ml})$. Once a week the biomass of the leeches was determined by blotting dry and weighing 50 specimens chosen at random after which they were returned to the stocks. At the beginning of each week five leeches were selected from each stock tank from each treatment and maintained individually in plastic pots (area=64 cm², volume=500 ml) for 7 d to determine prey ingestion

before being returned to the stock tanks.

ABSORPTION, RESPIRATION AND GROWTH COMPONENTS.

The remaining components of the energy budget (see Chapter 2 for details of methodology) were measured once every 10 weeks until the leeches reached sexual maturity and/or asymptote biomass after 38 - 39 weeks. Energy loss due to faeces. plus mucus production after each of two consecutive meals was measured using individuals maintained in pots (n=10). Resting and active respiration was measured (n=6) and five leeches sacrificed to determine total lipid concentration. To assess somatic and reproductive growth, 10 animals were selected from the stock every 2 weeks and dissected.

STATISTICS

Food consumption, absorption, gonad growth, lipid deposition and time of activity were analyzed using two-way ANOVA. To test for differences in food consumption between leeches of the same size a one-way ANOVA was used. Growth was analyzed using non-parametric two-way ANOVA, absorption efficiency using a non-parametric three-way ANOVA and respiration using a three-way ANOVA. When the null hypothesis of equality of population means was rejected, a Tukey multiple comparison test was used to determine among which population means the differences occurred.

RESULTS

GROWTH

In all treatments at both temperatures *N. obscura* showed a sigmoidal growth pattern (Fig 2a and b). Growth rates were calculated for each consecutive 10 week period and over the entire length of the experiment (life-time growth rate) (Table 1).

There was a highly significant effect of both temperature (H = 11.41, df = 1) and food availability (H = 212.27, df = 2) on life-time growth rate but with a nonsignificant interaction (H = 2.37, df = 2). Growth rates increased significantly from low to medium to high food availability at both temperatures (Table 2). In the high and medium food treatments *N. obscura* exhibited significantly higher growth at 15°C than at 20°C while in the low food treatments there was no significant difference in growth rate between temperatures (Table 2).

At 15°C asymptote biomass was reached in all treatments after 38 weeks, but because of the significant differences in growth rate the asymptote biomasses were significantly different (853 ± 222 mg in the high food, 740 \pm 150 mg in the medium food and 189 ± 69 mg in the low food treatment). Asymptote biomass was reached at week 39 in all treatments maintained at 20°C and were also significantly different amongst treatments (685 ± 157 mg in the high, 513 ± 118 mg in the medium and 129 ± 140 mg in the low food treatment). Figure 2. Mean (\pm SD) (n = 50) biomass (mg WWT) of *Nephelopsis obscura* over time (weeks) maintained in high (\blacksquare), medium (\checkmark) and low (\bullet) food treatments at (a) 15°C and (b) 20°C.





TIME [weeks]

,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	<u></u>	MEAN GROV	VTH RATE		
	0 - 10	10 - 20	20 - 30	30 - 40	life-time
15°C				******	
HIGH	25.16	101.00	150.20	22.95	76.55
	± 7.77	± 29.86	± 63.1	± 71.95	± 18.71
MEDIUM	19.49	56.92	120.90	46.13	64.32
	± 6.15	± 20.38	± 44.22	± 59.18	± 16.47
LOW	3.69	8.21	26.79	11.4	13.38
	± 2.26	± 4.83	± 15.12	± 17.89	± 4.55
20°C			<u>,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,</u>	····	
HIGH	30.21	67.64	124.10	26.83	64.20
	± 6.23	± 24.87	± 45.41	± 56.57	± 15.67
MEDIUM	17.63	37.81	95.76	32.74	48.44
	± 4.70	± 11.31	± 31.1	± 45.41	± 12.81
LOW	2.32	6.84	18.28	15.67	11.98
	± 2.44	± 5.01	± 11.93	± 16.13	± 4.46

Table 1. Mean (\pm SD) growth rate (J.week¹) of *Nephelopsis obscura* maintained in high, medium and low food treatments at 15°C and 20°C for weeks 0 - 10, 10 - 20, 20 - 30, 30 - 40 and for weeks 0 - 40 (life-time).

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Table 2. Results of Tukey multiple comparison test determining the effects of food availability (high, medium and low) and temperature (15°C and 20°C) on life-time mean growth rates (J.week⁻¹) of *Nephelopsis obscura*. Treatments connected with a solid line are not significantly different from one another.

+ · · · · · · · · · · · · · · · · ·	********************************	MEAN GRO	WTH RATE		
20 - L	15 - L	20 - M	15 - M	20 - H	15 - H
					<u></u>

INGESTION

Preliminary experiments showed that ingestion by an individual removed for 1 week from the stock was not significantly different from the mean ingestion in the stock tank (except for high food at 15°C and low food at 20°C during week 15) (Table 3).

Weekly food ingestion (mg WWT) increased over time in each treatment (Fig 3a and b). Feeding rates were calculated per 10 week period as the mean (n=10) of weekly means (n=10) and life-time feeding rates (n=40) calculated for the entire length of the experiment (Table 4). Variance was calculated as the sum of weekly variances following Walpole and Myers (1993).

There was no significant effect of temperature (F = 0.0125, df = 1) on life-time feeding rates but a highly significant effect of feeding frequency (F = 2686.51, df = 2) with a significant interaction (F = 12.55, df = 2). Regardless of temperature the feeding rate increased significantly from the low to medium to high food availability treatment (Table 5). There was no significant difference between temperatures at any food level (Table 5).

Size specific weekly food ingestion (mg WWT.mg leech WWT⁻¹) declined over time in all treatments (Fig 4a and b). At both temperatures the pattern of size-specific prey ingestion was similar in the high and medium food treatments and different in the low food treatments. In the low food treatments prey ingestion per mg leech was initially lower than in the other treatments, but after week 12 at 15°C and week 10 at 20°C it was higher and remained higher until the end of the experiment. At both temperatures the pattern of weekly prey ingestion (mg WWT) with increasing body size is similar in the high and medium food treatments but different in the low food availability treatments (Fig 5a and b). In the low food treatments there was a greater rate of increase in weekly prey ingestion with increasing biomass.

To determine if there were significant differences in food ingestion per week and per meal among animals of the same size from different treatments, times were chosen when the biomass of animals in the high and medium food treatments were not significantly different from the asymptote biomass attained by leeches in the low food treatments (Table 6). There was a highly significant difference among treatments at $15^{\circ}C$ (F = 7.56, df = 2) and at 20°C (F = 8.05, df = 2) in weekly food ingestion for animals of the same biomass. At 15°C there was no significant difference in weekly food ingestion between the low and the medium food treatment but food ingestion per week was significantly higher in the high food treatment (Table 6). At 20°C there was no significant difference in weekly food ingestion between the low and the medium food treatments or between the medium and high food treatments. Weekly food ingestion in the high food treatment was significantly higher than in the low food treatment.

Differences in mean food ingestion per meal among treatments were highly significant at both 15°C (F = 69.91, df = 2) and at 20°C (F = 9.20, df = 2). In the low food treatments leeches ingested significantly more food per meal than leeches in the medium and high food treatments at both 15°C and 20°C but there was no significant difference in food ingestion per meal between the medium and high food

treatments at either temperature (Table 6).

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Table 3. Mean (\pm SD) food ingestion (mg WWT) by *Nephelopsis obscura* maintained in high, medium and low food treatments at 15°C and 20°C for weeks 5, 15, 25 and 35 in pots (n = 10) and stock (n = 1) containers.

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15	5°C	LOW	MEDIUM	HIGH
	WEEK 5	5.5 ± 2.8	11.9 ± 3.7	23.2 ± 4.6
POTS	15	8.4 ± 3.4	32.1 ± 3.7	43.1 ± 6.1
1015	25 [.]	18.4 ± 5.1	54.3 ± 7.3	76.4 ± 15.4
	35	32.8 ± 5.3	48.7 ± 19.1	55.2 ± 11.1
	WEEK 5	7.0	10.3	20.6
STOCK	15	6.4	34.2	39.1
	25	15.6	50.2	67.7
	35	35.8	38.7	49.0
20	РС	LOW	MEDIUM	HIGH
	WEEK 5	3.2 ± 2.9	13.6 ± 3.3	21.5 ± 2.3
POTS	15	8.5 ± 4.5	26.7 ± 4.5	53.6 ± 7.1
1015	25	16.7 ± 4.4	43.8 ± 8.2	93.6 ± 12.4
	35	21.4 ± 6.4	54.8 ± 23.0	78.2 ± 24.7
	WEEK 5	4.7	15.4	20.5
STOCK	15	5.0	24.2	57.7
DICCIX	25	19.2	48.4	87.6
	35	24.9	67.6	91.8

Figure 3. Mean (\pm SD) (n = 10) weekly food ingestion (mg WWT) by *Nephelopsis* obscura over time (weeks) maintained in high (\blacksquare), medium (\checkmark) and low (\bullet) food treatments at (a) 15°C and (b) 20°C.

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	:	MEAN FEED	ING RATE		
	0 - 10	10 - 20	20 - 30	30 - 40	life-time
15℃	· · · · · · · · · · · · · · · · · · ·				
HIGH	116.84	200.10	301.30	270.94	222.30
	± 7.82	± 11.04	± 22.54	± 28.98	± 8.28
MEDIUM	75.90	129.72	202.86	203.32	152.95
	± 5.98	± 8.28	± 16.10	± 35.42	± 6.90
LOW	20.70	37.26	74.06	132.94	66.24
	± 3.22	± 4.60	± 6.90	± 8.74	± 4.14
20°C				•	
HIGH	124.20	196.42	334.42	330.74	246.44
	± 9.66	± 13.34	± 28.52	± 63.48	± 11.5
MEDIUM	78.66	120.06	178.94	236.44	153.53
	± 7.36	± 7.82	± 17.48	± 40.94	± 7.82
LOW	22.54	34.50	66.24	89.24	53.13
	± 3.68	± 4.60	± 5.52	± 9.20	± 4.14

Table 4. Mean (\pm SD) feeding rate (J.week¹) of *Nephelopsis obscura* maintained in high, medium and low food treatments at 15°C and 20°C for weeks 0 - 10, 10 - 20, 20 - 30, 30 - 40 and for weeks 0 - 40 (life-time).

Table 5. Results of Tukey multiple comparison test determining the effects of food availability (high, medium and low) and temperature (15°C and 20°C) on life-time mean feeding rates (J.week¹) by *Nephelopsis obscura*. Treatments connected with a solid line are not significantly different from one another.

MEAN FEEDING RATE					
20 - L	15 - L	15 - M	20 - M	15 - H	20 - H

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Figure 4. Regression of weekly food ingestion per unit biomass (mg WWT.mg leech WWT⁻¹) by *Nephelopsis obscura* over time (weeks) maintained in high (\blacksquare), medium (\checkmark) and low (\bullet) food treatments at (a) 15°C and (b) 20°C.



Figure 5. Regression of weekly food ingestion (mg WWT) by *Nephelopsis obscura* over biomass (mg WWT) maintained in high (■), medium (▼) and low (●) food treatments at (a) 15°C and (b) 20°C.



Table 6. Biomass (mg WWT), food ingestion (mg WWT), and food ingestion per meal (mg WWT.meal⁻¹) by *Nephelopsis obscura* in the high, medium and low food treatments at times when biomasses were similar to the asymptote biomass attained by leeches in the low food treatments at 15°C and 20°C. Treatments connected with a solid line are not significantly different from one another.

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15°C	LOW	MEDIUM	HIGH
TIME (week)	34	16	13
BIOMASS	139.9 ± 55.3	132.9 ± 42.4	125.9 ± 27.7
FOOD INGESTION	32.7 ± 5.3	30.7 ± 6.9	41.9 ± 8.0
FOOD INGESTION PER MEAL	32.7 ± 5.3	15.4 ± 3.5	14.0 ± 2.7
20 °C	LOW	MEDIUM	HIGH
TIME (week)	34	14	10
BIOMASS	96.4 ± 37.2	96.6 ± 17.1	88.9 ± 16.2
FOOD INGESTION	21.4 ± 6.4	30.4 ± 4.0	37.9 ± 13.9
FOOD INGESTION PER MEAL	21.4 ± 6.4	15.2 ± 2.0	12.6 ± 4.7

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As there was no significant effect of treatment (F = 0.971, df = 5) or time/age (F = 1.31, df = 3) on the energy value of faeces plus mucus data were pooled and a mean energy content of 21.0 J.mg DWT⁻¹ used in all calculations.

Absorption efficiency (AE) was calculated as:

$$AE = I - (Fe + Mu) \times 100$$

Where: I = food ingestion (J)

Fe + Mu = faeces plus mucus (J)

There was no significant temperature effect (H = 0.34, df = 1), a significant food effect (H = 54.92, df = 2) and a significant time/age effect (H = 44.27, df =3) with all interactions significant. In all but one of the treatments, absorption efficiency was lower at week 10 than at later weeks. Absorption efficiencies generally decreased as food availability increased (Table 7).

Mean rate of energy absorption (EA) (J.week¹) was calculated as:

$$EA = I x AE$$

Where: $I = food ingestion (J.week^{-1})$

Absorption rates were calculated for four consecutive 10 week periods and for weeks 0 - 40 (Table 8). There was no significant effect of temperature (F = 0.36, df = 1), a significant effect of food (F = 459.62, df = 2) and a significant interaction (F = 10.65, df = 2) on life-time absorption rates. Leeches in the low food treatments absorbed significantly less energy per week than leeches in the medium and high food treatments at both 15°C and 20°C (Table 9) but there was no significant difference in rate of energy absorption between the medium and high food treatments at either temperature.

Table 7. Mean (\pm SD) absorption efficiency (%) of *Nephelopsis obscura* maintained in high, medium and low food treatments at 15°C and 20°C after 10, 20, 30, and 40 weeks.

	MEAN ABSORPTION EFFICIENCY				
	10	20	30	40	
15°C					
HIGH	67.2	79.9	72.2	66.9	
	± 10.4	± 8.0	± 10.9	± 12.1	
MEDIUM	79.8	79.9	84.7	85.2	
	± 14.8	± 10.7	± 10.2	± 11.6	
LOW	54.7	93.1	90.1	94.0	
	± 19.8	± 5.4	± 3.9	± 2.6	
20°C					
HIGH	60.5	76.3	79.8	67.9	
	± 12.3	± 7.7	± 5.6	± 16.4	
MEDIUM	74.7	83.5	68.5	91.7	
	± 19.3	± 14.8	± 20.4	± 8.9	
LOW	65.9	96.5	85.2	96.7	
	± 24.1	± 1.6	± 5.7	± 1.9	

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Table 8. Mean (\pm SD) absorption rate (J.week ⁻¹) of Nephelopsis obscura maintained
in high, medium and low food treatments at 15°C and 20°C for weeks 0 - 10, 10 - 20,
20 -30, 30 - 40 and for weeks 0 - 40 (life-time).

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MEAN ABSORPTION RATE					
	0 - 10	10 - 20	20 - 30	30 - 40	Life-time
15℃					
HIGH	78.52	149.87	217.54	181.26	156.80
	± 5.26	± 8.27	± 16.27	± 19.39	± 13.57
MEDIUM	60.57	103.65	171.82	173.23	127.32
	· ± 4.77	± 6.62	± 13.64	± 30.18	± 17.05
LOW	11.32	34.69	66.73	124.96	59.43
	± 1.76	± 3.45	± 4.98	± 8.22	± 5.18
20°C	<u>, , , , , , , , , ,,,,</u> ,				<u>, 1994, 1997, 1997, 1997, 1997, 1997</u>
HIGH	75.14	149.87	266.87	224.57	179.11
	± 5.84	± 10.18	± 22.76	± 43.10	± 25.07
MEDIUM	58.76	100.25	122.57	216.82	124.60
	± 5.50	± 6.53	± 11.97	± 37.54	± 20.16
LOW	14.85	33.29	56.44	86.30	47.72
	± 2.43	± 4.44	± 4.70	± 8.90	± 5.63

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Table 9. Results of Tukey multiple comparison test determining the effects of food availability (high, medium and low) and temperature (15°C and 20°C) on life-time mean absorption rates (J.week⁻¹) of *Nephelopsis obscura*. Treatments connected with a solid line are not significantly different from one another.

