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ENERGY ALLOCATION STRATEGIES OF THE ZEBRA MUSSEL, 
*DREISSENA POLYMORPHA*

DISSERTATION

 Presented in Partial Fulfillment of the Requirements for 
the Degree of Doctor of Philosophy in the 
Graduate School of The Ohio State University

By
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* * * * *

The Ohio State University
1997

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ABSTRACT

The ability to adjust physiological parameters in response to environmental conditions, while simultaneously maintaining growth and reproduction, increases an organism's fitness. To study energy allocation in zebra mussels (*Dreissena polymorpha*), I measured individual energy budgets including oxygen consumption, ammonia excretion, tissue and shell growth, feces production, and collected gametes to measure reproduction.

I constructed a balanced seasonal energy budget (May to October) for western Lake Erie zebra mussels and combined with published length-frequency distributions and mussel densities, estimated seasonal population consumption. I estimated mussels average 3.16 cal ind⁻¹ day⁻¹. Metabolic costs accounted for > 90% of consumption. Mussels <15mm increased in mass, whereas mussels > 15 mm reproduced in lieu of growth. Population consumption was sensitive to size distribution with the most abundant size class responsible for the greatest proportion. Using published primary production estimates, I estimated that 10,000-50,000 mussels m⁻² consume an equivalent of 10-50% of primary production.

To examine energy allocation strategies, I altered energy demands. In multifactorial experiments I manipulated consumption and metabolic costs with four temperatures (12 - 30°C), three rations, and two diet qualities based on lipid content (Good, Poor) for 7 wks.

Increased temperature and ration increased metabolic costs but diet qualities did not. Assimilation efficiency, higher in Good Diet than Poor, decreased with ration. Shell growth, tissue growth, and reproduction responded similarly to temperature and ration. Between diets, reproduction was similar whereas growth and survival differed
the most. Good Diet mussels reproduced, grew, and survived. Poor Diet mussels reproduced but did not grow and died. Diet quality influenced reproductive effort with lower investment in body mass in the Poor Diet yielding higher reproductive effort. Thus, zebra mussels are flexible in energy allocation and in stressful conditions, most importantly food quality, reproduce at the expense of maintenance thereby increasing the probability of death.

Reproduction includes gamete production, maturation, and spawning (gamete release), processes that alter energy balance. I estimated spawning costs by inducing spawning and comparing oxygen consumption of spawning and non-spawning mussels and qualitatively scored spawning intensity. Metabolic costs increased with spawning (30%) and intensity indicating that spawning is energetically costly for zebra mussels.
DEDICATION

To my family:
my husband Stuart, daughter Marie, my Mother, and sister Kari.
For all their encouragement, support, sacrifice,
and love.
ACKNOWLEDGMENTS

I thank my advisors, David Garton and David Culver and committee member Roy Stein for their support, encouragement, and patience.

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FIELDS OF STUDY

Major Field: Zoology
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INTRODUCTION

Fitness, "central to the evolution paradigm" (Stearns 1976) is defined as an organism's genetic contribution to future generations. Because genes are contributed to future generations via reproduction, fitness is enhanced by maximizing reproduction and decreasing generation time and mortality. However, because available energy is finite, maximizing reproduction requires tradeoffs with other energetic components. Because body size is positively correlated to reproductive output, individuals should grow larger to increase lifetime reproduction (Gadgil and Bossert 1970). However, due to energy availability, somatic growth postpones reproduction. Conversely, if an individual reproduces, growth is delayed. Delayed growth results in smaller individual body size and reduces lifetime reproductive potential. Growth and reproduction cannot be maximized simultaneously and an organism's fitness is thus limited by conflicting needs to allocate energy between reproduction and growth (Gadgil and Bossert 1970).

In addition to energy invested into gametes, reproduction results in physiological costs affecting adult condition. Organisms adopt a variety of strategies in response to these costs (Calow 1979, Gadgil and Bossert 1970):

1. Allocating energy to reproduction reduces growth or results in competition for energy between growth and reproduction.

2. Metabolic demands increase during reproduction thereby increasing "costs" to the adult. These increased costs may divert energy that might otherwise be used for the accumulation of energy reserves. Lack of reserves, during times when available energy is limited, increases the probability of death. Therefore, increased costs associated with reproduction may reduce fitness by reducing adult survival.
3. Stressful environments invoke different strategies to ensure adult survival. In response, adults may delay the release of gametes or reabsorb gametes in stressful environments.

4. Aging reduces adult future survival. Therefore, to enhance fitness, allocation of energy to reproduction may increase as adults age.

An organism's response to competition between growth and reproduction for energy varies in different environments. It is variation in response to environmental fluctuations that makes the study of energy allocation between growth and reproduction interesting. Fisher (1958) appropriately summarized the problem:

"It would be instructive to know not only by what physical mechanisms a just apportionment is made between the nutriment devoted to the gonads and that devoted to the rest of the parental organism, but also what circumstances in the life-history and environment would render profitable the diversion of a greater or lesser share of the available resources towards reproduction."

Energy budgets: measuring growth and reproduction

The allocation of energy to growth and reproduction can be studied using individual energy budgets. An energy budget is calculated by measuring the energy acquired, lost and used by an organism where food consumed is partitioned into maintenance, growth, and reproduction. The balanced equation is:

\[ C = P + R + F + U \]

where, \( C \) = consumption (energy intake)
\( P \) = production (somatic growth \( P_g \)) and reproduction \( P_r \), \( P = P_g + P_r \)
\( R \) = respiration (metabolic demands)
\( F \) = energy lost as feces
\( U \) = energy lost as nitrogen excretion
A positive energy budget means energy consumed exceeds energy demands; energy is available for production or "scope for growth" (Warren and Davis 1967). If scope for growth is negative, reserve energy must be used to meet maintenance requirements.

Environmental factors, such as temperature and food quality and quantity, affect the physiological parameters (R, U, and F) and therefore the energy available for growth and reproduction. Surviving and maintaining fitness requires the ability to adjust physiological parameters in response to changing environmental conditions, thus minimizing effects on growth and reproduction. The total energy budget approach identifies which parameters are adjusted in response to specific environmental factors to maintain growth and reproduction. By identifying how each physiological parameter is adjusted, we can understand how organisms deal with the competition between growth and reproduction as a function of fluctuating environmental factors.

Novel species in novel habitats

The ability to adjust physiological parameters in response to environmental conditions is a characteristic of species with the potential to invade novel habitats (Ehrlich 1986). For example, the zebra mussel (*Dreissena polymorpha*), a bivalve mollusc native to the Black and Caspian seas, is a very successful invader. Zebra mussels spread through Europe following canal construction in the early 1800's, reaching Great Britain in 1824. Zebra mussels were introduced into the Great Lakes via ballast water discharge, probably in 1985; the first individuals were found in Lake St. Clair in 1988 (Hebert et al. 1989) and in Lake Erie shortly thereafter (Garton and Haag 1992). Zebra mussels continue to spread throughout the Mississippi River drainage and eastern North America and are of ecologic and economic importance. Assessing the impact of zebra mussels on native communities and developing control strategies requires a thorough understanding of the basic biology and ecology of zebra mussels across environmental gradients.
Considering the potential ecological impact of zebra mussels on aquatic ecosystems, very few studies have examined the relationship of environmental factors on physiological responses and allocation of resources to growth and reproduction in this species. Although a large number of studies on the energetics of marine bivalves exists, focused primarily on *Mytilus edulis*, the edible, blue mussel (Bayne and Worrall 1980, Hawkins et al. 1985, Thompson 1984, Widdows and Hawkins 1989), few have examined energy allocation in multifactorial experiments under varying environmental conditions.

Zebra mussels are ideal organisms for energy allocation experiments. Zebra mussels exhibit a short life span (< 5 years), early maturation, and high fecundity. Sexes are separate; gametes are shed into the water column where fertilization occurs. Zebra mussels possess a planktonic veliger larval stage. Veligers are planktotrophic for 2-3 weeks after which they settle and become benthic, sessile adults. Zebra mussel's sessile nature and the absence of etiological factors (parental care, mating, courtship and competition for mates) which can confound the estimation of reproductive costs, makes zebra mussels appropriate experimental organisms to study energy allocation.

The recent invasion of the Great Lakes by zebra mussels provides the opportunity to determine if this successful invader possesses characteristics of species with the potential to invade: the ability to adjust physiological parameters in response to changing environmental conditions by

1. determining the seasonal pattern of energy allocation,

2. examining how flexible zebra mussels are in energy allocation to growth and reproduction under different environmental conditions, and

3. given the importance of energy investment in reproduction, what additional costs are involved in spawning.
Accordingly, my dissertation examines these three aspects energy allocation in zebra mussels each in a separate chapter:

Chapter 1. A seasonal energy budget for zebra mussels (*Dreissena polymorpha*) in western Lake Erie.

I constructed a balanced energy budget for zebra mussels from the western basin of Lake Erie during the active growth and reproductive period (May to October). I measured metabolic costs (oxygen consumption and ammonia excretion), body mass change, and feces production biweekly and quantified shell growth in marked mussels. Costs of reproduction were measured by inducing spawning four times using serotonin and collecting and weighing gametes. After conversion to calories, I combined all energy budget components with published length-frequency distributions and mussel densities to estimate seasonal population consumption in Lake Erie.

Chapter 2. Flexible energy allocation strategies in zebra mussels (*Dreissena polymorpha*) in response to varying environmental conditions.

To determine energy allocation, I altered the stresses and energy available to zebra mussels over a wide range of temperature and food conditions in controlled multifactorial experiments. I manipulated combinations of temperature, ration, and diet quality to alter consumption and metabolic costs. I held zebra mussels at four temperatures (12, 18, 24, and 30°C), three ration levels (low, medium, and high), and two diet qualities (lipid rich and lipid poor) for 7 wks and measured metabolic costs (oxygen consumption and ammonia excretion), somatic tissue growth, shell growth, and feces production at the beginning and end of the experiment. To measure investment in reproduction, I induced spawning with serotonin and collected gametes twice during the experiment. I compared energy investment in reproduction with total
investment in the combination of reproduction and somatic tissue growth by calculating reproductive effort.

Chapter 3. The metabolic demands of spawning in zebra mussels (Dreissena polymorpha).