MEAN ABSORPTION RATE					
20 - L	15 - L	20 - M	15 - M	15 - H	20 - H
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TOTAL RESPIRATION

Total respiration (RT) (J.week⁻¹) for each treatment was estimated from:

$$RT = A - G$$

Where: $A = energy absorption (J.week^{-1})$

 $G = growth (J.week^{-1})$

Total respiration rates were calculated for four consecutive 10 week periods and for weeks 0 - 40 (Table 10). There was a significant effect of temperature (F = 7.10, df = 1), food (F = 46.1, df = 2) and a significant interaction (F = 6.18, df = 2) on life-time total respiration rates. Total respiration rates of leeches in the low food treatments were not significantly different from each other but were significantly lower than in leeches in the high food treatment at 20°C (Table 11). All other differences among treatments were not significant.

Table 10. Mean (\pm SD) total respiration rate (J.week ⁻¹) of Nephelopsis obscura
maintained in high, medium and low food treatments at 15°C and 20°C for weeks 0 -
10, 10 - 20, 20 - 30, 30 - 40 and for weeks 0 - 40 (life-time).

MEAN TOTAL RESPIRATION					
	0 - 10	10 - 20	20 - 30	30 - 40	life-time
15℃					
HIGH	53.36	48.87	67.34	158.31	81.97
	± 9.38	± 30.98	± 65.16	± 74.52	± 26.04
MEDIUM	41.08	46.73	50.92	127.1	66.46
	± 7.78	± 21.43	± 46.28	± 66.43	± 21.03
LOW	7.63	26.48	39.94	102.01	44.02
	± 9.38	± 5.94	± 15.92	± 19.69	± 6.91
20°C		<u></u>			
HIGH	44.93	82.23	142.77	197.74	116.92
	± 8.54	± 26.87	± 50.79	± 71.12	± 22.96
MEDIUM	41.13	62.44	26.81	184.08	78.62
	± 7.23	± 13.06	± 33.32	± 58.92	± 17.33
LOW	12.53	26.45	38.16	70.63	36.94
	± 3.44	± 6.69	± 12.82	± 18.42	± 5.92

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Table 11. Results of Tukey multiple comparison test determining the effects of food availability (high, medium and low) and temperature (15°C and 20°C) on mean life-time total respiration rates (J.week⁻¹) of *Nephelopsis obscura*. Treatments connected with a solid line are not significantly different from one another.

MEAN TOTAL RESPIRATION RATE					
15 - L	20 - L	15 - M	20 - M	15 - H	20 - H
To summarise, life-time rates of energy absorption, loss from faeces plus mucus and allocation to growth and total respiration were plotted for each treatment (Fig 6). As food availability increased life-time energy absorption, faeces plus mucus, growth and total respiration increased at both temperatures.

For each treatment the energy values for faeces plus mucus, total respiration and growth were converted to a percentage of energy ingested to determine the proportion of energy allocated to each component (Fig 7). As food availability decreased the proportion of energy lost in faeces plus mucus decreased from 28.4 ± 1.1 % in the high food treatments to 17.9 ± 1.1 % in the medium food treatments to 10.3 ± 0.1 % in the low food treatments. The proportion of energy allocated to total respiration was similar in the high (41.4 ± 5.3 %) and medium (45.4 ± 4.2 %) food treatments although it was greater at 20°C (48.1 ± 1.5 %) than at 15°C (38.7 ± 2.6 %). In the low food treatments the proportion of energy allocated to total respiration was considerably higher (68.4 ± 1.2 %) than in the other food treatments and was similar for both temperatures. A greater proportion of energy was allocated to growth in the medium food treatments (38.3 ± 3.8 %) than in the high (28.8 ± 2.7 %) and low food treatments (21.4 ± 1.2 %), i.e growth efficiency was highest in the medium food treatment followed by the high and low food treatments.

Figure 6. Rate of energy allocation (J.week⁻¹) to faeces plus mucus, total respiration and growth of *Nephelopsis obscura* maintained in high (H), medium (M) and low (L) food treatments at 15°C and 20°C.



- 🛛 Faeces plus mucus
- ☑ Total Respiration
- 🖾 Growth

Figure 7. Proportion of ingested energy (%) allocated to faeces plus mucus, total respiration and growth of *Nephelopsis obscura* maintained in high (H), medium (M) and low (L) food treatments at 15°C and 20°C.



- 🛛 Faeces plus mucus
- **Total Respiration**
- 🛛 Growth

COMPONENTS OF GROWTH

REPRODUCTION

After initiation, gonad biomass increased in all treatments at 15°C until week 42 after which there was a decline associated with the regression of the testisacs (Fig 8a). At 20°C gonad biomass continued to increase after initiation for the duration of the experiment (Fig 8b). To compare among treatments the rate of increase in gonad biomass was calculated using data up to and including week 42 (Table 12).

In each of the treatments there was a significant temperature effect (F = 18.87, df = 1), food effect (F = 1597.5, df = 2), and interaction (F = 31.78, df = 2) on the total energy allocation to gonads. Regardless of temperature the energy allocated to gonads increased with increasing levels of food availability and significant differences occurred amongst the food treatments (Table 12). In the low and the high food treatments the energy allocation to gonads was significantly higher at 15°C than at 20°C while in the medium food treatments it was highest at 20°C.

Timing of gonad development and the attainment of subsequent reproductive stages was based on 70 % of the individuals dissected exhibiting a particular stage (Biernacka 1994). In all treatments, regardless of temperature and food availability, gonad development began with the formation of testisacs which were always initiated at a biomass of 70-90 mg. However, due to differences in growth rates this biomass was attained at 9, 13 and 24 weeks at 15°C and at 9, 13 and 25 weeks at 20°C in the high, medium and low food treatments respectively (Table 13).

The first appearance of ovisacs occurred 3 weeks after the initiation of testisacs in

all treatments. Leeches in the high and medium food treatments at both temperatures reached the final mature stage of spermatogenesis at approximately the same time (43 - 45 weeks). In the low food treatments the second stage of oogenesis and the third stage of spermatogenesis were the highest levels of development observed and reproductive maturity was not attained although an asymptote biomass was.

Figure 8. Mean (\pm SD) (n = 5) biomass of gonads (mg WWT) of *Nephelopsis* obscura over time (weeks) maintained in high (\blacksquare), medium (\checkmark) and low (\bullet) food treatments at (a) 15°C and (b) 20°C.



Table 12. Results of Tukey multiple comparison test determining the effects of food availability (high, medium and low) and temperature (15°C and 20°C) on mean (\pm SD) rate of gonad biomass increase (J.week⁻¹) in *Nephelopsis obscura*. Treatments connected with a solid line are not significantly different from one another.

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RATE OF GONAD BIOMASS INCREASE							
20 - L	15 - L	20 - M	15 - M	20 - H	15 - H		
0.10	0.86	3.98	3.55	6.10	6.96		
± 0.03	± 0.04	± 0.058	± 0.24	± 0.047	± 0.046		

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Table 13. Time (weeks) to the initiation of the six spermatic and three oogenic stages in *Nephelopsis obscura* in the high, medium and low food treatments at 15°C and 20°C.

		F	OOD TREATMEN	Т
	STAGE	LOW	MEDIUM	HIGH
	1	24	13	9
SPERMATIC	2	30	17	13
15°C	3	37	20	16
	4	-	24	20
	5	-	41	41
	6	-	43 .	43
OOGENIC	1	27	16	12
15%	2	37	20	17
15 C	3	-	27	23
SPERMATIC	1	25	13	9
20°C	2.	34	16	14
	3	43	23	19
	4	-	29	23
	5	-	41	43
	6	-	43	45
OOGENIC	1	28	16	12
ാസംവ	2	43	20	18
20 C	3	-	33	26

LIPID STORAGE

Leeches in the high and medium food treatments showed a similar pattern in energy storage with percent lipid increasing with time until week 30 after which there was a decline in lipid concentrations prior to maturation (Fig 9a and b and Fig 10a and b). Lipid storage continued to increase with time throughout the experiment in the low food treatments. Life-time rates of lipid deposition up to, and including, week 40 were calculated for each treatment (Table 14).

There was a highly significant effect of temperature (F = 242.51, df = 1), food (F = 907.63, df = 2) and a highly significant interaction (F = 21.27, df = 2). Within each temperature there were significant differences among food treatments with the rate of lipid deposition increasing with increasing food availability (Table 14). Within food treatments, leeches maintained at 15°C allocated more energy to lipid storage that those at 20°C.

Figure 9. Mean $(\pm SD)$ (n = 5) total lipid content (%) in Nephelopsis obscura over time (weeks) maintained in high (\blacksquare), medium (\checkmark) and low (\bullet) food treatments at (a) 15°C and (b) 20°C.

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Figure 10. Mean $(\pm SD)$ (n = 5) total lipid content (%) in Nephelopsis obscura over biomass (mg WWT) maintained in high (\blacksquare), medium (\checkmark) and low (\bullet) food treatments at (a) 15°C and (b) 20°C.



Table 14. Results of Tukey multiple comparison test determining the effects of food availability (high, medium and low) and temperature (15°C and 20°C) on mean (\pm SD) rate of lipid deposition (J.week¹) by *Nephelopsis obscura*. Treatments connected with a solid line are not significantly different from one another.

RATE OF LIPID DEPOSITION						
20 - L	15 - L	20 - M	15 - M	20 - H	15 - H	
2.36	4.72	11.79	16.51	15.33	22.40	
± 0.35	± 0.20	± 0.71	± 1.02	± 1.38	± 0.67	
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Life-time rates of energy allocated to the separate components of growth, i.e. somatic tissue, reproductive tissue and lipid storage (Fig 11) show that as food availability increases the amount of energy allocated to all three components of growth increases.

The proportion of energy allocated to these components was calculated as a percentage of growth energy for each treatment (Fig 12). The average proportion of growth energy allocated to lipid storage was $26.4\% \pm 4.9$ (n = 6) with $26.6\% \pm 2.7$ in the high and $25.0\% \pm 0.7$ in the medium food treatments. Leeches in the low food treatment at 15° C allocated proportionally more energy to lipids (35.3%) while those at 20° C allocated less (19.7\%) than in the other treatments.

The average proportion of energy allocated to lipids was approximately four times higher than the average proportion allocated to reproductive tissue ($6.6\% \pm 2.9$ (n = 6)). Animals in the high food treatments allocated the highest proportion of energy to reproduction ($9.3\% \pm 0.2$) compared with the medium food treatments ($6.9\% \pm$ 1.4). Leeches in the low food treatment at 15°C allocated a similar proportion of energy to reproduction as in the high and medium food treatments (6.4%), but at 20°C the proportion of growth energy allocated to reproduction was considerably lower (0.8%).

In all treatments the greatest proportion of growth energy was allocated to somatic growth (67.0% \pm 6.6) (n = 6). At 15°C leeches in the low food treatment allocated proportionally less to somatic tissue (58.3%) while at 20°C leeches in the low food treatment allocated proportionally more (79.5%) as compared to the high (64.1% \pm

2.5) and medium (68.1% \pm 0.7) food treatments.

Despite differences in the amount of energy allocated to different components of growth the pattern of energy allocation was the same in the medium and high food treatments at both temperatures. Among these treatments the same proportions of growth energy were allocated to somatic tissue, reproductive tissue and storage. However, leeches maintained in the low food treatments deviated from this general pattern according to temperature. At 15°C leeches allocated proportionally more to lipid storage at the expense of somatic growth. In contrast, at 20°C proportionally more energy was allocated to somatic tissues with less to lipid storage and reproductive growth.

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Figure 11. Rate of energy allocation (J.week¹) to lipids, reproductive and somatic growth by *Nephelopsis obscura* maintained in high (H), medium (M) and low (L) food treatments at 15°C and 20°C.

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- ⊠ Reproductive
- Somatic

Figure 12. Proportion of growth energy (%) allocated to lipids, reproductive and somatic growth by *Nephelopsis obscura* maintained in high (H), medium (M) and low (L) food treatments at 15°C and 20°C.



- 🛛 Lipid
- ⊠ Reproductive
- 🛛 Somatic

COMPONENTS OF RESPIRATION

RESTING AND ACTIVE RESPIRATION

Size specific resting (Rm) and active (Ra) respiration for leeches in all treatments declined with increasing biomass (Fig 13a and b and Fig 14a and b). For all treatments and body sizes active respiration was significantly higher (approximately double) than resting respiration.

For resting respiration there was a highly significant temperature (F = 99.47, df = 1), food (F = 14.41, df = 2) and time effect (F = 165.58, df = 3) with highly significant interactions between temperature and time and between food and time. Interactions between temperature and food and temperature, food and time were not significant. At both temperatures the cost of resting respiration was significantly lower in the low food treatments than in the high food treatments while the medium food treatments were not significantly different from either the low or the high food treatments (Table 15). Resting respiration was significantly higher at 20°C than at 15°C.

A similar pattern was seen for active respiration with highly significant temperature (F = 61.76, df = 1), food (F = 18.60, df = 2) and time effects (F = 187.14, df = 3), highly significant interactions between temperature and time and food and time and non-significant interactions between temperature and food and temperature and food and time. At 15°C active respiration costs of leeches in the low food treatment were significantly lower than in the high and medium food treatments which were not significantly different from each other (Table 15). At 20°C leeches in the low food treatment had significantly lower active respiration than those in the high food treatment and respiration of leeches in the medium food treatment was not significantly different from either the low or high food treatments (Table 15). Active respiration was significantly higher at 20°C than 15°C for each respective food level.

Scope for activity (SfA) was calculated as:

$$SfA = Ra - Rm$$

Where: Rm = cost of resting respiration ($\mu l O_2.mg DWT^{-1}.h^{-1}$)

Ra = cost of active respiration (μ l O₂.mg DWT⁻¹.h⁻¹)

SfA declined with increasing biomass in all treatments (Fig 15a and b) and also declined with declining food availability. There was a highly significant effect of food (F = 7.61, df = 2), and time (F = 50.88, df = 3) but no significant effect of temperature (F = 3.82, df = 1). Significant interactions were found between temperature and time and food and time, but not between temperature and food and temperature, food and time. SfA of leeches in the low food treatments at 15°C was significantly lower than leeches in the high and medium food treatments at 20°C (Table 15). There was no significant difference in SfA among leeches maintained in the high and medium food treatments at 15°C or in any of the food treatments at 20°C. Figure 13. Mean (\pm SD) (n = 6) resting rate of oxygen consumption

(μ l O₂.mg DWT⁻¹.h⁻¹) for *Nephelopsis obscura* over time (weeks) maintained in high

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(\blacksquare), medium (\checkmark) and low (\bullet) food treatments at (a) 15°C and (b) 20°C.



Figure 14. Mean (\pm SD) (n = 6) active rate of oxygen consumption

(μ l O₂.mg DWT⁻¹.h⁻¹) for Nephelopsis obscura over time (weeks) maintained in high

(\blacksquare), medium (\checkmark) and low (\bullet) food treatments at (a) 15°C and (b) 20°C.



Figure 15. Mean (± SD) (n = 6) scope for activity (μl O₂.mg DWT⁻¹.h⁻¹) for
Nephelopsis obscura over time (weeks) maintained in high (■), medium (▼) and low
(●) food treatments at (a) 15°C and (b) 20°C.

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Table 15. Results of Tukey multiple comparison test determining the effects of food availability (high, medium and low) and temperature (15°C and 20°C) on resting and active rates of oxygen consumption (μ l O₂.mg DWT⁻¹.h⁻¹) and scope for activity for *Nephelopsis obscura*. Treatments connected with a solid line are not significantly different from one another.



TIME SPENT ACTIVE

Total number of hours spent active per day was calculated for each treatment (Table 16). There was a highly significant effect of food availability (F = 32.68, df = 2) but the effects of temperature (F = 0.14, df = 1) and the interaction between food and temperature (F = 1.46, df = 2) were not significant. There was no significant difference in activity time between leeches in the high and medium food treatments at either temperature (Table 17). Leeches in the low food treatments were significantly more active than those in the other food treatments at both temperatures (Table 17).

In the high and medium food treatments leeches were more active in the dark than in the light as shown by Davies and Kasserra (1989) and Anholt and Davies (1986). These differences were highly significant at 15°C (high food: t = 3.39, df = 18, medium food: t = 3.75, df = 18) and at 20°C (high food: t = 2.55, df = 18, medium food: t = 3.47, df = 18). However, no significant difference between light and dark activity occurred in leeches with low food availability (15°C: t = 1.30, df = 18, 20°C: t = 0.24, df = 18).

Table 16. Mean (\pm SD) total activity time in the light and dark and total per day (h)
by Nephelopsis obscura maintained in high, medium and low food treatments at 15°C
and 20°C.

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	TOTAL ACTIVITY TIME (h)			
15°C	LOW	MEDIUM	HIGH	
LIGHT	6.31 ± 2.70	1.15 ± 0.97	0.90 ± 0.73	
DARK	8.10 ± 3.12	4.38 ± 2.39	4.49 ± 3.10	
24 h	14.41 ± 4.13	5.53 ± 2.58	5.39 ± 3.18	
20°C	LOW	MEDIUM	HIGH	
LIGHT	5.86 ± 2.70	1.58 ± 0.91	1.39 ± 1.10	
DARK	6.16 ± 2.81	4.87 ± 2.70	4.50 ± 3.49	
24 h	12.02 ± 3.90	6.45 ± 2.85	5.89 ± 3.66	

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Table 17. Results of Tukey multiple comparison test determining the effects of food availability (high, medium and low) and temperature (15°C and 20°C) on total activity time per day (h) of *Nephelopsis obscura*. Treatments connected with a solid line are not significantly different from one another.

TOTAL ACTIVITY TIME					
20 - L	15 - L	20 - M	15 - M	20 - H	15 - H
		•			<u> </u>

DISCUSSION

Predators do not necessarily consume prey in direct proportion to their abundance in the environment (Kohler and Ney 1982; MacDonald and Green 1986). Whether a particular prey item is consumed by *N. obscura* depends on the probability that it will be encountered by the predator, the probability that once encountered the predator will attack the prey, and the probability that an attacked prey will be captured (Anholt 1986). For prey to appear in the diet, they must be available and accessible given the constraints of the morphology and sensory capacities of the predator. Consequently food ingestion reflects prey availability rather than absolute prey abundance in the environment. To ensure a consistent and super-abundant food supply an *ad libitum* quantity of prey (*T. tubifex*) was provided throughout each feeding period. In addition, the *ad libitum* supply of prey ensured that intra-specific exploitative competition was not a confounding factor.

In freshwater ecosystems, benthic prey distribution is patchy both spatially and temporally (Hildrew and Townsend 1982) and many invertebrates experience variable inter-feeding intervals including leeches (Wrona and Calow 1988), triclads (Calow 1980), and blood-sucking insects (Calow 1981). In Stephenson's Pond, benthic macroinvertebrate distribution is patchy horizontally and vertically within the substrate and also seasonally (Rasmussen 1983). This study contrasts the optimal pattern of energy acquisition and allocation in different environments, where prey patches are of the same type (i.e., quantity and quality of prey) but with differences in the frequency of encounter of the patches (i.e., meal periodicity).
GROWTH

The pattern of growth shown by N. obscura is the same regardless of food availability with asymptotic biomass reached after the same period of time in all treatments. Although N. obscura exhibits phenotypic plasticity in several life-history traits, including asymptotic biomass, the pattern of growth and time to asymptote biomass were consistent. Dratnal and Davies (1990) found that time to asymptote biomass was affected by exposure to winter stress with animals exposed to the stress taking 48 d longer to reach maturity than summer animals. However, since overwintered leeches were exposed to the stress before the start of the experiment they were not the same age and had different amounts of lipid storage than the summer animals at the start of the experiment (Dratnal and Davies 1990). Therefore the sigmoidal pattern of growth in N. obscura (with size decelerating onto a steadystate after an initial period of rapid growth) does not change and in the low food treatments asymptote biomass was reached even though sexual maturity was not attained. However, time to reach asymptote biomass is flexible and is affected by prior exposure to stress and is shorter for N. obscura maintained as individuals, compared to those in larger groups (Chapter 4).

Food availability in the medium food treatments was 33% lower and in the low food treatments was 66% lower than in the high food treatments. However, at 15°C leeches in the medium food treatment attained an asymptote biomass only 13.2% lower than in the high food treatment and in the low food treatment leeches had an asymptote biomass 78.0% lower than leeches in the high food treatment. At 20°C the asymptote biomass of leeches in the medium food treatment was 25% lower and in the low food treatment was 81% lower than *N. obscura* in the high food treatments. Leeches in the medium food treatments show some compensation in response to the reduction in food availability in terms of growth. However, the reduction in growth of leeches in the low food treatments is even greater than the reduction in food availability indicating that they are putting proportionally more energy into another component of the energy budget.

Leeches in the high and medium food treatments had higher growth rates and final biomasses at 15°C than at 20°C. These differences in growth among treatments could be due either to differences in energy acquisition and/or allocation of energy. FEEDING

At 15°C, weekly food ingestion rates for leeches in the medium food treatments were 31.2% lower and at 20°C 37.7% lower than in the high food treatments. These values are similar to the 33% reduction in food availability. Since the reduction in growth was not as high as the reduction in energy intake, *N. obscura* in the medium food treatments must allocate proportionally more energy to growth than leeches in the high food treatments. In the low food treatments there was a 66% reduction in food availability compared to the high food treatments with weekly food ingestion rates 70.2% and 78.4% lower at 15°C and 20°C, respectively. This partially accounts for the reduced growth rates of *N. obscura* in the low food treatments compared to the high food treatments compared to the high food treatments compared to the high food treatments of *N. obscura* in the low food treatments compared to the high food treatments, but since the reduction in growth was greater than the reduction in feeding, a smaller proportion of acquired energy is allocated to growth at

the lowest level of prey availability.

Although total food ingestion per week was greatest in the high food treatments, N. obscura ingested more prey per meal at the lowest levels of food availability which partially offset the lower number of meals per week. For animals of the same biomass, leeches receiving one meal ingested the same amount of food per week as leeches consuming two meals. For other invertebrates, as the time between consecutive meals increases, feeding time lengthens and meal size increases (Cook and Cockrell 1978; Calow 1981; Bailey 1986). Whether or not gut-filling is favoured as an optimal feeding strategy will depend on the prey exploited and the way in which the predator exploits it. For actively feeding freshwater invertebrate predators where prey encounter probabilities are high, gut-filling may not be the most optimal strategy (e.g. Notonecta (Hemiptera: Notonectidae) (Cook and Cockrell 1978; Kruse 1983) and Ranatra dispar (L.) (Heteroptera: Nepidae) (Bailey 1986). However, for organisms adopting a sit-and-wait foraging strategy, encountering periodic food shortages, a gut-filling strategy may be used as an adaptation to compensate for the infrequent but potentially large meals (Wrona and Calow 1988).

For sockeye salmon, *Oncorhynchus nerka* (Walbaum), food deprivation intervals of up to 25 h elicited larger meal sizes but deprivation beyond this do not (Brett 1971). The deprivation time associated with maximum meal size for sockeye salmon was about equal to the time to empty the stomach and Brett (1971) concluded that meal size consumed was dependent on the additional quantity of food that could be fit into the stomach (gastric emptying hypothesis). Tyler and Dunn (1976) found that in the flounder, *Pseudopleuronectes americanus* (Walbaum), the longer food was withheld, up to a 7 d deprivation interval, the more was consumed per meal. They suggested that for fish with a low scope for activity (i.e. low capacity for increasing respiration rate above resting) (Fry 1947; 1971) there could be limitations on meal size due to specific dynamic action (S.D.A. hypothesis). The importance of S.D.A. is that ingestion, digestion and post-digestive processes (such as deamination of proteins and synthesis of new tissue) impose a substantial metabolic cost which has to be met before other aerobic work can be done (Wootton 1990). When the difference between resting respiration and maximum respiration rate is small, the capacity for respiration rise due to S.D.A., and consequently meal size, will be restricted. Since the duration of S.D.A. is no more than 19 h in *N. obscura* at 20°C (Kalarani and Davies 1994), the increase in meal size with declining ration cannot be explained by either the gastric emptying hypothesis or the S.D.A. hypothesis.

Weekly size-specific food ingestion was lower in the low food treatments than in the other treatments until week 10 at 20°C and week 12 at 15°C after which time it was higher. This suggests that early on their life-history *N. obscura* in the low food treatments experience a foraging constraint and are unable to compensate for reductions in feeding frequency until a larger biomass is attained. During the period for which size specific food ingestion was lower than the other treatments, leeches in the low food treatments were less than 19.9 ± 6.7 mg WWT. *Nephelopsis obscura* less than 20 mg are not able to swallow prey whole since they have a smaller mouth diameter (approximately 0.5 mm) (Wrona 1982) than the width of the average sized T. tubifex (0.5 - 1.0 mm) (Brinkhurst and Gelder 1991). Therefore increased ingestion per meal cannot occur until a larger body size, and hence mouth diameter has been attained and prey handling efficiency increases. Greene (1974) noted that for *Erpobdella octoculata*, if prey are small and easily swallowed, the leech generally continued searching for food until two or three items were consumed within a period of 1 or 2 h. However, for smaller leeches swallowing prey was more difficult and required as long as 10 min before completion. When ingestion took longer than 1 min to complete, *E. octoculata* stopped foraging and remained inactive for many hours (Greene 1974).

Rates of food ingestion increase as temperature increases up to a maximum, with further increases in temperature resulting in a rapid decrease in consumption (Elliott 1975a, b, 1981). Kalarani and Davies (1994) found that energy ingested by small (40 mg WWT) *N. obscura* increased with increase in temperature from 11.6 ± 1.3 J.day⁻¹ at 5°C to a maximum of 41.9 ± 1.8 J.day⁻¹ at 15°C and decreased to 34.2 ± 1.7 and 19.0 ± 1.5 J.day⁻¹ at 20 and 25°C respectively indicating that the optimum temperature for feeding in *N. obscura* is between 15° and 20°C. In this study the rate of food ingestion was the same at both temperatures supporting the view that the optimum temperature for feeding lies between 15° and 20°C. The different growth rates of leeches maintained 15° and 20°C cannot be explained by differences in energy acquisition but must be due to differences in energy allocation.

ABSORPTION

When food availability decreased, absorption efficiency increased. An important

determinant of digestibility and hence how much energy is absorbed from prey is the rate of transport through the gut (Calow 1981; Townsend and Calow 1981). Sibly (1981) predicted that a reduction in food availability or food quality should result in a longer retention time of food in the gut and consequently a greater absorption efficiency. In response to changes in food supply the freshwater snail *Ancylus fluvatilis* Müller showed increased absorption efficiency (Calow 1977) as did *Philoscia musrorum* (Scopoli), a terrestrial isopod (Hassall and Jennings 1975). As the number of meals per week increases the rate of passage of food through the digestive system of *N. obscura* increases, leading to a reduction in efficiency.

In the early stages of the life cycle absorption efficiency was lower in all treatments. This could result from the inefficiency of enzyme secretions in juvenile leeches or from reductions in enzyme production relative to body size. Differences in enzyme production could also result from differences in food quantity and quality (Baker 1978). Absorption efficiencies were the same at both temperatures and since absorption efficiencies of many invertebrates tend to be independent of external temperature over the normal range (Calow 1981), this suggests that digestive enzymes may be adapted to the temperature regimes in which they operate and/or that several enzymes with different optimum temperatures may be involved in the digestion process.

Although the relative proportion of acquired energy allocated to growth and respiration increased, the actual amount of energy available for use decreased as food availability decreased. There was no difference in either the total amount or the relative proportion of acquired energy allocated to growth and respiration at different temperatures.

REPRODUCTION

In all treatments reproduction commenced with the development of testisacs followed by ovisacs confirming the sequential protandrous hermaphroditism recorded by Singhal, Davies and Baird (1985) and Davies and Singhal (1988). Testisacs first occurred in animals of similar biomass although for each treatment this was at a different time due to the different growth rates. The subsequent occurrence of ovisacs was not biomass dependent but time dependent, with ovisac formation following 3 weeks after testisac initiation. Sexual maturity was reached at different biomasses but at the same time in both high and medium treatments regardless of temperature. Leeches in the low food experiment failed to reach maturity by the end of the experiment, even though an asymptotic biomass was attained.

In some species maturity is associated with a minimal size rather than age (Moll 1979; Gibbons and Greene 1990) while in other species age is the determining variable and size is relatively fixed (Ford and Seigel 1994). Size or age at first reproduction will also be modified by environmental factors such as diet. For example, individual corn snakes on a low-energy diet grew more slowly, matured at a later age, and had smaller clutch sizes than did females on a high energy diet although size at reproduction was the same (Ford and Seigel 1994). However, Stearns and Koella (1986) suggested that most organisms mature neither at a fixed size nor a fixed age, but along an age-size trajectory. In *N. obscura* since testisacs first

developed in leeches of a similar biomass regardless of their age it seems that initiation of the reproductive organs requires a minimum size threshold and that age is not important. However, reproductive maturity was reached by leeches in the high and medium food treatments at the same time although at different biomasses, suggesting that maturity is age rather than size dependent.

The increase in gonad growth associated with the increase in ration from medium to high food availability was greater at 15°C (96% increase) than at 20°C (53% increase). Baird, Linton and Davies (1987) similarly found that at 15°C N. obscura allocated increasingly more energy to reproduction as ration levels increased but at 20°C the rate of energy allocation to reproduction declined at high rations. If all other environmental conditions are constant adult N. obscura has a greater probability of dying after cocoon production at 20°C than at 15°C (Baird, Linton and Davies 1986) and it might be expected that N. obscura at 20°C would put more energy into reproduction than at 15°C. However, the relative proportion of energy allocated to reproduction was the same at both temperatures not supporting the prediction above. The differences in mortality at 15° and 20°C are however likely the result of differential storage of total lipids by N. obscura at 15° and 20°C. At 15°C N. obscura behaves as predicted for a potentially iteroparous species, with a reduced rate of energy allocation to reproduction as ration declines (Baird, Linton and Davies 1987). In addition to the higher energy allocation to gonad production in the high food treatments the relative proportion of growth energy allocated to reproduction was also higher than in the medium food treatments at both temperatures.

In the low food treatments the total amount of energy allocated to reproduction was reduced particularly at 20°C where the proportion of energy allocated to reproduction accounted for less than 1% of total energy allocated to growth. By allocating less energy to reproduction, more is available for somatic growth and energy storage, thus increasing chances of survival.