Reproduction, an important component of fitness, includes processes that alter physiological parameters and overall energy balance. These processes, gamete production, maturation, and spawning, and their associated changes to physiological parameters, comprise "reproductive costs" in zebra mussels. In Chapters 1 and 2, I estimated the investment in gamete production and maturation. In this chapter, I present estimates of the costs for the spawning (release of gametes) by measuring metabolic costs (oxygen consumption) of mussels induced to spawn with serotonin three times during the active spawning period. I compared metabolic costs of three groups of mussels: 1) mussels not induced to spawn (Control), 2) mussels induced to spawn but did not release gametes (Non-spawners), and 3) mussels induced to spawn and released gametes (Spawners). Spawning intensity was qualitatively scored.
CHAPTER 1
A SEASONAL ENERGY BUDGET FOR ZEBRA MUSSELS
(DREISSENA POLYMORPHA) IN WESTERN LAKE ERIE

Introduction

The zebra mussel (Dreissena polymorpha) has recently colonized and become abundant in many North American freshwater ecosystems. This invader is currently the dominant benthic organism in Lake Erie where densities on western basin reefs reach 340,000 mussels m$^{-2}$ (Leach 1992). Their rapid population growth coupled with resultant high densities implies that zebra mussels, as new invaders, have the potential to negatively influence food webs by diverting energy from other species.

As filter-feeding bivalves, zebra mussels remove large quantities of phytoplankton from the water column (Stanczykowska et al. 1975, Reeders et al. 1989, Fanslow et al. 1995). Filtered phytoplankton may be either consumed or ejected as pseudofeces. Filtration rates measured on natural seston of Saginaw Bay, Lake Huron (4.0-40.7 ml mg$^{-1}$ h$^{-1}$) reveal that zebra mussels have the potential to filter the volume of the inner bay 0.2-1.3 times d$^{-1}$ (Fanslow et al. 1995). Based on a bioenergetics model, Lake Erie zebra mussel populations can remove 16-36% of primary productivity (Madenjian 1995). In addition, zebra mussel filtration is responsible for increasing water quality in Saginaw Bay while potentially shifting energy flow to the benthos (Fahnenstiel et al. 1995a,b; Johengen et al. 1995; Lowe and Pillsbury 1995; Skubinna et al. 1995). High filtering capacity coupled with high consumption reinforces the view that zebra mussels significantly alter energy flow in phytoplankton-based food web communities.
Reliable estimates of zebra mussel impact on energy flow depend on accurate measures of zebra mussel consumption. An energy budget approach is appropriate for estimating consumption in some species where environmental factors make direct measurements difficult. Filtration in zebra mussels takes place on the gill where oxygen uptake and pseudofeces production also occur. Therefore, environmental or physiological conditions that change gill activity will alter filtration. The energy budget approach circumvents difficulties associated with measuring filtration and instead, estimates consumption by measuring directly the energy used for metabolic costs (metabolism and excretion), growth, and reproduction.

Environmental factors, e.g., temperature and food conditions (Walz 1978a,b,c; Quigley et al. 1992; Fanslow et al. 1995; McMahon and Ussery 1995; Sprung 1995a,b,c) and physiological factors, e.g., reproductive condition (Sprung 1991), vary seasonally altering energetic demands and thus, energy budget components. Unlike studies using laboratory-acclimated mussels, a seasonal energy budget on field collected mussels not only accounts for variation in energy use, but also identifies which energy budget components vary seasonally in response to environmental factors and physiology.

To account for these seasonal effects, we measured energy budgets of zebra mussels from western Lake Erie regularly during the active growth and reproductive season (May through October). We use our energy budget to 1) estimate individual consumption, 2) determine the proportion of consumption devoted to each energy budget component, and 3) estimate consumption as a function of mussel size. We estimate population consumption by combining our individual energy budgets with published estimates of population density and size structure. Finally, as a potential application, we combine our energy budget with published estimates of phytoplankton productivity to estimate the potential impact of zebra mussels on Lake Erie phytoplankton.
Methods

We collected zebra mussels with SCUBA from 5 m depth near The Ohio State University's F.T. Stone Laboratory on South Bass Island, western basin, Lake Erie. The western basin averages 6 - 7 m deep and does not thermally stratify during summer. We estimated consumption by measuring the energy used for metabolic costs (metabolism and excretion), growth, and reproduction. In addition, a substantial proportion of consumption is not assimilated and is lost via feces. Total consumption was estimated by including assimilation efficiency (AE) in the formula as follows:

Equation 1: \[ C = \frac{(P + R + U)}{AE} \]

where, 
- \( C \) = consumption,
- \( P \) = production as somatic growth (\( P_g \)) and reproduction (\( P_r \)),
- \( R \) = energy lost through respiration,
- \( U \) = energy lost in excretion,
- \( AE \) = assimilation efficiency

At weekly intervals during May through October 1991, we collected mussels and held them for < 24 h in running Lake Erie water aquaria at the ambient temperature prior to measuring energy budget components. To remove suspended material, we filtered lakewater (glass fiber filter, Type AE, 1.0 μm pore size) for use when measuring energy budget components. We measured metabolic costs (\( R \) and \( U \)), growth (\( P_g \)), and feces production of zebra mussels weekly. Reproduction (\( P_r \)) was measured four times, at 2-wk intervals during the active spawning season (July - Aug.).

Energy budget components

Metabolic costs

Metabolic costs were measured via oxygen consumption (\( R \)) and ammonia excretion (\( U \)) rates. In filtered lake water at ambient lake temperature, we measured
Individual mussel oxygen consumption (n = 19, shell lengths 7-30 mm) using a Gilson Differential Respirometer and standard manometric techniques. Oxygen consumption (µl h⁻¹ at STP) was converted to calories using an oxy-caloric coefficient of 4.73 cal ml⁻¹ oxygen at STP (Crisp 1971). Immediately after determining oxygen consumption, we removed two 1-ml water samples from each respirometer flask and measured ammonia concentration with the phenol method (Parsons et al. 1984). Ammonia excretion rates (nM h⁻¹) were converted to calories by the relationship 5.94 cal mg⁻¹ ammonia N (Elliot and Davidson 1975). One respirometer flask containing only filtered lake water functioned as a control. Calories consumed via oxygen consumption and ammonia excretion were summed to determine metabolic costs.

Growth

To estimate individual growth, we measured initial shell lengths of 200 mussels (5 - 30 mm), collected in May, with vernier calipers (nearest 0.1 mm); mussels were uniquely tagged with a number. Marked mussels were allowed to reattach to rocks in running Lake Erie water aquaria for a week. These rocks with zebra mussels attached were returned to Lake Erie. We measured final shell lengths of 148 mussels retrieved in October (134 d).

To monitor change in body mass we measured soft tissue dry mass and shell lengths of mussels used in maintenance cost measurements. At weekly intervals, soft tissue mass (mg dry weight) was measured after drying at 65°C for 24 h, converted to calories by the relationship 3.87 cal mg dry wt⁻¹ (Schneider 1992). Because shell growth likely represents a small fraction of the energy budget, probably much less than the energy invested by bivalves in somatic growth and reproduction (Wilbur and Saleuddin 1983), we did not measure energy invested in shell growth.
Reproduction

Reproductive output of mussels was determined by weighing eggs and sperm collected following induced spawning. Mature mussels (12-25 mm, n = 24 per date) were induced to spawn four times, at about 2-wk intervals during July through August. Individual mussels were placed in beakers containing 1 mM serotonin (5-hydroxytryptophan) in 20 ml of filtered lakewater. After 6 h, we collected eggs and sperm on tared membrane filters (0.45 μm pore size). Filters were then dried at 65°C for 24 h. Final gamete dry mass was converted to calories via the conversion 5.5 cal·mg dry wt gametes⁻¹ (Bayne et al. 1978).

To determine when spawning actually occurred in the local population, we collected mussels weekly, preserving them in Bouin's Fixative for histological analysis. About 12 zebra mussels from each date were embedded in paraffin, thin sectioned, and stained with eosin red and hematoxylin. We assigned maturity status (0 = immature to 4 = mature) following Haag and Garton (1992).

Assimilation efficiency

Weekly, we placed three groups of 10 mussels (10-30 mm) in 80 ml of filtered lake water for evacuation of gut contents. Feces produced after an average of 6 h were collected either on membrane (0.45 μm pore size) or precombusted glass fiber filters (Type AE, 1.0 μm pore size). To measure seston content of lake water, we collected weekly samples with an integrated whole water column PVC tube sampler (3 m x 5 cm). After collecting the seston on the same type of filters as feces, feces, seston, and control filters were dried at 65°C for 24 h, weighed (nearest 0.1 mg), combusted for 1 h at 500°C, and reweighed. We estimated assimilation efficiency using the Conover ash ratio method (Conover 1966).
Consumption

Estimating seasonal consumption

We estimated consumption from metabolic costs, growth, reproduction, and assimilation efficiency at about weekly intervals for five size classes of mussels, 0-5, 6-10, 11-15, 16-20, and 21-25 mm. Based on regression equations for each date (Table 1.1, n = 19 for each date), we calculated the calories of each energy budget component using the log-transformed allometric relationships of the energy budget component and shell length in a no intercept regression model. We used the midpoint of each size class, at 5 mm intervals, to estimate the size-dependent energy budget component for each size class. The ESTIMATE function in GLM (SAS 1989) provided adjusted means and standard errors for each energy budget parameter. To estimate consumption, we divided the sum of the calories for all energy budget components for each size class by the assimilation efficiency.

We incorporated changes in shell length and body mass into the seasonal consumption estimate using mean shell growth of the marked mussels and weekly length-dry mass regressions. Size classes were the same for all dates. To calculate growth between dates, we estimated "initial" mussel size on the previous date. Mussels grew from an "initial" length (L_i) into each size class ("current" length, L_c).

"Initial" length was calculated by subtracting the estimated shell growth, based on the mean shell growth of the marked mussels (mm added d\(^{-1}\)) for the number of days between the sample dates, from the "current" length i.e., size class. Mean shell growth assumed growth was linear across the season and may underestimate shell growth early in the season but overestimate shell growth later in the season (Sprung 1995a).

"Initial" length was:

Equation 2:  \( L_i = L_c - (\text{days between sample dates} \times \text{mm added day}^{-1}) \).