Leeches in the high food treatments matured at a larger size and since body size is related to fecundity (Linton and Davies 1987) and survivorship (Davies, Yang and Wrona 1987; Cywinska and Davies 1989) presumably had the highest fitness. However, reducing the ration from three to two meals per week did not reduce mature size proportionally. Leeches in the medium food treatment showed compensation in terms of growth and timing of reproduction, but since gonad biomass and total lipid concentrations were significantly reduced, fecundity and fitness was presumably reduced. In the low food treatments growth and gamete development were severely reduced and *N. obscura* did not attain sexual maturity.

STORAGE

Animals store lipid primarily for when energetic demands exceed immediate availability from ingestion i.e., during reproduction, or when the external food supply is inadequate or unpredictable (Pond 1981; Dratnal and Davies 1990; Larson 1991). In *N. obscura* total lipid concentrations in the high and medium food treatments declined just prior to maturation when total lipid was presumably utilized for gonad maturation (Reddy *et al.* 1992). In the low food treatment at 15°C, *N.obscura* did not show a significantly lower lipid storage with increasing biomass indicating that during resource shortage leeches allocate a larger proportion of their total energy assimilated to storage than to somatic growth than when food was abundant, presumably in an attempt to reach the levels necessary for reproduction and/or as a hedge against the potential of further starvation.

However, at 20°C leeches in the low food treatments showed a different response, allocating proportionally less energy to storage and reproduction and more to somatic tissue. In the field the decreasing temperatures associated with the onset of autumn indicates the approach of winter conditions and in combination with the reduced levels of prey availability appears to be a stimulus to which *N. obscura* responds by switching energy allocation from somatic growth to storage and reproductive growth.

At 15°C total lipid concentrations were less than 10% until week 25 in leeches in the low food treatment and at 20°C were less than 10% throughout the entire life. Low lipid levels could be an explanation for restrained sexual development of these leeches. It is possible that a threshold level of total lipid and/or biomass must be attained before the start of the final stages of maturation in *N. obscura*, as shown for some fish species (Eliasson and Vahl 1982; Simpson 1992).

Some authors have proposed that organisms store energy only when it occurs in excess of maximum feasible growth (Cohen and Parnas 1976) and therefore within any one species, the organisms in the areas with the largest and most constant food supply would have the most storage material. Alternative evidence suggests that the opposite is true and that lipid storage may occur at the expense of other forms of growth, especially when prey is in short supply (Forsman and Lindell 1991). *Nephelopsis obscura* maintained in the low food treatment at 15°C allocated proportionally more growth energy to lipid storage and those at 20°C allocated proportionally less compared to leeches in the other treatments maintained at the same temperatures. Thus, in *N. obscura* energy is only allocated to lipid storage at the expense of somatic and reproductive growth at low prey availability in association with lower temperatures. In the medium and high food treatments although the total amount of energy allocated to lipid storage increased with increasing food ration the relative proportion of growth energy allocated to storage was constant.

RESPIRATION

Bioenergetic models often assume that the rate of catabolism is dependent only upon body weight and ambient temperature (eg Kitchell, Stewart and Weininger 1977; Adams, McLean and Parrotta 1982; Hewett and Johnson 1987; Beauchamp, Stewart and Thomas 1989). However, resting and active costs of respiration in *N. obscura* maintained at lower levels of food availability were significantly lower with leeches in the low food availability treatments allocating less energy to respiration per hour than animals of a similar biomass in the medium food treatment which similarly allocated less than leeches in the high food treatment. *Nephelopsis obscura* adapted to reduced levels of energy intake by lowering resting respiration rate. Thus, respiration rates are a function of feeding regime as well as body size and this should be incorporated into laboratory derived estimates of metabolism. Although leeches in the low food treatments allocated less energy to total respiration than leeches in the medium and high food treatments they allocated proportionally more in terms of percentage of ingested energy thus reducing the amount of available energy for growth.

Since leeches are obligate ectotherms, a rise in temperature increases respiration rate. Leeches in the medium and high food treatments maintained at 20°C had higher total respiration costs and allocated proportionally more acquired energy to respiration than those at 15°C, accounting for their slower growth. Kalarani and Davies (1994) similarly found that for *N. obscura* the amount of energy available for growth and activity was highest at 15°C when compared to 5°C, 10°C, 20°C and 25°C. In the low food treatments the proportion of energy allocated to total respiration was similar at both temperatures.

Although size-specific respiration costs were lowest in leeches maintained in the low food treatment, activity time was significantly higher than in the high and medium food treatments. In the latter two food treatments *N. obscura* adopts a sit-and-wait foraging strategy (Davies and Kasserra 1989). This type of feeding strategy minimizes the proportion of consumed energy used for active metabolic demands and maximise assimilated energy for growth. However, in the low food treatment when meals are scarce and infrequent an active foraging strategy is adopted (Davies and Kasserra 1989) with increased activity increasing the probability of finding prey. It has been suggested that for *N. obscura* longer starvation periods may result in the reduction of activity (McLoughlin and Davies 1992), but during the initial phase of food shortage increased locomotory activity might be a prerequisite for obtaining the

little food that is still available. Such a strategy has been described for larval cyprinids (Wieser et al. 1988) and a trade-off must be sought between these two options.

Scope for activity (SfA) (the difference between resting and active respiration) declined with declining food availability indicating a reduction in the amount of energy potentially available for aerobic activities, including active foraging, avoiding predation and location of suitable microhabitats, beyond the resting level.

SCOPE FOR GROWTH

Maltby and Naylor (1990) suggested that scope for growth (SfG = A - RT) is a good indicator of stress. However, to calculate SfG accurate measures of absorption (A) and total respiration (RT) are necessary. There are two methods of calculating RT, either as the difference between energy absorbed and energy utilized for growth (RT = A - G) or from hourly resting and active respiration costs and time of activity (RT = (Rm x Tm) + (Ra + Ta)).

By definition, SfG indicates the energy available for growth, and thus in experiments where growth of test organisms is directly measured, calculation of SfG based on life-time TR determined by difference is tautological. One proposed advantage of SfG over direct measurement of growth is that results can be obtained rapidly (hours or days depending on the species) negating the need for longer experiments (Naylor, Maltby and Calow 1989).

Maltby, Naylor and Calow (1990a, b) and Maltby and Naylor (1990) calculated RT with oxygen uptake, measured using a flow-through respirometer (after Wrona and Davies 1984), converted from a rate per h to a rate per d. Small errors in measuring the respiration rate would result in large errors in the energy balance and moreover the assumption that activity does not vary over the 24 h period is not likely valid. Indeed, light is important in initiating, controlling and orientating activity patterns (Zaret and Suffern 1976) and in the field many species are only active during limited periods of the diel cycle (Wootton 1990).

Studies of the relationship between short-term SfG and long-term whole organism responses (i.e. growth and fecundity) indicate that this relationship is complex. For example, Maltby and Naylor (1990) used brooding female *G. pulex* to evaluate how short-term changes in SfG related to longer term changes in reproduction after exposure to zinc stress for 3-4 weeks. Although there was a significant reduction in SfG over 6 d during exposure to 0.3 mg Zn.1⁻¹, there was no effect on either the size or the number of offspring released from the current brood. Offspring produced from subsequent broods were smaller but there was no significant difference in the number of offspring produced between previously stressed and non-stressed individuals. That short-term changes in SfG of test organisms accurately reflects growth or reproduction at later stages of the life-cycle is doubtful and has not been confirmed. Consequently, SfG is not a valid alternative to measuring actual growth, since important information on life-time growth pattern, size and number of offspring, etc. cannot be predicted. SUMMARY

When maintained at different levels of prey availability, *N. obscura* adjusted energy intake and differentially allocates energy to components of the bioenergetic

budget. As a result of higher absorption efficiency, and a lower proportion of absorbed energy allocated to total respiration leeches in the medium food treatments showed partial compensation to maintain growth and timing of reproduction. Although the amount of energy allocated to lipid storage, somatic and reproductive growth was lower, the relative proportion of energy allocated to each component of growth was the same as leeches in the high food treatments. This pattern was unaffected by temperature. Within the high and medium food treatments leeches had faster growth at 15°C than at 20°C resulting from a greater proportion of available energy allocated to respiration.

Although leeches in the low food treatment responded by ingesting more food per meal and adjusted several aspects of energy allocation, proportionally more energy was allocated to active metabolism at the expense of growth and potential fecundity. In the low food treatments the proportion of energy allocated to growth and respiration was the same regardless of temperature. However, different strategies were seen in energy allocation between components of growth. At 15°C energy allocation to total lipid storage was prioritized at the expense of somatic growth and based on other evidence resulted in individuals better able to survive further starvation or winter conditions. Low food availability in association with lower temperatures acts as a cue signalling the approach of winter conditions and results in *N. obscura* adjusting energy allocation and compensating to increase survival probability, thus maintaining the potential for future fecundity. However, at 20°C energy allocation to lipid storage and reproduction were minimal and a greater proportion was allocated to

somatic growth. These differences in allocation patterns could also be a result of differences in post-reproductive mortality at different temperatures with N. obscura having a much better chance of surviving breeding at 15°C than at 20°C.

CHAPTER FOUR

THE INFLUENCE OF GROUP SIZE ON THE BIOENERGETICS OF NEPHELOPSIS OBSCURA

INTRODUCTION

There is a tendency for some species to form temporary or permanent aggregations or groups (Pulliam and Caraco 1984; Packer and Ruttan 1988), with a group defined as any set of organisms belonging to the same species that remain together for a period of time interacting with one another to a greater degree than with other conspecifics (Wilson 1975). Group size can vary through time seasonally, daily or even hourly in many species (Puliam and Caraco 1984) and may or may not track environmental periodicities.

A diversity of both sessile and motile benthic taxa have been observed to live in groups (Stoner and Ray 1993) including freshwater leeches. *Helobdella stagnalis* (L.) and *Mooreobdella microstoma* (Moore) were observed in groups both in the laboratory, with as many as 50 or 100 individuals composing a single group, and in the field (Gee 1913). Sampling of the benthic macrofauna in a stony lake using 17 x 17.5 cm tiles (n = 16) showed that *Erpobedella octoculata* (L.), *H. stagnalis* and *Glossiphonia complanata* (L.) were most often found in single species clumps (Lang 1974). Mean group size measured during each of three summer months at various depths (6 - 25 m) ranged from a maximum 48 \pm 25 to a minimum 12 \pm 12 for *H. stagnalis*, from 34 \pm 20 to 14 \pm 11 for *G. complanata* and from 12 \pm 3 to 2 \pm 0.5

for *E. octoculata*. Group size generally declined with increasing water depth (Lang 1974). In the field *N. obscura* is typically found in groups of generally two to ten individuals under stones and in the laboratory *N. obscura* frequently form a single group, often several animals deep, usually in one corner of the aquarium (personal observations).

The potential advantages of group membership are diverse (Bertram 1978; Puliam and Caraco 1984; Folt 1987; Inman and Krebs 1987): it may reduce individual risk of predation due to enhanced detection and avoidance of, or escape from, predators; aggregation may also enhance mating and food intake, ameliorate physiological stress, coordinate and enhance dispersal, or synchronize development and emergence (Trowbridge 1991).

Although many studies have considered the consequences of group living in mammals with complicated social systems, the underlying mechanisms of aggregation are unclear and could result from individuals inadvertently responding in a similar way to a set of environmental conditions such as temperature, light or food (passive process) or from individuals responding behaviorally to one another (active process) or some combination of these (Wrona and Dixon 1990). Reasons for group formation in leeches have received little attention since Gee (1913) noted that the joint effects of positive thigmotaxis and negative phototaxis were important determinants of aggregation in *H. stagnalis* and *M. microstoma*. Aggregation in triclads was found to be mediated by visual and chemical processes (Reynierse, Gleason and Ottemann 1969) resulting from the combined effects of chemotaxis, photokinesis (Reynierse

1966) and distinctive morphology (Reynierse and Scavio 1968).

Several studies have shown that living in a group may not be beneficial for all individuals and can involve costs (Valone 1993). In the sea slug, Placida dendritica (Alder and Hancock) while small slugs always benefitted from the presence of conspecifics, large slugs benefitted only if conspecifics were comparable in size. The reduction in growth of large P. dendritica in the presence of small conspecifics was apparently due to reduced feeding effectiveness and not by prevention of feeding per se (Trowbridge 1991). Similarly, as group size of the triclad Dugesia tigrina Girard increased, daily per capita ingestion rates first increased but peaked at a group size of four then decreased as group size increased. Possible mechanisms for this include a reduction in activity and hence reduced encounter rates, and/or an increase in prey mortality with a subsequent reduction in prey availability at larger group sizes (Cash, Wrona and McKee 1993). Other potential costs of group living include increased conspicuousness to predators, competition for food and mates, higher incidence of contagious disease and parasitism or increased risk of predation on young by cannibalistic neighbours (Pulliam and Caraco 1984; Krebs and Davies 1987)

Despite the widespread occurrence of aggregative behaviour in many invertebrates, the reasons for the formation and maintenance of groups and their longterm consequences are not well understood. With the exception of Cash, McKee and Wrona (1993) who found that individual *D. tigrina* in groups of four produced significantly more and heavier tail buds than solitary individuals, and Trowbridge (1991) who examined the effects of group size and composition on growth of *P*. *dendritica*, studies have not established a link between group membership and correlates of fitness. The objective of this study was to investigate acquisition and allocation of energy by *N. obscura* in different group sizes to evaluate the physiological costs and/or benefits of group living.

Specifically, the following questions have been examined:

- (i) What are the physiological effects of aggregative behaviour in N. obscura?
 - (a) Which aspects of energy acquisition and allocation are altered as a result of changes in group size?
 - (b) Are there any benefits of living in a group?
 - (c) Are there any disadvantages of living in a group?
- (ii) Do the alterations in the energy balance change through the life cycle?
- (iii) Is there an optimum group size?

METHODS

The energy acquisition and allocation of N. obscura maintained individually has already been examined (Davies and Kalarani 1993; Oian and Davies 1994). Cocoons were collected immediately after deposition on macrophytes in late June, brought back into the laboratory and maintained at 20°C, 100% oxygen saturation and 12 h:12 h light:dark photoperiod regime until they hatched. Hatchlings were maintained under identical laboratory conditions with ad libitum prey (T. tubifex). Leeches randomly selected (mean size 11.0 ± 0.1 mg WWT) were individually maintained in plastic containers (30 cm²) with aerated pond water (no substrate), until the leeches reached sexual maturity (Qian and Davies 1994). Weekly measured WWT of leeches provided information on growth, and the difference between initial and final WWT of T. tubifex supplied for a 3 h period three times a week (corrected for prey losses due to handling) gave food ingestion (Qian and Davies 1994). Respiration (Rm, Ra and aerobic scope) of comparable animals previously starved for 48 h was measured using a flow-through respirometer (Chapter 2) at six stages in the life history (Davies and Kalarani 1993). Neither Qian and Davies (1994) nor Davies and Kalarani (1993) measured activity time for single N. obscura. Activity times for individual leeches determined by Anholt and Davies (1986) and Davies and Kasserra (1989) cannot be compared to results from this study because of differences in leech pre-history (feeding regime and body size) and experimental conditions (acclimation time, temperature and prey species) between studies. Total lipid was measured at the asymptotic growth stage (Qian and Davies 1994).

Experiments investigating the effects of group size greater than one were designed using the above experimental conditions and methodology as the standard. COLLECTION AND CULTURE

Nephelopsis obscura cocoons were collected from Stephenson's Pond shortly after they had been deposited on macrophyte leaves and maintained in aerated (100% oxygen saturation) pond water at 20°C with a 12 h:12 h light:dark regime. After approximately 4 weeks the cocoons hatched and the hatchlings were provided with *ad libitum T. tubifex* for 3 h three times a week until they reached approximately 10 mg WWT when they were transferred to experimental containers.

FEEDING AND GROWTH EXPERIMENTS

Five groups, based on the number of leeches per container (5, 10, 25, 50 or 100) were established with three replicates per treatment. Since container size and surface area were constant (area = 400 cm², volume = 4000 cm³), with the increase in the number of leeches there is a corresponding increase in density. Density of leeches in the treatments with 10, 25 and 50 leeches were 250, 625, and 1250.m⁻² respectively, representative of densities found in the field, while the treatment with 5 leeches was lower (125.m⁻²) and 100 leeches higher (2500.m⁻²) than densities encountered in the field (Rasmussen 1983; Gates *et al.* 1987).

As mud-dwelling *T. tubifex* withdraw rapidly into their tubes when disturbed by *N. obscura* (Dratnal, Dratnal and Davies 1992) *T. tubifex* were maintained without a substratum to ensure that foraging by individual *N. obscura* was not constrained by anti-predatory responses by prey resulting from the activities of conspecifics.

Leeches were fed an *ad libitum* supply of *T. tubifex* three times a week throughout their life-cycle. A ration of food, estimated to be at least twice the maximum amount that could potentially be ingested, was added to each container for 3 h. Food ingestion per individual leech was estimated from the total mass of *T. tubifex* ingested divided by the number of leeches present in the container. Corrections were made for changes in *T. tubifex* biomass caused by handling and exposure to experimental conditions (Chapter 2).

Each week the biomass of the leeches was determined by removing excess water and weighing (\pm 0.1 mg). In treatments containing 25 animals or less, all of the leeches were weighed each week but in treatments containing 50 or 100 animals, 25 leeches were randomly selected and weighed before being returned to the appropriate container.

RESPIRATION

Active and resting respiration (see Chapter 2) was measured collectively for all leeches from each replicate in each treatment i.e. 5, 10, 25, 50 or 100. Respiration per individual leech was estimated by dividing by the number of leeches in the respirometer chamber. Progressively larger chamber sizes were used as group size and biomass of *N. obscura* increased. Respiration was measured once every 8 weeks until animals reached asymptotic biomass or until the combined mass of the leeches could no longer be accommodated in the respirometer chamber (maximum size gastight glass syringe available was 50 ml). Activity of the animals was recorded using a video camera (Chapter 2). Total activity was calculated as the sum of locomotory

movement and random movement (Chapter 2).

LIPIDS

To determine energy storage in animals at the start of the experiment, lipids were measured for 20 individuals maintained in the same pre-experimental conditions and of the same initial size as the experimental animals. Five leeches from each container were sacrificed and analyzed for lipids (Chapter 2) at the end of the experiment when the leeches had attained asymptotic biomass.

STATISTICS

Statistical analyses for groups 5 - 50 were performed on treatment means (n = 3). Growth, coefficients of variation, food ingestion and lipids were analyzed using one-way ANOVA and two-way ANOVA used to analyze respiration and activity. When the null hypothesis of equality between means was rejected, a Tukey multiple comparison was used to determine among which population means the differences occurred.

RESULTS

GROWTH

In all groups *N. obscura* showed a sigmoidal growth pattern (Fig 16 and Fig 17). Growth rates (J.week⁻¹) for each consecutive 10 week period and for the entire length of the experiment (life-time) were calculated (Table 18).

There was a highly significant effect of group size (F = 26.90, df = 5) on total growth rates calculated over the life-time. Maximum growth rate occurred in groups of 5 and declined as group size increased from 5 - 100. There was no significant difference in total growth rate between groups of 1 and 10, 25 or 50 (Table 19). Growth of groups of 5 was significantly higher than groups 25, 50 and 100, groups of 10 significantly higher than 50 and 100 and groups of 25 significantly higher than groups of 100, with all other differences not significant (Table 19).

N. obscura maintained throughout their life cycle as individuals matured after 26 weeks at a biomass of 742 \pm 415 mg (Qian and Davies 1994) (Fig 16). As a result of the differences in growth rate asymptote biomass was reached at significantly different sizes (1926.7 \pm 82.4 mg, 1731.7 \pm 173.6 mg, 1543.0 \pm 56.5 mg, 1281.8 \pm 102.6 mg, and 1148.5 \pm 42.9 mg in groups of 5, 10, 25, 50 and 100 respectively) although the time to reach asymptote biomass was constant (38 weeks) (Fig 17).

Figure 16. Mean $(\pm SD)$ (n = 14) biomass (mg WWT) of Nephelopsis obscura over time (weeks) maintained as single animals (*) (after Qian and Davies 1994).

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Figure 17. Mean (\pm SD) (n = 3) biomass (mg WWT) of *Nephelopsis obscura* over time (weeks) maintained in groups of 5 (\bullet), 10 (\blacktriangle), 25 (\blacksquare), 50 (\diamond) and 100 (\bigstar) animals.



TIME [weeks]

Table 18. Mean (\pm SD) growth rate (J.week⁻¹) of *Nephelopsis obscura* maintained in groups of 1, 5, 10, 25, 50 and 100 animals for weeks 0 - 10, 10 - 20, 20 - 30, 30 - 40 and over the life-time (single animal = weeks 0 - 26, all other groups = 0 - 40 weeks).

MEAN GROWTH RATE							
	0 - 10	10 - 20	20 - 30	30 - 40	life-time		
1		,			152.7 ± 27.3		
5	125.8	156.6	308.9	115.9	182.9		
	•± 19.4	± 39.5	± 66.9	± 83.2	± 8.0		
10	114.0	150.9	265.2	128.4	168.7		
	± 13.7	± 29.6	± 26.2	± 89.3	± 16.7		
25	107.2	133.4	249.3	92.7	150.5		
	± 7.6	± 25.8	± 26.2	± 31.9	± 5.3		
50	97.3	131.1	183.2	91.2	128.4		
	± 8.4	± 14.4	± 21.3	± 48.6	± 10.0		
100	70.3	94.2	177.8	86.3	110.6		
	± 3.8	± 7.6	± 8.0	± 19.8	± 4.2		

Table 19. Results of Tukey multiple comparison test determining the effects of group size (1, 5, 10, 25, 50 and 100 animals) on life-time mean growth rates of *Nephelopsis obscura*. Treatments connected with a solid line are not significantly different from each other.

MEAN GROWTH RATE								
50	100							
<u></u>								
	<u></u>							

The coefficient of variation (CV) can be used a measure of the variability in the size of leeches within a group and was calculated for each replicate (n = 3) from:

mean weight

As there is no variation in the size of one leech within a container, CV for individual animals was not calculated. The average CV for groups (5 - 100) was $15.2\% \pm 4.4$ (n = 5) and there was no significant effect of group size (F = 2.24, df = 4) on the variability of growth of individual leeches (CV = $11.8\% \pm 3.7$, $13.0\% \pm 4.1$, $20.4\% \pm 3.2$, $16.7\% \pm 2.7$, $14.0\% \pm 2.1$ for group sizes of 5, 10, 25, 50, 100 leeches respectively).

INGESTION

Food ingestion (mg WWT) per week increased over time in each group (Fig 18). For each consecutive 10 week period and for the total experimental period (life-time) rate of food ingestion was calculated (Table 20).

There was a significant group effect (F = 406.3, df = 5) on life-time weekly feeding rates. Weekly food ingestion increased between group sizes of 1 and 5 animals then decreased as group size increased with all groups significantly different from each other (Table 21).

Size specific weekly prey ingestion (mg WWT.mg leech WWT⁻¹) decreased over time in all groups (Fig 19). For all group sizes the pattern of size specific food ingestion was the same. However, in single animals and in the largest group size (100), food ingestion per unit biomass was initially lower than in the other groups which were not different from each other.

The pattern of prey ingestion with increasing biomass is similar in all groups (Fig 20), with amount of weekly food ingestion lowest for single animals and groups of 100, highest for groups of 5 and declining with increasing group size from 5 - 100.

Figure 18. Mean $(\pm SD)$ weekly food ingestion (mg WWT) by Nephelopsis obscura over time (weeks) maintained in groups of (a) 1 (*) (n = 14) (after Qian and Davies 1994), 5 (•) and 10 (\blacktriangle) and (b) 25 (•), 50 (\blacklozenge) and 100 (\bigstar) animals (n = 3).

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Table 20. Mean (\pm SD) feeding rate (J.week¹) of *Nephelopsis obscura* maintained in groups of 1, 5, 10, 25, 50 and 100 animals for weeks 0 - 10, 10 - 20, 20 - 30, 30 - 40 and over the life-time (single animal = weeks 0 - 26, all other groups = 0 - 40 weeks).

MEAN FEEDING RATE								
	0 - 10	10 - 20	20 - 30	30 - 40	life-time			
1			· · · · · · · · · · · · · · · · · · ·		486.3			
					± 15.6			
5	565.3	694.1	1013.8	945.3	799.5			
	± 18.9	± 29.0	± 29.9	± 36.8	± 14.3			
10	517.5	651.4	931.0	899.8	743.8			
	± 13.3	± 37.3	± 28.5	± 23.9	± 13.3			
25	458.2	574.1	868.9	834.9	678.0			
	± 10.1	± 25.3	± 18.4	± 17.5	± 9.2			
50	407.1	521.6	719.9	703.3	583.3			
	± 11.0	± 13.8	± 18.4	± 18.9	± 7.8			
100	305.4	359.7	524.4	546.9	417.7			
	± 4.1	± 12.0	± 11.0	± 6.9	± 4.6			
Table 21. Results of Tukey multiple comparison test determining the effects of group size (1, 5, 10, 25, 50 and 100 animals) on life-time mean feeding rates (J.week⁻¹) of *Nephelopsis obscura*. Treatments connected with a solid line are not significantly different from each other.

	<u> </u>	MEAN FEE	DING RATE		
5	10	25	50	1	100

Figure 19. Regression of weekly food ingestion per unit biomass (mg WWT.mg leech WWT⁻¹) by *Nephelopsis obscura* over time (weeks) maintained in groups of (a) 1 (*) (n = 14) (after Qian and Davies 1994), 5 (•) and 10 (\bigstar) and (b) 25 (•), 50 (•) and 100 (\bigstar) animals (n = 3).



FOOD INGESTION PER BIOMASS (mg WWT · mg WWT⁻¹)

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Figure 20. Regression of weekly food ingestion (mg WWT) by Nephelopsis obscura over biomass (mg WWT) maintained in groups of (a) 1 (*) (n = 14) (after Qian and Davies 1994), 5 (•) and 10 (\bigstar) and (b) 25 (•), 50 (•) and 100 (\bigstar) animals (n = 3).

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GROWTH EFFICIENCY

Growth efficiency (GE) was calculated as:

$$GE = \frac{G}{I} \times 100$$

Where: $G = \text{growth} (J.\text{week}^{-1})$

 $I = food ingestion (J.week^{-1})$

In groups of 5 - 100 growth efficiency increased over time during weeks 0 - 30, however during weeks 30 - 40 growth efficiency declined to approximately half that of the previous measurement (Table 22). Life-time growth efficiencies were similar (22.0 to 22.9 %) among groups of 5, 10, 25 and 50 leeches but slightly higher (26.5 %) at the largest group size (100) and highest in individual animals (31.4%).

Table 22. Mean growth efficiency (%) of *Nephelopsis obscura* maintained in groups of 1, 5, 10, 25, 50 and 100 animals for weeks 0 - 10, 10 - 20, 20 - 30, 30 - 40 and over the life-time (single animal = weeks 0 - 26, all other groups = 0 - 40 weeks).

		GROWTH E	FICIENCY	<u> </u>	
	0 - 10	10 - 20	20 - 30	30 - 40	life-time
1		<u> </u>	*****		31.4
5	22.2	22.6	30.5	12.3	22.9
10	22.0	23.2	28.5	14.3	22.7
25	23.4	23.2	28.7	11.1	22.2
50	23.9	25.1	25.4	13.0	22.0
100	23.0	26.2	33.9	15.8	26.5

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The life-time rate of energy acquisition and allocation to growth were plotted for each treatment (Fig 21). Single individuals had lower food ingestion (486.3 J.week⁻¹) than animals maintained in groups sizes of less than 100 leeches. The highest rate of food ingestion was for leeches in a group size of five with the rate of energy acquisition declining from 799.7 to 417.7 J.week⁻¹ as group size increased from 5 to 100 animals.

The amount of energy allocated to growth of single animals was 152.7 J.week¹, similar to animals maintained in groups of 25 (150.5 J.week¹), and to total respiration was 333.7 J.week⁻¹, lower than animals maintained in group sizes of less than 100 leeches. The amount of energy allocated to growth and to total respiration and faeces plus mucus was highest for animals maintained in a group size of five and declined from 182.9 to 110.6 J.week⁻¹ and from 616.8 to 307.1 J.week⁻¹ respectively in group sizes between 5 - 100 animals (Fig 21).

For each treatment the energy values were converted to a percentage of energy ingested (Fig 22). For single *N. obscura* the proportion of energy allocated to growth was 31.4%. The proportion of energy allocated to growth was the same ($22.4\% \pm 0.3$) among groups of 5, 10, 25 and 50 leeches. However, as group size increased to 100 leeches, the proportion of energy allocated to growth increased slightly to 26.5%. At lower levels of food ingestion in the treatments with single animals and with groups of 100 animals, the proportion of energy allocated to growth was higher than in the other treatments i.e. they had higher growth efficiency.

Figure 21. Rate of energy allocation (J.week⁻¹) to total respiration and faeces plus mucus and growth by *Nephelopsis obscura* maintained in groups of 1, 5, 10, 25, 50, and 100 animals.



Total Respiration and faeces plus mucusGrowth

Figure 22. Proportion of ingested energy (%) allocated to total respiration and faeces plus mucus and growth by *Nephelopsis obscura* maintained in groups of 1, 5, 10, 25, 50 and 100 animals.

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Total Respiration and faeces plus mucusGrowth

LIPID

At the start of the experiments (week 0) lipid storage from a representative sample of leeches was $8.48\% \pm 0.71$ (n = 5). After asymptote biomass was attained (week 26 for single and week 39 for animals in groups > 5) the average percent lipid content of leeches from all treatments was $12.72\% \pm 1.40$ (n = 6) and there was no significant effect of group size on lipid storage (F = 1.54, df = 5) (Table 23).

Table 23. Results of Tukey multiple comparison test determining the effects of group size (1, 5, 10, 25, 50 and 100 animals) on mean (\pm SD) (n = 5) total lipid content (%) of *Nephelopsis obscura* at week 26 (single animal) or week 39 (all other groups). Treatments connected with a solid line are not significantly different from one another.

		LIPID CONTENT			
1	5	10	25	50	100
12.67	10.94	12.50	13.04	11.72	15.44
± 0.49	± 3.42	± 2.24	± 3.54	± 2.50	± 3.50

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RESPIRATION

Size-specific (per unit leech weight) resting and active respiration in all treatments declined with increasing biomass (Fig 23 and Fig 24).

For resting respiration there was a highly significant effect of time (F = 87.74, df = 2) and group size (F = 10.07, df = 5) with a non-significant interaction (F = 1.68, df = 10). The same pattern was seen for active respiration with highly significant time (F = 70.78, df = 2) and group size effects (F = 10.49, df = 5) although the interaction was not significant (F = 2.18, df = 10). There was no significant difference in either resting or active respiration between groups of 10, 25 and 50, and leeches in groups of 100 had significantly lower resting and active respiration than all other groups (Table 24). Resting respiration was significantly higher costs than groups of 5 and 10 which were not significantly different from each other (Table 24).

Scope for activity declined with increasing biomass in all groups (Fig 25) and also declined with increasing group size. There was a highly significant effect of time (F = 18.09, df = 2) and a significant effect of group size (F = 3.83, df = 5) with a non significant interaction (F = 1.28, df = 10). Groups of 1 and 5 had higher scope for activity than groups of 100 but groups of 10, 25 and 50 were not significantly different from either the group of 5 or the group of 100 (Table 24). Animals maintained individually had significantly higher SfA than all other groups.