The change in soft tissue body mass (cal) between dates was included in the consumption estimate as \( P_g \). Body mass at the beginning of the season was estimated from the length-dry mass regression derived from a separate sample of mussels.
<table>
<thead>
<tr>
<th>Date</th>
<th>Oxygen Consumption (cal)</th>
<th>Ammonia Excretion (cal)</th>
<th>Body Mass (cal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>b (SE)</td>
<td>$r^2$</td>
<td>adj mean (SE)</td>
</tr>
<tr>
<td>11May</td>
<td>0.113 (0.029)</td>
<td>0.54</td>
<td>1.23 (0.177)</td>
</tr>
<tr>
<td>16May</td>
<td>0.413 (0.024)</td>
<td>0.94</td>
<td>3.38 (0.151)</td>
</tr>
<tr>
<td>25May</td>
<td>0.379 (0.023)</td>
<td>0.94</td>
<td>3.09 (0.155)</td>
</tr>
<tr>
<td>5June</td>
<td>0.207 (0.015)</td>
<td>0.92</td>
<td>1.89 (0.155)</td>
</tr>
<tr>
<td>18June</td>
<td>0.431 (0.022)</td>
<td>0.95</td>
<td>3.53 (0.147)</td>
</tr>
<tr>
<td>29June</td>
<td>0.363 (0.032)</td>
<td>0.88</td>
<td>2.94 (0.151)</td>
</tr>
<tr>
<td>6July</td>
<td>0.392 (0.024)</td>
<td>0.94</td>
<td>3.23 (0.155)</td>
</tr>
<tr>
<td>18July</td>
<td>0.408 (0.022)</td>
<td>0.95</td>
<td>3.29 (0.151)</td>
</tr>
<tr>
<td>28July</td>
<td>0.323 (0.021)</td>
<td>0.93</td>
<td>2.44 (0.155)</td>
</tr>
<tr>
<td>7Aug</td>
<td>0.345 (0.022)</td>
<td>0.94</td>
<td>2.60 (0.155)</td>
</tr>
<tr>
<td>16Aug</td>
<td>0.367 (0.025)</td>
<td>0.92</td>
<td>2.61 (0.152)</td>
</tr>
<tr>
<td>26Aug</td>
<td>0.317 (0.021)</td>
<td>0.92</td>
<td>2.36 (0.151)</td>
</tr>
<tr>
<td>9Sept</td>
<td>0.289 (0.024)</td>
<td>0.89</td>
<td>2.13 (0.155)</td>
</tr>
<tr>
<td>28Sept</td>
<td>0.191 (0.018)</td>
<td>0.87</td>
<td>1.50 (0.157)</td>
</tr>
<tr>
<td>11Oct</td>
<td>0.268 (0.015)</td>
<td>0.94</td>
<td>2.12 (0.147)</td>
</tr>
</tbody>
</table>

Table 1.1: Regression coefficients, adjusted means, and standard errors for oxygen consumption, ammonia excretion, and body mass of Lake Erie zebra mussels (Dreissena polymorpha) for each date in 1991. Regressions were log(dependent variable) vs log(shell length) through the origin. Adjusted means for a 17.8 mm mussel were not log-transformed. All regressions were significant at p < 0.001. (b = slope, n = 19 for each date)
collected prior to the date when energy budget measurements began. For each date thereafter, we calculated "initial" body mass by applying \( L_1 \) in the log-transformed length-dry mass allometric relationship from the previous date. "Current" body mass was estimated from the log-transformed regression for the current date using size class as the "current" length \( (L_2) \).

To include reproduction in the consumption estimate, the number, dates, and relative intensity of spawning events were determined from histology and weekly length-dry mass regressions. We induced spawning in size classes \( \geq 11-15 \) mm and included reproduction calories in the total of the energy budget components for these size classes.

Estimating Population Consumption

Changes in a population's size frequency distribution reflect natural processes of mortality, recruitment, and growth. We did not measure recruitment or mortality but incorporated seasonal change in size frequency distribution by summarizing the frequency of each size class from a representative size distribution from Mackie (1992) for three times: late May, mid July, and late September. We applied these frequencies to our energy budget in the following manner: late May frequencies to May and early June dates, mid-July frequencies to dates during June through early August, and late September frequencies to the remaining dates. To calculate consumption, we weighted total calories consumed on a size class basis. We calculated total population consumption as the caloric sum for all size classes during the season multiplied by population density for representative population densities of 10,000 and 50,000 mussels m\(^{-2}\).
Results

Metabolic costs

Metabolic costs varied across the season without tracking ambient water temperature (Fig 1.1). In addition, costs and their fluctuation across the season increased with size class. In May - early June, costs fluctuated widely whereas water temperature steadily increased. In early June, costs declined to one of the season's lowest levels followed by an increase to the highest costs in mid-June. During June through August, water temperatures were high and relatively constant, but costs varied and declined an average of 20% for mussels > 6 mm over this period. In contrast, costs remained relatively unchanged varying only an average of 4% during September to October when water temperatures decreased 10°C.

Based on size classes, oxygen consumption, corrected for soft tissue mass, was not correlated with temperature (Fig 1.2, p < 0.351, r² = 0.001, n = 89) and the slope of the equation was small (O₂ = 0.0519 + (0.003*temp)). However, analyzing the entire data set (n = 274), temperature influenced oxygen consumption (p < 0.0001, r² = 0.17, O₂ = -0.013 + (0.002*temp)). Oxygen consumption and gametogenic index also were related, though the regression explained little of the variability (p < 0.004, r² = 0.03, n = 274, O₂ = 1.29 + (0.161*reproductive condition)). Combining these variables in a multiple regression, temperature had a greater influence on oxygen consumption than did reproductive score (temperature: p < 0.0001, reproductive score: p = 0.387, n = 274).

Growth and reproduction

We calculated shell growth for our five size classes from the shell length added per day (SLA d⁻¹) of marked mussels (SLA d⁻¹ = 0.0795 - 0.00347(initial length), r² = .54, p < 0.0001, n = 148). Mean shell growth declined with increasing size class in the following order: 0.071, 0.053, 0.036, 0.019, and 0.001 mm d⁻¹.
Fig 1.1. Metabolic costs (oxygen consumption plus ammonia excretion) in cal·ind⁻¹·d⁻¹ for zebra mussel (*Dreissena polymorpha*) size classes and ambient lake temperature (°C) during May - October, 1991 (South Bass Island, Western Lake Erie). Adjusted means and standard errors for the midpoint of each size class are plotted and were calculated from weekly metabolic cost measurements (n = 19 per date, shell lengths 7-30 mm) using the ESTIMATE function in GLM for the regression of log(metabolic costs) against log(shell length) in a no intercept model.
Fig 1.2. Weight-specific oxygen consumption (cal·cal⁻¹·d⁻¹) plotted vs ambient lake temperature for zebra mussel (*Dreissena polymorpha*) size classes. Oxygen consumption and temperature were not correlated (p < 0.351, r² = 0.001, slope = 0.003).

As size class increased, absolute body mass fluctuated more through the season (Fig 1.3a). Body mass of zebra mussels < 11 mm remained constant whereas those > 11 mm fluctuated markedly twice during summer. In June, larger mussels lost about 40% of their body mass, regaining this mass during July, and then losing an additional 30% during mid-July - August. Figure 1.3a depicts absolute change in
body mass. When recalculated as proportion of original body mass, body mass of smaller size classes fluctuated no more than 10% of their original mass whereas body mass of the 3 largest size classes fluctuated 50%. Relative change in body mass paralleled the changes depicted in Fig 1.3a.

Two peaks in spawning activity were observed near June 18 and July 19 (Fig 1.3b), thus we included reproduction calories in the energy budget on these two dates. Maximum reproductive output typically occurs with the first spawning event (Walz 1978b, Sprung 1991) and body mass changes reflect reproductive events (Garton and Haag 1992, Nalepa et al. 1993). However, the first time we induced spawning was after the first spawning event (June 18). Therefore, to better estimate reproductive output of the first spawning event, we increased the maximum reproduction calories 10% (because 10% more body mass was lost on June 18 than on July 19) and added these calories to the energy budget on June 18. Because we induced spawning on July 19, coinciding with the second natural spawning event, we did not adjust spawning output and added those reproduction calories in the energy budget on July 19. Reproductive output was higher with the first induction and increased with mussel size (Table 1.2). However, reproductive effort, expressed as a percentage of dry mass, declined with increasing size class.

Proportion devoted to each energy budget component

Metabolic costs (oxygen consumption + ammonia excretion) comprised the largest proportion of the seasonal energy budget for all sizes and overall averaged 95% (Fig 1.4). Calories used in oxygen consumption were the highest of all energy budget
Fig 1.3. (a) Soft tissue body mass (cal·ind⁻¹) for western basin Lake Erie zebra mussel (Dreissena polymorpha) size classes during May - October, 1991. Adjusted means and standard errors for the midpoint of each size class are plotted and were calculated from weekly length-dry mass regressions (n = 19 per date, shell lengths 7-30 mm) using the ESTIMATE function in GLM for the regression of log(dry soft tissue mass) against log(shell length) in a no intercept model. (b) Mean gametogenic index and standard errors determined from histological sections. Indices range from 0 = immature to 4 = mature (Haag and Garton 1992). Arrows (↑) indicate dates of spawning events in the lake population and dates when reproduction calories were included in the energy budget. Check marks (✓) indicate dates spawning was induced and reproductive output measured.
Figure 1.3
Table 1.2. Reproductive output of zebra mussel (*Dreissena polymorpha*) size classes from western basin, Lake Erie measured by inducing spawning with serotonin (n = 24 per date, 12-25 mm) on the dates when reproduction calories were added to the total consumption estimate. Reproductive output is in cal of gametes ind⁻¹ and the percentage of soft body mass calories lost. Also shown are the total percentages of body mass lost with both spawning events in units of cal and mg dry soft body mass. Values are means ± SE for the midpoint of each size class.

<table>
<thead>
<tr>
<th>Size Class (mm)</th>
<th>18 June</th>
<th>19 July</th>
<th>Total Percent of Soft Body Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cals ind⁻¹</td>
<td>Percent of body mass</td>
<td>cals ind⁻¹</td>
</tr>
<tr>
<td>11-15</td>
<td>3.6 ± 1.6</td>
<td>13 ± 6</td>
<td>3.3 ± 1.5</td>
</tr>
<tr>
<td>16-20</td>
<td>4.3 ± 1.7</td>
<td>10 ± 4</td>
<td>3.9 ± 1.5</td>
</tr>
<tr>
<td>21-25</td>
<td>4.8 ± 1.8</td>
<td>8 ± 3</td>
<td>4.4 ± 1.6</td>
</tr>
</tbody>
</table>

components, averaging 88% of the energy budget. The smallest proportion of the energy budget was devoted to growth and reproduction. When combined, growth and reproduction averaged 5% of the seasonal energy budget.