Figure 23. Mean (\pm SD) resting rate of oxygen consumption (μ l O₂.mg DWT⁻¹.h⁻¹) for *Nephelopsis obscura* over time (weeks) maintained in groups of (a) 1 (*) (n = 5) (after Davies and Kalarani 1993), 5 (•) and 10 (\blacktriangle) and (b) 25 (=), 50 (•) and 100 (\bigstar) animals (n = 3).



Figure 24. Mean (\pm SD) active rate of oxygen consumption (μ l O₂.mg DWT⁻¹.h⁻¹) for Nephelopsis obscura over time (weeks) maintained in groups of (a) 1 (*) (n = 5) (after Davies and Kalarani 1993), 5 (•) and 10 (\blacktriangle) and (b) 25 (=), 50 (\diamond) and 100 (\bigstar) animals (n = 3).

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Figure 25. Mean (\pm SD) scope for activity (μ l O₂.mg DWT⁻¹.h⁻¹) for *Nephelopsis* obscura over time (weeks) maintained in groups of (a) 1 (*) (n = 5) (after Davies and Kalarani 1993), 5 (•) and 10 (\blacktriangle) and (b) 25 (=), 50 (\diamond) and 100 (\bigstar) animals (n = 3).



Table 24. Results of Tukey multiple comparison test determining the effects of group size (1, 5, 10, 25, 50 and 100) on mean (\pm SD) resting and active rates of oxygen consumption (μ l O₂.mg DWT⁻¹.h⁻¹) and scope for activity for *Nephelopsis obscura*. Treatments connected with a solid line are not significantly different from one another.

		RESTING RI	ESPIRATION		
1	5	10	25	50	100
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			· · · · · · · · · · · · · · · · · · ·		
<u></u>		ACTIVE RE	SPIRATION		
1	5	10	25	50	100
				<u>,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,</u>	<u>, , , , , , , , , , , , , , , , , , , </u>
		SCOPE FOR	R ACTIVITY		
1	5	10	25	50	100
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ACTIVITY TIME

Although there was no significant difference in the time spent in locomotory activity among groups (F = 1.03, df = 4), within groups there was a highly significant light/dark effect (F = 30.27, df = 1) with leeches spending a greater time in locomotion in the dark compared to the light (Table 25). The interaction was not significant (F = 0.29, df = 4).

In contrast, for the time spent engaged in random activity, there was a significant effect of group size (F = 4.21, df = 4). The group of 100 animals spent significantly more time in random activity than leeches in the other groups which were not significantly different from each other (Table 26). Within groups there was no significant effect of light or dark (F = 0.59, df = 1) and a non significant interaction (F = 2.15, df = 4).

These data were used to calculate the total time spent in activity. There was a significant group effect (F = 5.42, df = 4) with the highest group size exhibiting significantly higher total activity than the other groups, amongst which there was no significant difference (Table 26). Within groups activity was higher in the dark than in the light and these differences were highly significant (F = 22.98, df = 1). The interaction between the covariates was not significant (F = 2.25, df = 4).

Table 25. Mean (\pm SD) locomotory, random and total activity time in the light and dark and per day (h) by *Nephelopsis obscura* maintained in groups of 5, 10, 25, 50 and 100 animals.

GROUP	5	10	25	50	100
		LOCOMO	TORY		
LIGHT	1.00	1.60	1.09	2.58	2.81
	± 0.74	± 1.00	± 0.84	± 2.38	± 2.29
DARK	4.08	5.29	4.42	4.99	4.94
	± 2.05	± 2.82	± 1.43	± 2.14	± 1.91
TOTAL	5.08	6.89	5.51	7.57	7.75
	± 2.18	± 2.99	± 1.66	± 3.20	± 2.98
		RAND	M	•	
LIGHT	1.46	2.38	2.22	1.40	5.58
	± 1.96	± 1.61	± 1.15	± 0.66	± 2.52
DARK	2.63	1.73	2.02	2.03	2.93
	± 1.13	\pm 1.55	± 0.72	± 1.31	± 2.16
TOTAL	4.09	4.11	4.24	3.43	8.51
	± 2.26	± 2.23	± 1.36	± 1.47	± 3.32
		TOTAL AC	TIVITY		
LIGHT	2.46	3.98	3.31	3.98	8.39
	± 2.10	± 1.90	± 1.42	± 2.47	± 3.41
DARK	6.71	7.02	6.44	7.02	7.87
	± 2.34	± 3.22	± 1.60	± 2.51	± 2.88
TOTAL	9.17	11.00	9.75	11.00	16.26
	<u>+</u> 3.14	± 3.74	± 2.14	± 3.52	± 4.46

Table 26. Results of Tukey multiple comparison test determining the effects of group size (5, 10, 25, 50 and 100) on mean locomotory, random and total activity time (h) by *Nephelopsis obscura*. Treatments connected with a solid line are not significantly different from one another.

		LOCOMOTORY		
5	10	25	50	100
		RANDOM	<u> </u>	
5	10	25	50	100
		TOTAL ACTIVIT	Y	
	10	25	50	100

DISCUSSION

In Stephenson's Pond simultaneous sampling of both macrophytes in the water column and the sediment has shown that *N. obscura* moves between these strata (Gates *et al.* 1987) and also undergoes seasonal migration between deep water and the macrophyte zone (Davies and Everett 1977; Gates and Davies 1987). During most of the ice-free season leeches are confined to the shallow water zones but during fall they migrate to water deeper than 1.5 m down to a maximum depth of 3 m (Davies and Everett 1977). Density of *N. obscura* in the field ranges from approximately 200 m^2 (Rasmussen 1983) to a maximum of 1764 m^2 (Gates *et al.* 1987) and varies according to location within the pond (i.e. mud or macrophytes), depth of sediment, and season. Vertically within the sediment leeches are generally found within the top 2-3 cm where average leech densities are approximately $500-1000 \text{ m}^2$ (Rasmussen 1983).

Sampling the littoral zone of Stephenson's Pond using a compartmentalised sampler (625 cm²) along a 30 m transect showed that *N. obscura* has an aggregated distribution (Gates *et al.* 1987) but a random distribution was recorded when sampled using a core sampler (20 cm²) in enclosures (3600 cm²) filled with sieved mud (Rasmussen 1983; Rasmussen and Downing 1988). These results suggest that *N. obscura* forms groups only when stones and macrophytes compose the majority of the substrate (i.e., in the littoral zone) and support observations that in the field leeches are found in groups under stones. Distribution of *E. octoculata*, *H. stagnalis* and *G. complanata* is also contagious in stony littoral zones (Randall, Spelling and Young 1985).

Since all ecological systems exhibit heterogeneity and patchiness on a broad range of scales (Kotlier and Weins 1990) the ability to detect distribution patterns depends on the extent (the overall area covered) and the grain (size of individual units sampled) of the investigation (Wiens 1989) and the outcome will vary according to the scale of the study. For example, a species could be patchily distributed over the area of an entire pond, but within individual patches distribution could be random, aggregated or regular. Thus, inferences about group living can only be made if the scale of the investigation is ecologically relevant. A tacit assumption often made in analyses of spatial data obtained by sampling is that organisms are randomly distributed. However, the application of non-random sampling designs to ascertain spatial distribution of species can often fail to detect contagious distributions.

Aggregated distributions may result from individuals actively seeking out and joining conspecifics and/or individuals responding in the same way to a suite of environmental conditions (Wrona and Dixon 1991). Aggregation in *N. obscura* is likely a consequence of the joint effects of positive thigmotaxis and negative phototaxis, as suggested by Gee (1913) for *H. stagnalis* and *M. microstoma*. Gee (1913) noted that groups of leeches form in the darkest part of the aquaria, and that leeches in groups, with their thigmotactic inclinations satisfied, were less responsive to changes in light intensity than those leeches which were not in direct contact with conspecifics. Most leeches show a negative phototactic reaction (Mann 1961; Greene 1974; Blinn, Davies and Dehdashti 1987, Davies and Kasserra 1989) and in the field

N. obscura seeks shelter under stones and other suitable substrata during daytime and forages primarily at night (Davies *et al.* 1982). In the laboratory leeches tend to form single groups several leeches deep which present the smallest surface area to volume ratio. Since individuals inside the perimeter of the group are sheltered from the light, aggregative behaviour under homogeneous external conditions could also be explained by phototactic behaviour.

GROWTH

Regardless of size or time to asymptote biomass the pattern of growth exhibited by *N. obscura* was always sigmoidal. In groups of 5 - 100 asymptote biomass was reached after 38 - 39 weeks in all groups. However, leeches maintained individually reached an asymptote biomass 12 to 13 weeks earlier (Qian and Davies 1994).

Growth rate and asymptote biomass were lower in single leeches compared to N. obscura maintained in groups of five and ten animals suggesting that there is some advantage to living in a group. The reduction in growth rates and asymptote biomass between group sizes of 10 - 100 leeches shows that there is also a cost to living in groups larger than ten.

Competition can be defined as an interaction between individuals, brought about by a shared requirement for a resource in limited supply, leading to a reduction in survival, growth and/or reproduction of the competing individuals (Begon, Harper and Townsend 1990). The mechanism of competition is often unclear (Tilman 1987). However, in these experiments with *N. obscura* food was provided in excess at each meal and therefore exploitative competition, where each individual is affected by the amount of resource that remains after it has been exploited by the others, does not occur. Consequently, the reduced growth in larger group sizes may have been caused by interference competition with the behaviour of conspecifics interfering directly with the ability of an individual to acquire that resource. In this interaction there need not be an absolute shortage of the resource, but the behaviour of the competitor creates a relative shortage (even though prey may be abundant they may not be available).

Density-dependent stress can have behavioral effects that lead to increased variation in the growth of individuals (Brown 1957; Ricker 1958; Arthur and Dixon 1994). This process, termed growth depensation (Ricker 1958) is often attributed to the effects of size-hierarchical behaviour (Brown 1957). The dominant individuals consume an unequal proportion of food and so grow faster than they would if the resource was distributed equally amongst all individuals, consequently subordinate individuals grow more slowly. The faster growth of the dominant individuals usually enhances their dominance and so allows them to gain access to prey even more effectively and further increases the difference in growth rate. In *N. obscura* statistical analyses showed there was no significant difference in variances of the five populations and thus the reduction in growth rate with increasing group size affected all individuals equally and there were no size-hierarchy effects operating.

Differences in growth at different group sizes could be due to differences either in the rate of food consumption and/or in the rate of energy expenditure (i.e., total respiration).

FEEDING

An increase in energy intake is frequently one of the major benefits derived from aggregative behaviour in invertebrates (Trowbridge 1991; Cash, McKee and Wrona 1993). Single N. obscura had lower feeding rates than leeches maintained in groups of less than 100, showing that there is an advantage for individuals in groups in terms of increased prey ingestion. Group feeding has been observed in several liquidosomatophagous leech species (Sawyer 1986). Individual H. stagnalis and G. complanata are commonly joined by other leeches while in the process of feeding (Sawyer 1986) and any offspring attached to a brooding parent may also participate in the feeding process (Sawyer 1986; Kutschera and Wirtz 1986a, b). These leeches are also attracted to prey which have been previously attacked by other leeches or by. triclads and in the laboratory are attracted to crushed prey items (Sawyer 1986). The presence of adult H. stagnalis and G. complanata individuals increases the feeding success, growth and survival of solitary young leeches on all prey types, possibly resulting from the adult leaving some uneaten food which the young could obtain and/or the simultaneous sharing of the food on capture by the adult (Young, Seaby and Martin 1995). In these situations it is generally concluded that predators are attracted by the release of body fluids from damaged prey and the possibility that conspecifics are attracted directly by the presence of the foraging competitor is not considered. In macrophageous species such as N. obscura, individuals could be simulated to feed as a result of the release of prey body fluids or from the behaviour of other feeding conspecifics.

Weekly feeding rates for individual leeches within each group declined with increasing group sizes above five. Although at each meal food was provided in excess, results from this study suggest that *N. obscura* does not ingest prey in direct proportion to its abundance and that in group sizes larger than five there were restrictions in prey availability resulting from the behaviour of conspecifics. Similarly in large groups per capita ingestion rates of the triclad *D. tigrina* decreased with increasing group size (Cash, McKee and Wrona 1993).

Even though food may be abundant within the immediate environment of the forager, prey may avoid or evade the predator (Edmunds 1974) and/or the predator can reject or is unable to gain access to potential prey (Curio 1976). Since prey escape behaviour and potential evasion from *N. obscura* by *T. tubifex* was not a confounding variable, the reduction in food consumption with increasing group size must have resulted from the activities of conspecifics. Group members may impede different stages of food procurement of conspecifics and observations suggest that a possible mechanism could be a reduction in the ability to initiate a feeding response. Since *N. obscura* requires direct contact with the prey to initiate feeding (Davies *et al.* 1982; Dratnal, Dratnal and Davies 1992) some individuals belonging to large groups will likely not come into contact with prey and consequently feed. Similarly *E. octoculata* successfully captures prey only when random probing brings the leeches mouth into chance contact with the prey (Elliott 1973; Greene 1974).

Maximum life-time growth and feeding rates occurred in groups of five animals. However, in groups of one and 100 leeches the reduction in growth rate from the maximum (16.5 and 39.5% respectively) was not as large as the reduction in feeding rate (39.2 and 47.8%) suggesting that compensation was occurring. However, in groups of ten, 25 and 50 the reduction in growth (7.8, 17.7, 29.8% respectively) was comparable to the reduction in feeding (7.0, 15.2 and 27.0% respectively).

Although the total amount of energy ingested and allocated to growth processes was different in each group, the relative proportion of energy allocated to growth was the same regardless of group size between group sizes of five - 50 leeches. In group sizes of one and 100 leeches a greater proportion of acquired energy is allocated to growth and the highest growth efficiency occurred in these groups i.e. growth efficiency increased with decreasing food ingestion.

STORAGE

Leeches in all groups had the same percent lipid content at the end of the experiment showing that leeches allocated the same proportion of growth energy into storage materials. However, as animals in groups of one and 100 allocated a greater proportion of ingested energy to growth they therefore allocated a greater proportion of acquired energy to lipid storage than those in groups of five, ten, 25 and 50 amongst which there was no difference.

RESPIRATION

Group sizes of one, five and ten had the highest hourly resting respiration costs per unit weight and therefore allocated more energy to maintenance respiration than animals of a similar biomass in the other groups. Groups of one had higher active respiration than groups of five and ten which were higher than larger group sizes. Groups of 100 had the lowest resting and active respiration costs per unit weight and thus allocated less energy to maintenance respiration. However, leeches maintained individually allocated proportionally less energy to total respiration than individuals in groups and had the greatest efficiency of conversion of ingested food to body tissues.

Although aggregation has been shown to result in the reduction of respiration rate in endotherms (Trune and Slobodchikoff 1976; Herreid and Schlenker 1980) this study provides the first evidence of alterations in respiration as a result of aggregation in ectotherms. As also shown in Chapter 3, hourly costs of Rm and Ra are not purely a function of body size and temperature. Thus, respiration rates show a high degree of flexibility in response to numerous environmental and biological variables and should not be considered as a fixed trait responding only to body size and temperature changes. Bioenergetic models used by fishery and aquaculture management to predict growth or feeding rates of fish (Wootton 1990) presently rely heavily on empirically derived relationships. If the flexibility in metabolism of *N. obscura* is indicative of similar flexibility in other ectotherms this could result in gross miscalculation of growth or feeding rates. Energy budgets constructed from data collected from laboratory animals reared in isolation similarly should not be applied to aggregated field populations living under very different conditions.

The same pattern of size specific hourly respiration costs in resting respiration was seen for active respiration. Scope for activity (SfA) in the group size of 100 was significantly lower than SfA in groups of one or five which had the most energy for potential aerobic activities such as searching for food, escape or avoidance of predation and movement away from unfavourable environmental conditions e.g. toxicants.

Leeches in the largest group size (100) had the highest total activity time due to greater random activity, however locomotory activity was the same regardless of group size. Leeches move between patches of prey by swimming and/or crawling predominantly in the dark indicating that *N. obscura* tend to seek shelter and restrict swimming and crawling when light intensity is high. Anholt and Davies (1986) and Davies and Kasserra (1989) also found greater activity of *N. obscura* in the dark than in the light and similar results have been shown for other erpobdellid species (Elliott 1973; Greene 1974; Blinn, Davies and Dehdashti 1987; Davies *et al.* 1988).

Random activity where the leech remains in one place includes activities such as dorso-ventral undulations for ventilation, and probing of the immediate environment. The amount of time engaged in this form of motion was highest for a group size of 100 and unlike locomotory activity was independent of light intensity. In the largest group size the lower hourly costs of Rm and Ra compensated for the increased time spent in activity, and the overall cost of total respiration was lower. Leeches in the group of 100 also allocated a lower proportion of ingested energy to respiration than leeches in the other groups.

Living in a group may provide a fitness advantage in situations where the effects of predation are significant (Wrona and Dixon 1991; Stoner and Ray 1993). There are two distinct mechanisms that can be involved: reduction in the probability of encountering the predator, and dilution of the predator's attack efficiency once encountered (Wrona and Dixon 1991). Considering the very high densities of potential invertebrate predators in Stephenson's Pond and the evidence that invertebrate predation is high on *N. obscura* less than 30 mg (Cywinska and Davies 1989) group living may well be advantageous for hatchlings and small *N. obscura*. However, predation pressure on *N. obscura* decreases with increasing body size (Cywinska and Davies 1989), a trend also found in other leeches (Spelling and Young 1987; Young 1987) due to the inability of some potential predators to physically capture and handle large leeches or to the ability of larger leeches to produce large amounts of mucus and so impede the movements of potential predators. It is possible that mucus produced by leeches living in groups is a more effective impediment than mucus produced by solitary leeches.

In situations where larger organisms such as fish, birds, and salamanders are important predators (Cywinska and Davies 1989) there would be little safety in numbers for small leeches since these predators are able to consume several prey at one time. However, in prairie pothole lakes winter kill eliminates fish and apart from foraging water fowl, which take only the occasional individual, large *N. obscura* in Stephenson's Pond have no major predators (Davies and Kasserra 1989).

To summarize, in terms of growth, asymptote biomass and subsequently fitness, group sizes greater than one and less than ten leeches are optimal. As group size increases from ten - 100 animals *N. obscura* has slower growth and lower weekly food ingestion, even though an *ad libitum* supply of food was provided suggesting interference competition. Single leeches had lower food consumption than group sizes

of less than 100 animals. Lipid deposition was unaffected by group size. Size and time specific respiration rates showed flexibility and declined with increasing group size. For group sizes between five and 50 leeches the same relative proportion of ingested energy was allocated to respiration. Above this threshold group size, leeches allocated a higher proportion of ingested energy to growth i.e. had higher growth efficiency, as did single leeches. In the largest group size, time spent in total activity was the highest (reflecting the high degree of interference between conspecifics) but as a result of the lower hourly costs of respiration they allocated a lower proportion of ingested energy to total respiration.

Optimum group size of *N. obscura* in the laboratory is within the typical range of group sizes found in the stony littoral zone of Stephenson's Pond. Advantages of group living in the field are apparent in terms of increased feeding success, likely due to the behaviour of other feeding conspecifics or to the release of fluid from captured prey. Another proposed advantage, at least for small individuals, is increased survival due to reduced predation rates.

The results of this study have important implications for studies of anthropogenic toxicants on aspects of the energy budget. Growth is a common endpoint used to study the toxicity of pollutants to aquatic organisms (CCREM 1987, USEPA 1989, Environment Canada 1992). One problem in interpreting results of growth under toxicant stress may be related to density-dependent competition for food and space. The results presented here show that knowledge of natural variation in growth is fundamental otherwise, growth differences resulting from uncontrolled variables (e.g.
group size) could enhance or diminish true changes which result from the variable being tested (e.g. toxicant exposure).

Arthur and Dixon (1994) have shown that group size can also directly affect the nature of the relationship between toxicant exposure and response of juvenile fathead minnow (Pimephales promelas Rafinesque). The effect of exposure to pentachlorophenol (PCP) and 2,4,5-trichlorophenol (TCP) was reduced as the number of fish increased and different rearing densities resulted in as much as twofold differences in chronic toxicity estimates based on growth studies. Arthur and Dixon (1994) rejected the possibility that density-related effects were due to a reduction in the toxicant concentration in the immediate vicinity of the fish at high densities as their system provided adequate replenishment and good circulation of the toxicant. However, concentrations of a toxicant within an organism (dosage) may not always be directly correlated with the external concentration. In the case of N. obscura metabolic rates declined with increasing group size which would result in lower quantities of toxicant taken up by individuals in larger group sizes. This in turn would lead to less apparent toxicity at high densities even though external toxicant concentrations remain unchanged. It is clear that rearing density should be carefully considered in the development of standard protocols for chronic toxicity tests.

CHAPTER FIVE

STRESS RESISTANT SYNDROME OF NEPHELOPSIS OBSCURA

A common suite of traits characteristic of most plants living in high stress environments first identified by Grime (1977) includes low rates of growth, photosynthesis, and nutrient absorption, high root:shoot ratios, low rates of tissue turnover, and high concentrations of secondary metabolites. It has been suggested that this suite of traits, termed Stress Resistant Syndrome (SRS), represents an adaptive strategy enabling plants to survive in stressful environments. Although fixed responses have been documented for plants, a common adaptive strategy has not yet been identified either for an individual animal species or for heterotrophs in general. Consequently, the aim of this chapter was to compare the results from this study with other work in the literature to see if N. obscura produces similar responses to several different types of natural stress and also to compare its response to anthropogenic stresses not previously encountered. If organisms exhibit a common suite of traits in response to stress, then the response to a novel environmental stress will be the same as the response to a natural stress, although it may not be adaptive. Conversely, does N. obscura show compensation in traits when exposed to anthropogenic stress not exhibited when exposed to natural stresses? The responses of N. obscura to natural and anthropogenic stress will also be compared to other aquatic species which experience similar kinds of stress to see if a general stress response can be identified.

Several studies have examined the physiological responses of *N. obscura* to natural stresses. Dratnal and Davies (1990), Reddy, Dratnal and Davies (1992), and Davies and Kalarani (1993) studied the patterns of energy acquisition, allocation and storage and changes in life-history traits in *N. obscura* exposed to winter stresses of low food, temperature and oxygen. Leeches which had overwintered in the field and then maintained in the laboratory in summer conditions were compared with initially similarly sized leeches that had hatched in and experienced only summer conditions. Monita (1994) examined changes in energy acquisition and allocation patterns in *N. obscura* during and after exposure to starvation stress. Leeches with the same prehistory were maintained under summer conditions in the laboratory from hatchling to mature adults and the effects of starvation experienced during either the somatic growth phase or the reproductive phase, examined. Following 3, 4 or 5 weeks starvation (corresponding to approximate biomass losses of 20, 30 and 40 % respectively) leeches were returned to optimal conditions until maturity was attained.

In this study leeches were exposed to different levels of food availability (based on the number of feedings per week) at two temperatures (15°C or 20°C) over the entire life-time and several components of the energy budget examined. Changes in energy acquisition and allocation in *N. obscura* maintained in different group sizes (1 - 100 animals) were also examined to assess the effects of stress resulting from the presence or absence of conspecifics.

The physiological responses of N. obscura exposed to natural stress can be compared to its responses when exposed to anthropogenic stress. Monita (1994) and

Wicklum (1994) studied the effects of cadmium (Cd) on patterns of energy acquisition and allocation in *N. obscura*. Monita (1994) studied the effects of exposure for 35 d to 50, 100 or 200 μ g.1⁻¹ Cd during either the growth phase or the reproductive phase in *N. obscura*. Before and after the stress period until maturity was attained, leeches were maintained in uncontaminated water. Energy acquisition and allocation patterns were examined before the stress and during both the stress and recovery periods. Wicklum (1994) exposed leeches 200 mg WWT to 5, 10 or 50 μ g.1⁻¹ Cd over the remainder of the life-cycle until maturity was attained and measured energy acquisition and allocation before and during the stress.

GROWTH

In the laboratory, *N. obscura* acclimating to winter conditions had decreased growth and since consumption was the same as unstressed individuals, Dratnal *et al.* (1993) showed that the reduction in somatic growth was the result of a greater proportion of energy being allocated to lipid storage. Leeches that had survived overwintering in the field had equal growth rates to individuals which had never experienced winter, although they had higher rates of energy ingestion (Dratnal and Davies 1990), indicating that unstressed leeches allocated a greater proportion of ingested energy to growth. *Nephelopsis obscura* experiencing starvation also had decreased growth rates when stressed during the growth or reproductive phases (Monita 1994). However, when the stress was alleviated during the recovery period *N. obscura* overcompensated and growth rates were significantly higher than the

controls.

In this study *N. obscura* showed reduced growth as feeding became less frequent (Chapter 3). Leeches in the medium food treatments showed compensation since growth rates were not reduced as much as the reduction in food availability. However, leeches in the low food treatments had growth rates even lower than the reduction in food availability suggesting that a greater proportion of energy was allocated to total respiration and/or that a higher proportion of energy was allocated to storage in the form of lipid. Growth rates at 15°C were higher than at 20°C due to the higher costs of total respiration at 20°C. Leeches maintained in group sizes of five and ten had higher growth rates than those maintained individually or in group sizes of 25, 50 or 100 (Chapter 4).

Exposure of *N. obscura* to natural stresses results in a reduction in the proportion of ingested energy allocated to growth and if energy acquisition does not increase, a reduction in absolute growth during the stress period. Reduced growth can be the result of a reduction in food consumption, reduction in absorption, increased total respiration costs and/or an increase in the proportion of ingested energy allocated to lipid storage. When exposed to starvation stress in the laboratory leeches exhibit over compensation during the recovery period and have higher growth rates than the controls that did not experience the stress.

Exposure to 100 or 200 μ g.l⁻¹ Cd during both the growth and reproductive phase resulted in significantly higher growth rates of *N. obscura* than the controls or leeches exposed to 50 μ g.l⁻¹ Cd (Monita 1994). However, it is not known whether this

increase in growth is a result of the direct or indirect effects of exposure to Cd. During the growth phase, the growth rate of leeches exposed to 50 μ g.1⁻¹ Cd was not significantly different from the controls but during the reproductive phase growth rate of leeches exposed to 50 μ g.1⁻¹ Cd was less than the controls (Monita 1994). However, by the end of the recovery period, leeches in the control group were significantly larger than those previously exposed to 50 μ g.l⁻¹ Cd, which were significantly larger than those exposed to 100 μ g.l⁻¹ Cd and all leeches previously exposed to 200 μ g.1⁻¹ Cd had died. Wicklum (1994) found no significant difference in growth between the control, 5 or 10 μ g.l⁻¹ Cd but at 50 μ g.l⁻¹ growth was significantly lower than controls. Once N. obscura was returned to uncontaminated conditions growth rates in the Cd treatments were lower than in the controls (Monita 1994) and leeches did not respond by overcompensating as they did after exposure to natural starvation stress. Thus, exposure to anthropogenic stress in N. obscura usually results in a reduction in growth during the stress period although under certain circumstances an increase in growth in response to Cd stress occurs. Leeches did not exhibit compensatory growth during the recovery period after exposure to Cd, even though they were returned to optimal conditions.

Several studies have found that growth of other aquatic species is reduced during exposure to stress and over compensation occurs during the recovery period. No other study in the literature has reported an increase in growth rate for organisms during exposure to stress. Forbes (1991) studied stress response of the estuarine gastropod, *Hydrobia ventrosa* (Montagu) to a natural stress (changes in salinity) and an anthropogenic stress (Cd). Exposure to Cd concentrations of 100 - 200 μ g.l¹ significantly reduced growth rate and snails previously acclimated to 23 ‰ salinity sea-water (S) showed reduced growth rates at 13 ‰ and 33 ‰ S relative to snails grown at 23 ‰ S. Compensatory growth in the juveniles of three cyprinid species occurred after periods of starvation (Wieser, Krumschnabel and Ojwang-Okwor 1992) and the response is similar to that described for *N. obscura* by Monita (1994) and the colonial hydroid *Campanularia flexuosa* by Stebbing (1981) and discussed by Sibly and Calow (1986). During exposure for 16 d to a range of copper concentrations (1 - 25 μ g.l⁻¹) *C. flexuosa* growth was inhibited, but when colonies were returned to normal sea water they showed compensatory growth. In these studies the rate of compensatory growth increased with the amount of stress (duration of starvation or concentration of copper).

CONSUMPTION

Overwintering leeches maintained in the laboratory under optimal conditions showed a similar response to leeches previously exposed to starvation stress and had feeding rates twice as high as summer leeches which had not experienced the stress (Dratnal and Davies 1990). Since food was constantly available and food intake was measured at weekly or biweekly intervals, it is not known whether the higher food ingestion was from a higher number of meals and/or increased food ingestion per meal. When experiencing starvation at either the growth or reproductive phase, weekly food consumption of *N. obscura* increased once the stress was alleviated and food was available (Monita 1994). The longer the starvation period, the greater the subsequent increase in feeding when the stress was removed.

In this study weekly food ingestion of *N. obscura* decreased as number of meals per week decreased (Chapter 3). When time between successive meals was increased to 7 d leeches showed compensation and increased food consumption per meal. However, *N. obscura* were only able to increase food ingestion at sizes larger than 19.9 ± 6.7 mg due to a physical limitation of mouth size. Single *N. obscura* had lower weekly feeding rates than group sizes of 5 - 50 indicating that the presence of conspecifics increases food consumption (Chapter 4). Maximum life-time feeding rates were found for leeches in groups of five and as group size increased to 100 animals, weekly consumption rates declined due to interference competition.

The general trend in *N. obscura* is for reduced weekly food consumption with increasing levels of natural stress. However, compensation does occur at low levels of food availability since food ingestion per meal is greater than for unstressed animals of a comparable size. During recovery periods after leeches have experienced low prey availability or starvation, animals over compensate and have higher consumption rates relative to individuals that are reared continuously on liberal feeding regimes.

Monita (1994) reported increased daily consumption rates (per unit biomass) of *N*. obscura stressed during the growth phase in treatments exposed to 50 or 100 μ g.l⁻¹ Cd compared to the control, while leeches exposed to 200 μ g.l⁻¹ Cd had decreased ingestion rates. Wicklum (1994) reported no significant difference in consumption between 5 or 10 μ g.l⁻¹ Cd, but at 50 μ g.l⁻¹, consumption was significantly lower than

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controls. When stressed at the reproductive phase consumption rates were lower in all treatments exposed to Cd during the stress period (Monita 1994). In contrast to the response to natural stress, size specific consumption rates of *N. obscura* were always lower than controls during the recovery period regardless of Cd concentration or phase of development during exposure. Thus, in some circumstances *N. obscura* showed increased consumption and in others decreased consumption in response to anthropogenic stress and always had lower consumption rates than the controls during the recovery period.