Although the investment in somatic growth declined with size class, reproductive investment was equal (Fig 1.4). Mussels ≤ 15 mm invested in somatic growth, whereas those > 15 mm lost body mass during May through October. Growth and reproduction were equal in mussels 11-15 mm, whereas mussels in the two largest sizes (16-20 and 21-25 mm) reproduced but did not invest in somatic tissue growth.
Fig 1.4. Percentage of total calories and standard errors invested in each energy budget component for western basin Lake Erie zebra mussel (*Dreissena polymorpha*) size classes, Total consumption was the sum of the calories for all energy budget components divided by assimilation efficiency for all dates during May - October, 1991.
Assimilation efficiency

Like metabolic costs, assimilation efficiency (AE) varied during May through October (Fig 1.5). AE tracked seston organic content ($r^2 = 0.695$, $p < 0.0001$), whereas feces organic content was not correlated to seston ($r^2 = 0.164$, $p = 0.379$) and did not vary across the season ($p < 0.099$). AE averaged 74% in May, declined in June, reaching its lowest value (48%) in July. Following a steady increase, AE averaged 88% through September and declined again in October. The overall seasonal average was 72%.

Fig 1.5. Average assimilation efficiencies for western basin Lake Erie zebra mussels (*Dreissena polymorpha*), organic content of feces and seston, and standard errors during May - October, 1991. We estimated assimilation efficiency using the Conover ash ratio method (Conover 1966) and measured organic content of feces and seston using ash free dry weights.
Seasonal consumption estimate

Because population length-frequency distributions change during the season, we applied a representative distribution to our energy budgets to estimate population consumption. We summarized length-frequency distributions from Mackie (1992) for three periods that represented typical changes in a zebra mussel population (Fig 1.6). The May sample reflected a population following overwintering, individual growth but minimal recruitment of smaller size classes occurred in July, and September reflected the major recruitment period.

Fig 1.6. Size class frequencies for May, July, and August summarized from a zebra mussel \textit{(Dreissena polymorpha)} size distribution representing a typical seasonal change in population distributions (Mackie 1992).
Total calories consumed during the season by individuals within a size class increased with size (Table 1.3). Zebra mussels consumed an average of 3.16 (± 0.04) cals ind⁻¹ d⁻¹ over 152 d. However, when corrected for frequency within a population, smaller size classes had a greater proportion of total seasonal consumption. The proportion of consumption by larger mussels was much lower because of their lower frequency in the population.

<table>
<thead>
<tr>
<th>Size Class (mm)</th>
<th>Calories per individual</th>
<th>Percent of population consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 5</td>
<td>357.5 ± 11.5</td>
<td>33.6 ± 0.1</td>
</tr>
<tr>
<td>6 - 10</td>
<td>491.8 ± 38.1</td>
<td>27.0 ± 2.0</td>
</tr>
<tr>
<td>11 - 15</td>
<td>580.5 ± 65.2</td>
<td>21.2 ± 2.5</td>
</tr>
<tr>
<td>16 - 20</td>
<td>623.2 ± 91.8</td>
<td>12.1 ± 1.8</td>
</tr>
<tr>
<td>21 - 25</td>
<td>670.4 ± 118.4</td>
<td>6.1 ± 1.1</td>
</tr>
</tbody>
</table>

Table 1.3. Total seasonal individual consumption (± SE) by zebra mussels (*Dreissena polymorpha*) from the western basin, Lake Erie and the percentage of total population consumption (± SE) estimated using a length-frequency distribution (summarized from Mackie 1992) for the midpoint of each size class.

The sizes of zebra mussels responsible for the greatest proportion of consumption varied across the season, tracking the changes in the length-frequency distribution (Fig 1.7a). In spring and fall, 0-5 mm mussels averaged 40 and 65%, respectively. By mid-season, the highest consumption had shifted to middle sizes (maximum of 32%).
Fig 1.7 (a) Percentage of total consumption and standard errors for western basin Lake Erie zebra mussel (*Dreissena polymorpha*) size classes weighted by frequency using the size distribution summarized from Mackie (1992). Total consumption was the sum of the calories for all energy budget components divided by assimilation efficiency for the interval between dates. (b) Total consumption for zebra mussel population densities of 10,000 and 50,000 mussels m\(^{-2}\) weighted by size frequency.
Total population consumption fluctuated during May through October and varied with the density of mussels applied to the consumption estimate (Fig 1.7b). Total consumption at 10,000 mussels m\(^{-2}\) remained constant and averaged 317.7 (± 27) kcal m\(^{-2}\). Consumption peaked in July for both densities when middle sized mussels had the highest consumption. At 50,000 mussels m\(^{-2}\) consumption averaged 1600 (± 136) kcal m\(^{-2}\). In addition, the absolute magnitude of fluctuation in consumption is very different at higher density where consumption ranged from a minimum of 470 kcal m\(^{-2}\) in May to a maximum of 3729 kcal m\(^{-2}\) in July.

Discussion

Energy budget components

Metabolic costs

Metabolic costs comprised the highest proportion of calories consumed by zebra mussels in our seasonal energy budget. Oxygen consumption alone averaged 88% (± 4%). Metabolic costs, and, thereby the majority of energetic demands, fluctuated across the season. Environmental and physiological factors such as temperature, food conditions, and reproduction alter energetic demands (Walz 1978a,b,c; Sprung 1991, Sprung 1995a,b,c; Quigley et al. 1992; McMahon and Ussery 1995) and likely are responsible for the seasonal variation in metabolic costs documented in Lake Erie zebra mussels.

Temperature alters metabolism in mussels and oxygen consumption rates can follow temperature changes (Bayne and Newell 1983, Quigley et al. 1992, Alexander et al. 1994, McMahon and Ussery 1995, Sprung 1995a,b,c). Oxygen consumption in zebra mussels has been correlated with temperature (Quigley et al. 1992, McMahon and Ussery 1995, Sprung 1995c), though the influence of temperature on a seasonal
basis has not been clearly established. Though ambient water temperature often
influences oxygen consumption, mass-specific oxygen consumption by zebra mussels
and seasonal water temperatures were not strongly correlated in our study.

One possibility for the lack of a strong correlation of oxygen consumption and
temperature on a seasonal basis is acclimation ability. Acclimation allows some
bivalves to stabilize oxygen consumption rates. Following 2 wks acclimation,
*Mytilus*, an intertidal marine bivalve, adjusts oxygen consumption rates to a new level
(Bayne and Newell 1983). Similarly, zebra mussels require 12-14 days in the
laboratory to acclimate to new temperatures (A. M. Stoeckmann, unpublished data).
Acclimation ability thus is influenced by exposure time, and in addition, by the rate of
change and the temperature range.

Rate of temperature change influences acclimation ability. Acclimation
temperature and rate of temperature increase alters upper lethal temperatures of zebra
mussels (McMahon and Ussery 1995). Metabolic costs, while not highly correlated to
temperature, fluctuated across the season and were most variable during May and
June. Rapidly increasing temperature without acclimation could be responsible for
these variations. During May and June, temperature increased 9°C in 45 days, equal
to a rate of change of 0.2 degrees per day. This rate of temperature change should be
well within the acclimation abilities of zebra mussels, negating rapid warming as a
causal factor for increasing metabolic costs.

Temperature range influences acclimation. Over a 10°C temperature
difference (10-20°C), oxygen consumption rates in laboratory-acclimated, unfed
mussels do not differ (Quigley et al. 1992). Similarly, during May through October,
oxygen consumption rates did not differ over the slightly higher temperature range
(15-25°C) in our study. The lack of a strong correlation of oxygen consumption and
temperature reveals that zebra mussels can adjust oxygen consumption within normal
summer water temperature patterns.

Other factors such as food and reproductive condition likely contribute to
variation in oxygen consumption. Specific dynamic action (SDA), the physiological

Physiological changes due to reproduction, specifically gametogenesis and spawning, increase oxygen consumption in mussels (Bayne et al. 1978, Sprung 1991) and therefore contribute to fluctuations in metabolic costs. Although seasonal oxygen consumption is not correlated to gonad volume (Sprung 1995c), reproduction in zebra mussels is an oxygen demanding process (Sprung 1991) and oxygen consumption rates during the reproductive period are greater than respiration rates measured at other times of the year (Lyashenko and Karchenko 1989). In laboratory experiments, oxygen consumption doubles during spawning (Stoeckmann and Garton, unpublished data). Lake Erie zebra mussels were actively undergoing gametogenesis and spawning during May and June, the time of greatest fluctuation in metabolic costs. Thus, as Lyashenko and Karchenko (1989) found, reproductive state can be correlated to, and is implicated as a factor responsible for, observed variation in metabolic costs.

Overall, no single factor explains the seasonal variation in metabolic costs in Lake Erie zebra mussels. The combination of physiological and environmental factors, reproductive state, temperature, and food conditions, drive variation in metabolic costs and thus energy demands in zebra mussels.

Growth and reproduction

Reproductive and food conditions are factors also responsible for variation in body mass observed during May through October. Body mass changes in zebra mussels were tightly linked to reproductive condition; greatest decreases in body mass occurred during spawning. Zebra mussels lose 25-33% of body mass over summer
and losses coinciding with spawning range from 33% of wet body mass (Nalepa et al. 1993) to 50% of dry body mass (Garton and Haag 1992, Haag and Garton 1992). Mass of gametes released equals 30% of initial body mass (Sprung 1991). We found that although body mass loss (40%) fell within published ranges, reproductive output (mean = 17%) fell below other studies and was less than observed body mass loss. In addition, largest mussels did not invest calories equally into growth and reproduction.

The lack of balance between growth and reproduction suggests reproductive output may have been underestimated. To control for and minimize errors in measuring reproductive output we: 1) kept the time allowed for spawning constant, 2) induced spawning several times during the season, 3) incorporated results of histological gametogenic indices, and 4) compared reproductive output with changes in body mass. In larger mussels, changes in body mass associated with spawning are comprised of both reproductive output and additional body mass used as reserves (posed by Nalepa et al. 1993). During mid to late summer, a clear water phase occurs in Lake Erie, and available food may be scarce (Makarewicz 1993). Reproduction increases metabolic costs (Bayne et al. 1978, Sprung 1991) which requires the use of body reserves at times of high costs and insufficient food. Thus, under these conditions, a negative energy balance occurs and loss of body mass exceeds reproductive output.

Net negative growth of larger zebra mussels (>15 mm) during summer further confirms that zebra mussels use energy reserves. Total calories invested in reproduction and growth revealed that small zebra mussels grew while large mussels only reproduced. Even if reproduction in larger mussels was underestimated by 10%, increasing the total to 30% of body mass (near the range of reported body mass losses (Garton and Haag 1992, Haag and Garton 1992, Nalepa et al. 1993)), reproductive output would still be much less than body mass loss. Excess body mass loss indicates zebra mussels experience a period of negative energy balance and must use body mass reserves to survive. Greater reproductive investment and use of reserves may cause
high mortality and explain the scarcity of large mussels in Lake Erie populations (Garton and Berkman unpublished data).

Consumption estimate

The total energy available for metabolic costs, growth, and reproduction depends on assimilation efficiency. Food conditions change physiological components of assimilation efficiency and are reflected in changes in ingestion capacity (Sprung 1995b), absorption efficiency (Bayne et al. 1989), and assimilation efficiency (Stanczykowska et al. 1975, Bayne and Newell 1983) allowing mussels to take advantage of current food conditions. Assimilation efficiency of Lake Erie zebra mussels closely tracked seston organic content. Seasonal variations in seston quality correspond to changing phytoplankton species composition (some taxa being more nutritious than others) and with levels of inorganic matter (increase in inorganic material reduces quality). When exposed to changes in their food source, assimilation efficiency in zebra mussels parallels the changes thus implying ingestion rates of natural seston are never at maximum.

Estimates of total population consumption were influenced strongly by the frequency distribution with the most abundant size class contributing the most to consumption. During the season, the smallest size class often had the highest proportion of population consumption. This pattern reflects the fact that in this sample population smaller sized individuals were most common and is consistent with a population that lives < 2 years, with mussels rarely reaching lengths > 30 mm (Mackie 1992). At zebra mussel densities of 10,000-50,000 per m², seasonal patterns of consumption were exaggerated at high densities, reinforcing the observation that consumption and therefore impact on phytoplankton varies across the season and is density dependent.

A seasonal energy budget permits estimating total consumption and potential impacts of zebra mussels on phytoplankton. Two models estimating zebra mussel growth (Schneider 1992) and phytoplankton impact in Lake Erie (Madenjian 1995)
have been published. Using Madenjian's estimates for both primary production (24.8 million tonnes) and inhabited area of western Lake Erie (based on mussels occupying hard bottom areas, 13% of 3276 km$^2$ (total western basin area)), assuming 1000 cal g wet wt algae$^{-1}$ and our energy budget consumption estimate of 3.16 cal ind$^{-1}$d$^{-1}$, then 10,000-50,000 mussels m$^{-2}$ consume 10-50% of primary production. Our energy budget estimates consumed calories and does not include the additional proportion of primary production removed by zebra mussels in pseudofeces (about 22%, Madenjian 1995). Total primary production impact increases to 12-60% when pseudofeces production is included. Our energy budget estimates thus brackets Madenjian's model estimate of 26 ± 10% of primary production removed.

Although these estimates imply a significant impact on phytoplankton by zebra mussels, phytoplankton and zooplankton are still present in western Lake Erie. Impact on phytoplankton depends on several factors including delivery rates of phytoplankton to the benthos and on zebra mussel population densities. Due to its shallow and turbulent nature, delivery rates in western Lake Erie may be high. In addition, zebra mussel populations may still be increasing via colonization of soft substrates (Nalepa et al. 1995, Dermott and Munawar 1993) at densities up to 30,000 mussels m$^{-2}$ (D. Garton, unpublished data). An increasing population implies zebra mussel impacts on phytoplankton will likely continue to increase, unless mussels ingest a substantial proportion of required calories from non-planktonic sources (Cotner et al. 1995, Lavrentyev et al. 1995).

This study clearly identifies the importance of metabolic costs in zebra mussel energy budgets. Because > 90% of the calories consumed are used in metabolic costs, the accuracy of this measurement is paramount for constructing energy budgets. Seasonal variation in metabolic costs was influenced more by reproduction than by temperature, hence consumption estimates will be more sensitive to physiological condition than to temperature. Likewise, changing seston composition changes assimilation efficiency, directly influencing consumption estimates. Thus, any zebra
mussel energy budget or bioenergetics model must incorporate seasonal metabolic, reproductive, and phytoplankton cycles to accurately estimate all energetic components.
CHAPTER 2
FLEXIBLE ENERGY ALLOCATION STRATEGIES IN ZEBRA MUSSELS
(*DREISSENA POLYMORPHA*) IN RESPONSE TO
VARYING ENVIRONMENTAL CONDITIONS

Introduction

Allocating energy to growth and reproduction is a challenge for organisms experiencing variable environmental conditions. Because available energy is finite, both growth and reproduction cannot be maximized simultaneously (Gadgil and Bossert 1970, Stearns 1976, Reznick 1992). Thus, in variable environments, organisms experience conflicting demands for energy by growth and reproduction (Gadgil and Bossert 1970). In addition, lifetime influences an organism’s options to delay reproduction. For example, short-lived bivalves (e.g., zebra mussels live 3 - 4 yrs) have fewer reproductive years, and thus fewer options to delay reproduction, than do long-lived species (e.g., unionids) that may have 15 yrs of reproductive opportunities.

Reproduction is an energy demanding process that alters adult condition. In addition to energy invested into gametes, reproduction is accompanied by physiological costs, diverts energy that might otherwise be invested in reserves, and thereby potentially reduces adult survival (Calow 1979, Gadgil and Bossert 1970). Stressful environmental conditions additionally tax energy reserves and invoke tactics, such as delay in gamete release or gamete reabsorption, that ensure adult survival but reduce reproductive investment (Calow 1979, Gadgil and Bossert 1970). As a consequence, organisms continually adjust physiological processes and energy
allocation in response to varying environmental conditions to minimize negative effects on growth, reproduction, and survival.

Organisms allocate energy according to basic principles of energy exchange. A portion of the energy acquired by the organism (consumption, $C$) is lost to metabolic demands ($M$). Metabolic demands include energy used in metabolism, energy lost in excretion, and energy lost as unassimilated food in feces. If consumption minus metabolic losses results in a positive energy balance, energy is available for somatic growth ($G$) and reproduction ($R$) as described by the expression:

$$C - M = G + R.$$

Herein lies the energy allocation challenge. All energy balance components vary in response to environmental conditions, such as temperature and food conditions, and thus net energy balance fluctuates, changing the energy available for somatic growth and reproduction. Surviving and maintaining positive growth and reproduction requires the ability to adjust physiological functions in response to environmental conditions.

We examined energy allocation strategies in an invading freshwater bivalve, the zebra mussel (*Dreissena polymorpha*). Zebra mussels, were introduced in mid 1980's (Hebert et al. 1989), rapidly spread to all the Great Lakes, and continue to spread throughout the Mississippi River drainage. Zebra mussels grow rapidly (maximums range 38 (Sprung 1995a) to 75 mm d$^{-1}$ (Bj de Vaate 1991)), mature quickly (in first year (Nichols 1996)), have high fecundity ($10^6$ eggs, $10^{10}$ sperm · yr$^{-1}$ (Sprung 1991)), and devote an average of 5% of their seasonal energy budget to growth and reproduction (see Chapter 1). Successful invasions enhanced by rapid growth and reproduction, occurring in a variety of habitats, imply zebra mussels are physiologically adaptable. Physiological adaptability and the absence of etiological factors (e.g., parental care, mating, courtship, and competition for mates) which can confound the estimation of reproductive costs, make zebra mussels an ideal organism to examine energy allocation between growth and reproduction under different environmental conditions.
Our multifactorial experimental design incorporates the interaction of temperature and food conditions to examine how these environmental factors alter physiological parameters and energy allocation in zebra mussels. In laboratory experiments, we controlled consumption and metabolic costs by manipulating combinations of temperature, diet quality, and ration. We measured maintenance costs, somatic growth, and reproduction to determine if zebra mussels have flexible energy allocation into growth and reproduction.

Methods

Experiment design

We used a multifactorial design to test how temperature, diet quality, and ration influenced energy allocation in zebra mussels. We chose four temperatures within the range of seasonal temperatures in Lake Erie and that encompass the extremes for vital physiologic functions: 1) 12°C (early spring and the lower threshold necessary for spawning initiation (Sprung 1991)), 2) 18°C (late spring and fall), 3) 24°C (mid - late summer), and 4) 30°C (extreme high summer temperature but below lethal temperature).

We provided diets at two quality extremes and that were readily consumed by zebra mussels. In addition to possible texture and palatability differences, diet quality was based on the relative proportion of long-chain polyunsaturated fatty acids (20:5ω3 and 22:6ω3) in the diets as derived from literature values (Whyte and Ginther 1989 unpublished report to Coast Oyster Co., Volkman et al. 1989, Ahlgren et al. 1990). "Good" quality diet was a prepared marine algae mixture used in oyster culturing (Thalassiosira and Skeletonema, Diet B, Coast Seafood Co., Bellevue, WA) that contained substantial percentages of the two fatty acids. "Poor" quality diet was a dried green alga (Chlorella) that did not contain either fatty acid.
We fed mussels at three rations (Low, Medium, and High) based on the percentage of estimated dry body mass of a mean sized mussel (20 mg dry mass), respectively: 2.5, 5, and 25% of estimated dry soft tissue mass mussel\(^{-1}\) \text{day}^{-1}. Low ration (2.5%) was the minimum ration necessary to support metabolic costs of an average mussel (20 mg dry soft tissue, Chapter 1). Because carbon content was 9% greater in *Chlorella*, diet rations were adjusted to equal organic content. We pulse fed mussels 2-3 times per day.

Zebra mussels were collected in late spring after gametogenesis had begun but prior to the onset of spawning (early June 1992 and 1993) from a 5 m site near Ohio State University's F. T. Stone Laboratory, South Bass Island, western basin, Lake Erie. We tested the Good Diet for 7 wks during June - August, 1992 and the Poor Diet during June - August 1993. All temperatures and rations were tested each year; however, in 1992, the 18°C Good Diet treatment was lost mid-experiment due to a recirculating water chiller failure. To test for a tank effect, all three ration levels were replicated; one randomly assigned per temperature during 1993: 1) Low ration was replicated at 24°C, 2) Medium at 30°C, and 3) High at 12°C.