Other studies have also demonstrated a decrease in food consumption with increasing stress. Naylor, Maltby and Calow (1989) reported a decrease in energy absorbed by *Gammarus pulex* with increasing concentration of zinc or decreasing pH and Naylor *et al.* (1990) monitored consumption in *G. pulex* exposed to four different stresses (zinc, 3,4 dichloroaniline (DCA), oxygen and ammonia) and all four stresses resulted in a reduction in food consumption. Brett (1971) and Tyler and Dunn (1976) found that meal size increased with increasing duration of food deprivation up to a maximum of 25 h in sockeye salmon *Oncorhynchus nerka* and 7 d in the flounder *Pseudopleuronectes americanus*, similar to the trend found for *N. obscura* exposed to low levels of prey availability.

ABSORPTION EFFICIENCY

Energy losses in faeces plus mucus were less in winter animals (Dratnal and Davies 1990) and similarly Dratnal, Dratnal and Davies (1992) found that N. obscura

experiencing a foraging constraint responded by increasing absorption efficiency. After periods of starvation, regardless of phase of development, absorption efficiency increased when food became available (Monita 1994).

In this study the proportion of ingested energy lost in faeces and mucus production decreased as food availability decreased i.e. stressed leeches were more efficient (Chapter 3). As faeces production was not measured, the effects of group size on absorption efficiency could not be determined.

Natural stress always results in a lower proportion of ingested energy lost in faeces production both during the exposure period and during recovery indicating that increased absorption efficiency is a common response of *N. obscura*.

Absorption efficiency of *N. obscura* also increased during stress at all concentrations of Cd (except 200 μ g.l⁻¹ Cd) either at the growth stage or at the reproductive stage (Monita 1994). Data were not collected during the recovery period.

Keckeis and Schiemer (1992) found that assimilation efficiency of three cyprinid fish species at low food concentrations was approximately twice that of the well-fed groups. Although exposure to stress (zinc, DCA, low oxygen or pH) resulted in *G*. *pulex* producing less faeces, they also had lower ingestion and the relationship between food ingested and faeces production remained unchanged (Maltby, Naylor and Calow 1990a) with no significant effect on absorption efficiency.

ENERGY STORAGE

Comparison of lipid content showed that winter N. obscura had lower levels than

similar sized summer animals, indicating that lipids are utilized during winter when food is in short supply (Dratnal and Davies 1990). After experiencing winter stress, leeches had a faster and higher rate of lipid deposition, indicating that they allocated absorbed energy surplus primarily to stored energy rather than to somatic growth (Dratnal and Davies 1990). During acclimation to winter conditions in the laboratory *N. obscura* also increased energy allocation to lipids (Dratnal *et al.* 1993) which in the event of discrepancy between energy supply and demand, will enhance survivorship since leeches with the highest lipid storage have the lowest rates of mortality. During starvation stress at the growth phase, lipids were not utilised except in the leeches starved for 35 d, suggesting that *N. obscura* only utilise lipids towards the end of longer periods of starvation. Monita (1994) concluded that lipids were presumably being saved for later use in reproduction.

In this study total lipid concentrations for *N. obscura* in the high and medium food treatments (Chapter 3) declined just prior to maturation, indicating the importance of lipid utilization for gonad maturation (Reddy *et al.* 1992). In the low food treatment at 15°C a greater proportion of energy was allocated to lipid storage at the expense of somatic and reproductive growth (Chapter 3), suggesting that a reduction in prey availability in conjunction with lower temperatures indicates the onset of winter conditions, to which *N. obscura* responds by storing energy. On the effects of group size, although there was no significant difference in lipid content among animals after attaining asymptote biomass, the proportion of ingested energy allocated to lipid storage was highest in groups of one and 100, in which feeding rates

were lowest.

Freshwater leeches rely on the breakdown of storage materials during winter months when energy acquisition is restricted. Exposure to pre-winter conditions (declining temperatures, reduced prey availability) results in increased lipid storage in *N. obscura* (Dratnal *et al.* 1993). Lipids also have a very important role in maturation and the general strategy in *N. obscura* seems to be to store as much energy as possible to use for reproduction and thus only exposure to long-term or very high levels of stress results in utilisation and reduction of lipid storage.

During Cd stress at the growth phase, lipid content in leeches in each treatment was higher than in the controls and significantly higher in animals exposed to 200 μ g.l⁻¹ Cd suggesting that when leeches are stressed during the early stages of development they prioritize energy allocation to lipid storage. During stress at reproductive phase, lipids were significantly lower in leeches exposed to 100 and 50 μ g.l⁻¹ Cd but there was no significant difference between leeches exposed to 200 μ g.l⁻¹ Cd and the controls. Monita (1994) concluded that the decline in lipids in leeches exposed to 200 μ g.l⁻¹ Cd during the reproductive phase was associated with onset of maturity in these animals.

Leeches respond to conditions simulating the approach of stressful conditions in the field by storing energy and a similar pattern was found when leeches were exposed to anthropogenic stress.

Many aquatic organisms which must endure harsh winter conditions store energy in late summer and fall for overwintering and also for reproduction in spring (Reznick and Braun 1987; Meffe and Snelson 1993). Although there have been no other studies on the effects of anthropogenic stress on lipid storage in freshwater organisms, results from N. obscura suggest that organisms which have developed responses to deal with natural winter stresses respond in a similar way to anthropogenic stresses. However, marine organisms which live in more stable environments (in terms of temperature, oxygen, ionic content and food availability) do not respond to anthropogenic stress by prioritising or maintaining energy storage. Interferences in lipid storage have been reported in marine crustaceans exposed to organic xenobiotics and, unlike N. obscura exposed to Cd stress, the general pattern seems to be that lipids are utilized during the stress period. For example, lower levels of lipid have been observed both in a marine amphipod (Lee, Macko and Nicol 1981) and in lobster larvae (Capuzzo, Lancaster and Sasaki 1984) exposed to petroleum hydrocarbons. Since optimization in storage of energy reserves and efficient utilization of these reserves are critical to the developmental and reproductive success of organisms (McKenney and Matthews 1990), it has been suggested that alterations in lipid storage may be a mechanism for disruption of these processes by environmental contaminants (Cappuzzo, Moore and Widdows 1988).

RESPIRATION

Davies and Kalarani (1993) found that Rm in overwintered leeches was higher than in summer leeches, probably reflecting the higher metabolic costs of tissue repair and maintenance after winter stresses. Although Ra and SfA in winter leeches were initially lower than in summer animals, rapid respiratory recovery occurred and mature overwinter leeches had higher Ra and SfA than summer leeches. During starvation stress at the growth and reproductive phases, Rm and Ra decreased (Monita 1994). During the recovery period, respiration of *N. obscura* increased and it was suggested that the higher Rm and Ra was due to increased costs of repair (Monita 1994). However, after the stress period when leeches were provided with food they exhibited over compensation and had significantly faster growth rates compared to the controls (Monita 1994) and thus elevated Rm could also be the result of increased costs of tissue synthesis.

In this study, size-specific hourly costs of resting (Rm) and active (Ra) declined, both with reductions in prey availability (Chapter 3), and with increasing group size (Chapter 4). Thus, *N. obscura* shows flexibility in terms of respiratory costs and the general trend is to show decreased costs during exposure to stress. However, during the recovery period following exposure to natural stress, costs of Rm and Ra are generally higher than in unstressed individuals.

Rm and Ra of animals during exposure to Cd at the growth phase was higher than controls but after the recovery period, only Rm for leeches previously exposed to 200 μ g.1⁻¹ Cd was significantly different from the controls (Monita 1994). After the recovery period, Ra was significantly lower in the treatments than controls so that SfA is significantly lower in previously stressed animals. Cd stress during the reproductive stage significantly reduced Rm and Ra in leeches in each of the treatment groups below that of Rm and Ra in the control group but there were no significant differences amongst the three treatment groups (Monita 1994). Rm and Ra remained significantly lower during recovery and SfA was significantly lower in leeches exposed to Cd. Wicklum (1994) found that Rm of *N. obscura* exposed to 5, 10, 50 μ g.1⁻¹ Cd remained the same as the controls throughout the experiment and thus there are no consistent patterns in changes in Rm and Ra of *N. obscura* in response to exposure to anthropogenic stress.

In other organisms Rm has been reported to either stay the same, increase or decrease in response to stress and a general response does not emerge. Wieser, Krumschnabel and Ojwang-Okwor (1992) found that maintenance respiration rate decreased with starvation in three fish species, similar to the response of *N. obscura*, and it was suggested that starving fish saved energy by reducing costs of maintenance functions. Naylor, Maltby and Calow (1989) found no significant change in hourly respiration costs of *G. pulex* with increasing concentration of zinc or decreasing pH. Significant increases in hourly size-specific respiration occurred in most clones of *Daphnia magna* exposed to 5 p.p.b. Cd and 50 p.p.b. DCA (Barber, Baird and Calow 1990).

Oxygen consumption is not independent from other aspects of physiology (Clarke 1993) and respiration rates may either be positively or negatively related to changes in other components in the energy budget. For example, if Rm decreases during exposure to Cd it cannot be concluded that this is a direct result of Cd. In many situations exposure to stress results in a significant decrease in food ingestion and consequently several components of Rm associated with the reduction in feeding (i.e. cost of growth, faeces plus mucus etc) will automatically be lower. Components of Rm not affected by consumption may increase, decrease or remain the same in response to the stress. If no relationship between stress and Rm is found, since some components of Rm have decreased as a result of decreased consumption, other components of Rm such as repair costs due to detoxification mechanisms must have increased to counterbalance this. Under different circumstances if repair costs increase more than the decrease in other components, Rm will be higher. Thus, the apparent inconsistencies in the respiratory response of organisms to stress may partially be explained by confounding factors such as differences in food ingestion and growth during exposure. To determine if changes in Rm due to anthropogenic stress are direct (due to the effects of the stress itself) or indirect (due to reductions in growth and/or food ingestion), the relationships between these components of the energy budget and respiration must be compared. If there are direct effects of anthropogenic stress on respiration the relationships between respiration and other components of the energy budget (growth and/or food ingestion) will change.

ACTIVITY TIMES

Singhal and Davies (1987) found an initial increase in activity followed by periods of inactivity in *N. obscura* exposed to hyperoxic water. Anholt and Davies (1986) examining the activity of starved and fed *N. obscura* found greater activity before feeding than 1 d after feeding. Conversely, Davies and Kasserra (1989) found that the activity pattern was unchanged during either starvation of 1 to 10 d, or after refeeding. No other studies have examined the effects of natural stress on activity time of *N. obscura*.

Total activity time of animals in the low food treatments was greater than that of *N. obscura* in the high and medium food treatments and although during the initial phase of food shortage increased locomotory activity might be a prerequisite for obtaining the little food that is available, longer starvation periods may result in the reduction of activity (McLoughlin and Davies 1992). In groups of 100, time of activity was significantly higher than smaller group sizes resulting from longer periods of random movement due to interference between conspecifics.

There are no general trends in time of activity of *N. obscura* in response to stress. However, as discussed in Chapter 3 there are a number of variables such as body size, nutritional state and gut fullness which affect activity and may confound any changes in activity resulting directly from exposure to stress.

Activity patterns in response to anthropogenic stress are likely determined by the ability of *N. obscura* to detect and avoid the stress, with low concentrations of Cd leading to the initiation of an escape response and hence increased activity while high concentrations immobilize animals due to narcotization resulting in very low activity levels (Wicklum 1994). Thus behavioral responses to anthropogenic stress are also inconsistent with leeches increasing and/or decreasing activity time during exposure according to toxicant concentration.

Literature on the effects of anthropogenic stress on activity of fish (reviewed by Atchison, Henry and Sandheinrich 1987; Døving 1991) shows that stress can affect

the locomotor activities of organisms in many ways. Organisms may elicit movement toward (attractance) or away from (avoidance) a contaminated area, show hyper- or hypo-activity in the presence of contaminants, alter locomotory patterns such as turning frequency or orientation, or show reduced swimming endurance. For some species, no observable change or reduction in activity may result from the inability to make the adjustments necessary for appropriate reaction to the toxicant due to lack of, or malfunctioning, sensory apparatus (Døving 1991). Consequently, behavioral modifications in response to anthropogenic stresses should be interpreted with caution as changes in activity may not necessarily represent the optimum strategy in terms of net energy gain or survival and subsequently fitness.

SUMMARY

Growth of *N. obscura* is highly flexible and the proportion of ingested energy allocated to somatic growth declines during exposure to both natural and anthropogenic stresses, in many instances resulting in reduced growth rates. During the recovery period after exposure to starvation, leeches exhibit over compensation and have higher growth rates relative to organisms that do not experience the stress. Exposure to anthropogenic stress usually results in a reduction in growth during the stress period although an increase in growth in response to Cd stress was reported it is not known whether or not this is due to the direct effect of Cd. Leeches did not exhibit compensatory growth during the recovery period after exposure to Cd, even though they were returned to optimal conditions. Growth rates of other species are

also reduced during exposure to stress and overcompensation has been reported after exposure to both natural and anthropogenic stress.

Prey ingestion also shows flexibility in response to stress. The general trend in *N. obscura* is for reduced weekly food consumption with increasing levels of natural stress. However, compensation does occur since food ingestion per meal is greater than for unstressed animals of a comparable size. During recovery periods after leeches have experienced low prey availability or starvation, animals over compensate and have higher consumption rates relative to individuals that are reared continuously on liberal feeding regimes. In studies on the effects of anthropogenic stress, *N. obscura* in some circumstances showed increased consumption and in others decreased consumption in response to stress. *Nephelopsis obscura* always showed lower consumption rates than controls during the recovery period. Food consumption by other species are reduced during exposure to both natural and anthropogenic stress. Increased feeding rates occur after exposure to anthropogenic stress has not been recorded.

In *N. obscura* natural and anthropogenic stress results in a lower proportion of ingested energy lost in faeces production and thus absorption efficiency increases both during the exposure period and during recovery. Although increased absorption efficiency occurs in other species as a result of exposure to low prey availability, changes in efficiency in response to anthropogenic stress has not been reported for other species.

Exposure to pre-winter conditions (declining temperatures, reduced prey availability) results in increased lipid storage in *N. obscura* and a similar pattern was found when leeches were exposed to anthropogenic stress during the growth phase. Lipids have a very important role in maturation of *N. obscura* and only after exposure to long-term or very high levels of stress do reductions in lipid content occur. The decline in lipids during reproductive phase under natural and anthropogenic stress was associated with onset of maturity in these animals.

Nephelopsis obscura also showed flexibility in terms of respiratory costs. During periods of natural stress size specific hourly costs of resting (Rm) and active (Ra) respiration declined, similar to results found for other species. However, during the recovery period costs of Rm and Ra are generally higher than in unstressed individuals. There are no consistent patterns in changes in Rm and Ra of *N. obscura* or any other species in response to exposure to anthropogenic stress. These inconsistencies are likely due to the confounding effects of food ingestion and growth rates which influence Rm.

It is clear that the responses of animals (i.e., magnitude and duration of plasticity and compensation) when exposed to stresses are not fixed and vary with the life-stage of the animal as well as the concentration, duration and type of stress. If certain components of the energy budget change in response to a stress, the response may change with the duration of the stress and the concentration of the stress and may not be consistent between different life-stages. Conversely, some aspects of the energy budget may not show flexibility but this does not necessarily preclude them from being part of a stress resistant syndrome as with higher intensity or longer duration of stress different responses may emerge. It is also apparent that previous exposure to natural stress will affect an organisms response to anthropogenic stress. Further studies might involve comparison of flexibility of organisms from variable environments (high natural stress) to those from stable environments (low stress) in response to novel types of stress. Furthermore organisms which have been previously exposed to a particular type of stress might be expected to show a different response when re-exposed to the same stress than naive individuals. Further studies might also consider different combinations of stresses to address issues such as how an organism responds to heavy metal pollution when it is simultaneously exposed to starvation stress.

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