Each treatment had 200 mussels (5-30 mm shell length) held in 10 l aquaria containing filtered lake water (glass fiber filters, type AE, 1.0 \(\mu\text{m}\)) or aged tap water, undergravel filters, and 15 cm long air bubble bars. Insulated coolers held the aquaria, one for each ration level, with recirculating water chillers maintaining water temperature. Aquaria were cleaned every 2-3 days.

**Energy Budget Components**

To determine the relative importance of temperature, food quantity and quality on zebra mussel energetics, we measured all components except consumption and accounted for the energy lost as unassimilated food in feces by calculating
assimilation efficiency. The balanced equation is

Equation 1: \[ C = \frac{(R + U + P_g + P_r)}{A_E} \]

where,
- \( C \) = consumption
- \( R \) = energy lost in respiration
- \( U \) = energy lost as excretion (ammonia)
- \( P_g \) = somatic growth
- \( P_r \) = reproduction
- \( A_E \) = assimilation efficiency

After acclimating mussels for 10-14 days to temperature, diet, and ration, all energy budget components were measured of each treatment group in filtered water (glass fiber filters, type AE, 1.0 \( \mu \)m) and at the treatment temperature.

Metabolic costs (\( R \) and \( U \))

We measured oxygen consumption (\( R \)) and ammonia consumption (\( U \)) twice: 1) after the initial 2 week acclimation period and 2) at the experiment's end. We measured oxygen consumption on 19 mussels (shell lengths 7-30 mm) using a Gilson Differential Respirometer and standard manometric techniques. Following oxygen consumption measurements, two 1-ml water samples were removed from each respirometer flask and ammonia concentration was quantified with the phenol method (Parsons et al. 1984). One respirometer flask containing only filtered water functioned as a control. Oxygen consumption (\( \mu \)l h\(^{-1}\)) and ammonia excretion (nM h\(^{-1}\)) were converted to Joules (0.01979 \( J \mu l^{-1} \) oxygen at STP (Crisp 1971), 0.000348 \( J \) mg\(^{-1} \) ammonia N (Elliot and Davidson 1975) and combined to estimate metabolic costs.

Growth (\( P_g \))

To measure shell growth over the 7 wks, we uniquely marked 35 mussels (shell lengths 5-30 mm) with numbered tags at the beginning of the experiment and measured initial shell lengths with vernier calipers (nearest 0.1 mm). At the end of the
experiment we measured final shell lengths of marked mussels. Individuals were dried at 65°C for 24 h and weighed (nearest 0.1 mg). We also measured lengths and dry soft tissue mass of mussels used in metabolic cost measurements. Body mass was converted to Joules by the relationship 16.192 J/mg dry soft tissue¹ (Stanczykowska 1976).

Assimilation efficiency (AE)

We measured fecal production once, at about the fifth week of the experiment. We placed three groups of five mussels from each treatment in 250 ml of filtered water for about 6 h. After guts were empty, we returned mussels to the treatments. We collected feces produced on precombusted, tared, glass fiber filters (type AE, 1.0 μm). Filters were dried at 65°C for 24 h, cooled in a dessicator, and reweighed. To determine the organic proportion of the feces, we combusted filters at 500°C for 1 h, cooled, and reweighed. We also determined the organic proportion of the diets by similarly combusting a known weight of diet. We used the organic proportions of feces and diets to calculate assimilation efficiency using the Conover ash ratio method (Conover 1966).

Reproduction (Pᵢ)

Because zebra mussels mature and spawn gametes in successive cohorts, we induced spawning in 24 mussels (shell lengths 12-30 mm) from each treatment, twice during the experiment at about 3 wk intervals (after 3 and 6 wks). We induced spawning by placing individuals in 20 ml of 1 mM serotonin (5-hydroxytryptophan) in filtered water. Gametes were collected on membrane filters (0.45 mm pore size), dried (65°C for 24 hrs), cooled in a dessicator, and reweighed (nearest 0.1 mg). After the first induction, we uniquely marked spawned mussels with tags and returned them to the treatments. Reproductive output was the total gamete mass an individual released in the two spawning inductions.
To determine differences in reproductive condition, we evaluated gametic maturation in mussels from each treatment (n = 12) using histological sections. At experiment’s end, mussels from each treatment were preserved in Bouin's Fixative, dehydrated in ethanol and toluene, embedded in paraffin, thin sections fixed on slides, and stained with eosin red and hematoxylin. We assigned gametogenic maturation using an index (0 = immature to 4 = mature) following Haag and Garton (1991).

Reproductive effort (RE)

We calculated reproductive effort (RE) as the proportion of total energy (soft tissue growth and reproduction) invested in reproduction:

Equation 2: \[ RE = \frac{\text{Reproductive output}}{\text{Final soft tissue mass} + \text{Reproductive output}} \]

RE was calculated in joules. Gamete mass was converted to joules (22.999 J mg dry gamete mass\(^{-1}\), Bayne et al. 1978) and dry soft tissue mass as explained earlier. Because soft tissue mass declines with spawning, to estimate final soft tissue mass of mussels induced to spawn, we applied the shell lengths of mussels induced to spawn in length - dry soft tissue body mass regressions from mussels used to measure final metabolic cost for each treatment. Reproductive output was the total of the two spawning inductions.

Condition Factors

We used four indices, protein catabolism (O:N ratios), lipid and carbohydrate reserves, and survival to assess overall mussel condition at the experiment end. Protein catabolism, calculated as the ratio of moles of oxygen consumed to moles of ammonia excreted (O:N ratio), was based on metabolic cost measurements at the experiment end. As indices of energy reserves, we measured lipid and carbohydrate content of mussels from each treatment (n = 12, shell lengths 10 - 30 mm). Total lipids (mg) were measured by the vanillin-phenol method (Van Handel 1985) and total
carbohydrates (mg) by the phenol - sulfuric acid method (Montgomery 1957). Soft tissue body mass for individuals was estimated from their measured shell lengths (mm) and the length - dry soft tissue body mass regressions from mussels used to measure final metabolic cost for each treatment. We determined survival by inspecting aquaria daily, removing all dead mussels (gaping and unresponsive to touch), and calculating mortality as the total marked mussels dead at the experiment end.

**Statistical analysis**

Energy budget components vary allometrically; therefore, we used ANCOVA to control for body mass effects in all statistical analyses (SAS 1989). The contribution of among-tank variance on each component was tested with a nested ANOVA model. LSMeans for all variables were used in correlations (SAS 1989).

**Results**

The response of each energy budget component, reproductive effort, and condition factor to the experimental conditions of temperature, ration, and food quality are each examined separately. Because there was no difference between replicated tanks for oxygen consumption, ammonia excretion, final soft tissue mass, shell growth, and reproductive output, (P > 0.05 for all) results from replicated tanks were combined. In addition, six treatments were omitted from some results: 1) due to a recirculating water chiller malfunction, all rations in the 18°C Good Diet were lost and 2) due to early, high mortality, all rations of 30°C Poor Diet were discontinued at 3 wks.
**Metabolic Costs (oxygen consumption and ammonia excretion)**

Metabolic costs did not differ between the beginning and end of the experiment (Table 2.1, Time as main effect). Thus, to quantify treatment effects, we averaged metabolic costs from the beginning and end of the experiment. All three treatments, diet quality, temperature, and ration, strongly influenced metabolic costs as did all combinations of these effects (Fig. 2.1, Table 2.1). In both diets, metabolic costs increased with temperature. High Ration also increased metabolic costs and the increase was greater at higher temperatures (24 and 30°C). Metabolic costs, at a particular temperature, were generally lower in Poor Diet than in Good Diet and the increase at High Ration was less pronounced in the Poor Diet.

**Assimilation efficiency (AE)**

Diet quality significantly influenced AE, with AE generally higher in the Good Diet (Fig. 2.2, Table 2.1). The interaction of diet quality and temperature also influenced AE but the main effects of temperature and ration did not. Although accompanied by high variation, AE differed with temperature in the Poor Diet and was lower at 12°C than in the Good Diet.

**Growth**

Marked mussels' ability to grow in shell length was strongly influenced by diet quality with mussels fed Good Diet growing in shell length, whereas mussels fed Poor Diet reabsorbed shell (Fig. 2.3, Table 2.1). Shell growth also differed by temperature and by temperature x ration (Table 2.1). However, ration did not influence shell growth except at High Ration, Good Diet. Shell growth was correlated with assimilation efficiency (shell length added = (-0.198) + 0.00365 AE, r² = 0.33, p<0.05, n = 18).
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NS P > 0.05, * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001

Table 2.1. Significance levels from ANCOVA for metabolic costs (MC, oxygen consumption and ammonia excretion), assimilation efficiency (AE), shell growth (SG), final soft body mass (FBM), reproductive output (RO), reproductive effort (RE), gametogenic index (GI), O:N, lipid, and carbohydrate (CHO) levels for Lake Erie zebra mussels held for 7 wks at different temperatures, rations, and diet qualities. Blank cells were untested effects.
Figure 2.1. Metabolic costs (J h⁻¹) (± SE) averaged across the beginning and end of the experiment for Lake Erie zebra mussels held for 7 wks at different temperatures, rations, and diet qualities. Mean metabolic costs, derived from ANCOVA, for the mean zebra mussel (15.1 mg) are plotted.
Figure 2.2. Assimilation efficiency (AE) (± SE) for Lake Erie zebra mussels held for 7 wks at different temperatures, rations, and diet qualities.
Figure 2.3. Shell growth for individually marked Lake Erie zebra mussels held for 7 wks at different temperatures, rations, and diet qualities. Mean shell growth (± SE) derived from ANCOVA for the mean mussel (18.5 mm) are plotted.
Diet quality also influenced final soft tissue mass with mussels fed Good Diet being heavier at the experiment end than those fed Poor Diet (Fig. 2.4a,b, Table 2.1). In addition, temperature negatively influenced soft tissue mass in both diets. Body mass also differed by ration and the two-way interactions of temperature with quality and ration (Table 2.1) but ration influenced body mass more in the Good Diet than in the Poor Diet. Zebra mussels fed High Rations, Good Diet were heavier at 24 and 30°C whereas ration did not influence body mass at 12°C - Good Diet nor at any temperature in the Poor Diet.

Reproduction

Total reproductive output was influenced by temperature, but not by diet quality or ration (Fig. 2.4c,d, Table 2.1). In the Good Diet, reproductive output declined with temperature as did body mass, but temperature did not similarly influence reproductive output in Poor Diet. Temperature altered reproductive output through its influence on metabolic costs resulting in a negative correlation between reproductive output and metabolic costs (reproductive output = 3.04 - 2.11 (metabolic costs), $r^2 = 0.29$, $p < 0.05$, $n = 18$).

Reproductive output generally did not change with ration except at 12°C - Poor Diet where output increased with ration. Although diet did not influence reproductive output, diet quality strongly influenced zebra mussels' ability to spawn a second time (3 wks later). Mussels fed Good Diet released gametes during the second induction whereas mussels from the Poor Diet did not spawn a second time.

Reproductive effort (RE)

Diet quality and temperature both influenced RE, but RE was not influenced by ration (Fig. 2.4e,f, Table 2.1). Although RE differed by temperature in the Poor Diet, temperature did not influence RE in the Good Diet. Whereas body mass increased with ration, RE decreased with increasing ration in the Good Diet, but
Figure 2.4. Final soft tissue body mass (panels a, b), reproductive output (panels c, d), and reproductive effort (panels e, f) for Lake Erie zebra mussels held for 7 wks at different temperatures, rations, and diet qualities. All data are means (± SE) derived from ANCOVA for the mean-sized mussel. Final soft tissue body mass was the soft tissue mass at the end of the experiment for the mean mussel (19.4 mm). Reproductive output was the total output of two spawnings induced with serotonin for the mean mussel (20.4 mm). Reproductive effort (RE), calculated in joules as $RE = \frac{\text{reproductive output}}{(\text{soft tissue growth} + \text{reproductive output})}$ is plotted for the mean mussel (20.4 mm).
Figure 2.4

Reproductive effort (%)  Reproductive output (mg dry mass)  Final soft tissue (mg dry mass)

Low  Medium  High

Ration

Low  Medium  High

Good Diet

Poor Diet
RE increased with ration in the Poor Diet at 12°C only. At all rations in 24°C, RE was higher in the Poor Diet than in the Good Diet. At 12°C, increasing ration resulted in decreased RE. RE increased with ration in the Poor Diet whereas RE decreased with ration in the Good Diet. In addition, RE and shell growth were negatively correlated (RE = 18.2 - 21.5(shell growth), $r^2 = 0.40$, $p<0.01$, $n=18$).

Gametogenic maturation

Maturating of gametes was most strongly influenced by temperature, generally decreasing at higher temperatures (Fig.2.5, Table 2.1). Within a temperature, gamete maturation did not differ by ration or quality.

Condition Factors

Protein catabolism ($O:N$, the ratio of moles of oxygen consumed to moles of ammonia nitrogen excreted) was strongly influenced by food quality, temperature, and their interaction (Table 2.1, Fig. 2.6a,b). At 24°C Low and Medium rations, protein catabolism did not differ between diet qualities, whereas it was higher in Poor Diet at 12°C. At 12°C O:N was 3 - 5 times higher in the Good Diet than in the Poor Diet indicating lower levels of protein catabolism. O:N was correlated to final soft tissue mass ($O:N = (-11.4) + 2.71$(soft tissue mass), $r^2 = 0.33$, $p<0.05$, $n = 18$) indicating larger zebra mussels were catabolizing less protein.

Zebra mussel lipid content differed by diet quality, temperature, and ration. The two-way interactions of temperature with diet quality and ration were also significant (Table 2.1, Fig. 2.6c,d). Lipid content differed little between temperatures or rations for mussels fed the Poor Diet. However, in Good Diet - Low Ration treatment, lipid content differed by temperature with highest lipid content in mussels held at 30°C. As with reproductive output, lipid content (not mass standardized) was negatively correlated with metabolic costs ($lipid = 0.873 - 0.552$(metabolic costs), $r^2 = 0.22$, $p < 0.05$).
Figure 2.5. Mean gametogenic index (± SE) for Lake Erie zebra mussels held for 7 wks at different temperatures, rations, and diet qualities. Gametogenic index was assigned following Haag and Garton (1991).
Figure 2.6. Mean O:N ratio (panels a,b), percentage lipid content (panels c,d), and percentage carbohydrate content (panels e,f) for Lake Erie zebra mussels held for 7 wks at different temperatures, rations, and diet qualities. All data are means (± SE) derived from ANCOVA for the mean sized mussel. O:N (moles of oxygen consumed to moles of ammonia nitrogen excreted) was calculated from metabolic cost measurements at the end of the experiment for the mean sized mussel (15.1 mg). Lipid content, measured by the vanillin-phenol method (Van Handel 1985) and carbohydrate content, measured with the phenol-sulfuric acid method (Montgomery 1957) are plotted for the mean sized mussel (17.5 mm) at the end of the experiment.
Figure 2.6
Total carbohydrate differed by temperature, ration, and their interaction, but unlike protein catabolism, and lipid content, total carbohydrate did not differ between diet qualities (Table 2.1, Fig. 2.6e,f). As with lipid content, total carbohydrates were similar across treatments except for high carbohydrates levels of mussels in 30°C Good Diet receiving Low and High rations.

Diet quality strongly influenced mortality especially at higher temperatures (Table 2.2). All mussels in 30°C - Poor Diet died by 3 wks. In addition, for each temperature, mortality was higher in the Poor Diet than in the Good Diet. However, mortality did not vary predictably with ration. Mortality was negatively correlated to final soft tissue mass (mortality = 66.7 - 3.91(soft tissue mass), $r^2 = .506$, $p < 0.001$, $n = 18$, final soft tissue mass was measured on live mussels) and lipid content (mass standardized) (mortality = 12.956 - 9.224(lipid), $r^2 = .769$, $p = .0003$, $n = 18$).

Discussion

In this experiment, ration not only determined available energy but both ration and temperature altered metabolic costs thereby changing energy demands. The equal influence of temperature and ration on energy budget parameters between diet qualities was not unexpected because zebra mussels minimize energy demands by maintaining relatively constant metabolic costs across seasonal changes in temperature and food conditions (Chapter 1). Thus, temperature, ration, and their associated costs were not solely responsible for energy available for allocation to growth and reproduction.

Reproductive effort, a function of somatic growth and reproduction, differed most between diets. Although gonad volume increases when food is highly available and decreases with temperature (Borcherding 1995), reproductive output and gamete maturation were about equal between diet qualities. Consequently, differences in
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<td>12</td>
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<td></td>
<td>Medium</td>
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<tr>
<td></td>
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</table>

* 18°C Good Diet lost due to heater malfunction
b 12 and 24°C Poor Diet, High Rations terminated after 5 wks.

30°C Poor Diet terminated due to high mortality: Low Ration at 2.5 wks, Medium Ration at 3 wks, High Ration at 2 wks

Table 2.2. Total percentages of marked Lake Erie zebra mussels dead at the end of 7 wk experiment. Zebra mussels were examined and dead removed from each treatment daily.
reproductive effort between diet qualities were due to differences in somatic growth. Seasonally, growth is more highly correlated to food than temperature (Sprung 1995) and when food is limited and costs are high, somatic and shell growth may cease or mussels may reabsorb their shells (Sprung 1995, Chapter 1). Both somatic and shell growth were negatively affected by the Poor Diet. Poor Diet mussels weighed less and shells were reabsorbed indicating energy available was insufficient to maintain growth without additional diversion from other physiological components.

Physiological demands determine the net energy available for allocation to somatic growth and reproduction. Tradeoffs in these components can only occur if energy allocation is flexible. In zebra mussels, reproductive effort was not constant across environmental gradients. Rather, energy allocation was flexible, with investment in reproduction determined more by food quality than by temperature or ration. In stressful conditions, zebra mussels are "reckless" reproducers (Calow 1979), reproducing at the expense of self-maintenance and survival.

Both growth and survival were compromised in the Poor Diet suggesting Poor Diet conditions were more stressful. Because diet quality was the primary factor influencing growth, diet quality must be responsible for the differences in reproductive effort with Poor Diet increasing stress to a level that cued changes in energy allocation.

Diet quality likely influenced survival by altering energy reserves and increasing the probability of starvation and death. Lipid and carbohydrate, important energy reserves in bivalves, fluctuate with the seasonal reproductive cycle (Gabbott 1983). Low lipid content at experiment end is consistent with the mussels' post-spawning state (Bruner et al. 1994, Nalepa et al. 1993, Sprung 1995, Walz 1978). Low lipid content is also correlated to starvation (Sprung and Borcherding 1991, Walz 1979, Gabbott 1983). Zebra mussels use lipids early in starvation whereas carbohydrates are used only in small percentages (Sprung and Borcherding 1991). Low lipid content at experiment end suggests energy reserve depletion. In addition, low O:N ratios in Poor Diet and at higher temperatures in the Good Diet indicating
protein catabolism (Bayne and Newell 1983) also occur during summer when food is low, costs are high, and mussels may be starving (Quigley 1992, Sprung 1995). The Poor Diet (dried Chlorella, chosen because it lacked essential fatty acids), resulted in high adult mortality, also does not support development of larval zebra mussels (Wright 1996). By lacking essential fatty acids, Poor Diet may have additionally taxed already low reserves increasing overall stress and the probability of death.

Energy reserve depletion, starvation, and death could have been combated in Good Diet mussels by reabsorbing gametes (Tourari et al. 1988, Bielefeld 1991, Sprung and Borcherding 1991) because gametes are energy rich (Bayne et al. 1975) and represent about 3% of the seasonal energy budget of zebra mussels (Chapter 1). This experiment began in mid-gametogenesis so energy investment in reproduction had already begun. We expected experiment conditions would influence additional investment in gamete maturation or that gametes may be reabsorbed and used as an energy source to combat starvation or stressful conditions (Tourari et al. 1988, Bielefeld 1991, Sprung and Borcherding 1991). If gametes were used as an additional energy source, starvation and death may be delayed. Using gametes for energy decreases reproductive output and, if accompanied by continued somatic growth, would decrease reproductive effort.

Good Diet mussels had lower reproductive effort than mussels fed the Poor Diet. Reproductive outputs were similar between diets, but because body mass was higher with the Good Diet, reproductive effort was lower. In addition, mussels in the Good Diet had better survival than those in Poor Diet even when body reserves were at equally low levels. The lower reproductive effort, maintained body mass, and survival in mussels in the Good Diet implies they may have used gametes as an additional energy source. Although reproductive outputs were similar between diets, Poor Diet mussels released their total reproductive output with the first spawning induction; they were unable to spawn a second time (3 wks later). Poor Diet mussels likely did not reabsorb gametes but maintained reproductive output, sacrificing somatic growth and survival resulting in higher reproductive effort with that diet.
Equal reproductive outputs, in light of differing reproductive efforts, could be explained if the timing of the experiments were such that Good Diet mussels were unable to increase investment in reproductive output. Investment in reproduction is a lengthy process in zebra mussels. Gametogenesis begins in the fall, resumes in the spring, and successive cohorts of gametes mature and are released several times across the season (Borcherding 1991, Garton and Haag 1992, Haag and Garton 1992, Neumann et al. 1992). The timing of nutrient stress relative to developmental stages is also important because of prior energy allocations (summarized in Boggs 1992). Our experiment, began in mid-gametogenesis after initial energy investment in reproduction, may have been too short for additional investment in reproduction or the initiation of the experiment may have been after some critical date, a "window of opportunity," after which investment in additional gametes could not occur. In either case, Good Diet mussels would be unable to increase energy investment in gametes and reproductive output would be unaffected by diet.

Reproductive effort in bivalves is highly variable and depends on maturity (e.g. Bayne et al. 1983, MacDonald and Bayne 1993), habitat (e.g., Bayne et al. 1983, MacDonald and Bayne 1993, Sprung 1995a), and degree of environmental stress (e.g., Bayne et al. 1983, Sprung 1995a). For example, *Mytilus edulis* and zebra mussels increase reproductive effort with age (Bayne et al. 1983, Sprung 1995a). *Mytilus* also increases reproductive effort in stressful environments (Bayne et al. 1983). When exposed to stressful conditions in our experiment, zebra mussels responded like *Mytilus*, increasing reproductive effort.

Optimality theory predicts that energy will be diverted from growth to reproduction when conditions are stressful (Sebens 1982, cited in Bayne et al. 1983). Stressors can fall within a "zone of tolerance" where energy allocation is unaffected or within a "stress zone" where noticeable changes occur (Koehn and Bayne 1989). Our treatment combinations spanned this range with Good Diet and low temperatures in the "zone of tolerance" and Poor Diet and high temperatures in the "stress zone". Our results confirm that zebra mussels are highly flexible in energy allocation. Zebra
mussels use this flexibility to exploit good conditions thereby increasing their reproductive effort when survival is assured or they may simply reproduce when environmental conditions threaten survival. In either case, zebra mussels optimize energy allocation.

Maintaining reproduction in adverse conditions when chances for survival are low is an advantage for zebra mussels. Early reproduction in zebra mussels, often within the first year under normal conditions, coupled with flexible energy allocation not only produces the next generation, but likely is responsible for this species' invasion success in a variety of habitats. Unlike the longer-lived bivalve, *Plactopecten magellanicus* (15-20 yrs) that sacrifices reproduction for maintenance in poor habitats (MacDonald and Bayne 1993), short-lived zebra mussels have neither the reserves nor the time to delay reproduction. When reserves are low and survival is questionable, maintaining reproduction, in lieu of persisting through the bad times only to die before reproducing later, is a definite advantage for zebra mussels.

Energy allocation tradeoffs are "dynamic-linkages" (Stearns 1989) and stressors interact to constrain growth and reproduction. When resources are reduced, the general response is to decrease lifetime fecundity, but the short-term response may be to increase reproduction and die (summarized in Boggs 1992). Zebra mussels appear to have adopted the short-term response: increase reproduction and die. When stressed by temperature, ration, and most importantly, diet quality, zebra mussels sacrifice body mass growth and survival for reproduction.
CHAPTER 3
METABOLIC COSTS OF SPAWNING
IN ZEBRA MUSSELS (*DREISSENA POLYMORPHA*)

Introduction

Reproduction is an important component of an organism’s life and a necessary event for the perpetuation of the species. Reproduction, as a component of the energy budget, requires energy investment that must be balanced with other energy demands to ensure the organism’s survival.

Energy demands for reproduction in zebra mussels (*Dreissena polymorpha*) includes energy invested in the production, maturation, and release of gametes. Gamete production and maturation includes energy used to synthesize the gametes and for increased metabolism during gamete maintenance and maturation. Reproductive energetic demands also includes energy used during the release of gametes (spawning). The total cost of reproduction in zebra mussels can be quantified only when estimates of all three components, production, maturation, and release of gametes, are included.

Zebra mussels produce abundant gametes (> 1 million gametes/individual^1 yr^-1, Borcherding 1991) comprising 30 - 50% of pre-spawning body mass (Sprung 1991, Borcherding 1992, Garton and Haag 1992). However, energy invested in gametes represents only 5% of zebra mussels’ seasonal energy budget (Chapter 1) and more body mass is lost during spawning than can be explained by the mass lost via gametes (Nalepa et al. 1993, Chapter 1). Because the majority of seasonal energy expenditure
(95%) is required for metabolism, metabolic costs of reproduction may help explain a portion of the additional body mass lost during spawning.

Other studies have estimated increases in metabolic costs during gamete maintenance and maturation in bivalves by correlating seasonal changes in metabolism with the reproductive cycle (Bayne et al. 1983, Iglesias and Navarro 1991, Sprung 1991). Metabolic costs of gametogenesis in *Mytilus* are high and temperature dependent (Bayne 1984, from Sprung 1991). In zebra mussels, oxygen consumption by gametes alone is moderate and unrelated to temperature (Sprung 1991). Correlations between seasonal changes in metabolic costs and events in the reproductive cycle provide estimates of the cost of gamete production and maintenance, but they do not include costs of spawning. To date, no estimates of bivalve reproductive costs have included the metabolic costs of spawning.

The omission of metabolic costs of spawning may be based on the assumption that because spawning is brief and temporary, metabolic costs are minimal. However, because metabolic costs represent such a large proportion of the seasonal energy budget of zebra mussels, any increase, even temporary, could substantially influence an individual’s short-term energetic demands and, when combined with limited energy reserves (Nalepa et al. 1993), become highly stressful for zebra mussels. Temporary, high physiological stress due to spawning, limited energy reserves, and low available resources may increase post-spawning mortality.

To estimate the energy demands during the release of gametes, I induced spawning in zebra mussels and measured oxygen consumption while they spawned. Because oxygen consumption comprises the majority of metabolic costs (88% of 95% total, Chapter 1) any increase in oxygen consumption during spawning provides an estimate of metabolic costs of gamete release. I then relate the costs to the seasonal energy budget of zebra mussels (Chapter 1), and speculate on the impact of short-term depletion of energy reserves.
Methods

Zebra mussels attached to rocks were collected three times during the spawning season (July 1991) from 5 m depth with SCUBA near The Ohio State University’s F. T. Stone Laboratory on South Bass Island, western Lake Erie. Mussels were removed from the rocks and held in running lake water aquaria at ambient lake temperature for <24 h before measuring oxygen consumption.

Oxygen consumption was measured for a range of reproductive-sized mussels (12-25 mm) in two treatments: 1) mussels induced to spawn (n = 40) and 2) mussels not induced to spawn (Controls, n = 20). I measured oxygen consumption in filtered lake water (glass fiber filter, Type AE, 1.0μm pore size) at ambient Lake Erie temperature using a Gilson Differential Respirometer. To induce spawning, respirometer flasks contained 1 mM serotonin (5-hydroxytryptophan) in filtered lake water. All mussels were held in the flasks for 30 min prior to measuring oxygen consumption. Oxygen consumption (μl/h) was converted to Joules (0.01979 J/μl氧气 at STP, Crisp 1971).

To account for the effect of serotonin on oxygen consumption, I separated the mussels induced to spawn (i.e., exposed to serotonin) into two groups: 1) Spawners and 2) Non-spawners, based on presence or absence of gametes in the respirometer flasks after oxygen consumption was measured. I also determined sex of Spawners.

To account for the effect of spawning intensity on oxygen consumption and to track the reproductive condition of the population on the sampling date, I visually inspected the respirometer flasks and qualitatively scored the intensity of spawning. Sperm released by males remained in solution so water cloudiness scores were assigned by comparing with dilutions of skim milk (cow’s milk, 0%fat). The egg mass released by females was scored as the percent of the respirometer flask bottom
Scores for males and females were assigned as:

0 = no spawning (These mussels became the Non-spawners.)
1 = low quantity of gametes produced (males - cloudiness < 0.5% skim milk solution, females - egg mass <10% of flask bottom)
2 = medium quantity of gametes produced (males - cloudiness near 1.5% skim milk solution, females - egg mass 20 - 50% of flask bottom)
3 = large quantity of gametes produced (males - cloudiness near 3% skim milk solution, females - egg mass at least 50% of flask bottom).

I measured lengths of all mussels with vernier calipers (nearest 0.1 mm). Soft tissue body mass of Control mussels was dried at 65°C for 24 h. Because body mass is lost with spawning, soft tissue body mass of Spawners and Non-spawners (mg dry mass) was estimated from the length-dry mass regression of Control mussels. Because oxygen consumption varies with mussel size, I used ANCOVA (SAS 1989) to control for body mass in all statistical analyses.

Results

Spawning increased oxygen consumption of zebra mussels (Figure 3.1). However, oxygen consumption of Spawners was significantly higher than Non-spawners or Controls (ANCOVA, p<0.0001) on only two dates (July 8 and 24). Serotonin increase was calculated as the percentage increase in oxygen consumption of Non-spawners (induced to spawn but did not release gametes) over Controls (not induced to spawn). Increase in oxygen consumption due to spawning, not the effect of serotonin, averaged 58% of the oxygen consumption of Controls and decreased with date (Table 3.1). High variability in percentage increase and the decrease with date is accounted for by differences in spawning intensity (Table 3.2).
Oxygen consumption of Spawners declined on 31 July to equal the oxygen consumption of Non-spawners (Figure 3.1). The decline was reflected in the number, intensity, and sex of Spawners on that date. Fewer mussels spawned on 31 July than on the other dates and no mussels spawned at high intensity (Table 3.2). The decline in oxygen consumption also was reflected in the sex of mussels that spawned; no females spawned and of the few spawning males, the majority (86%) spawned at low intensity.

Figure 3.1: Oxygen consumption (J·h⁻¹) of Lake Erie zebra mussels (*Dreissena polymorpha*) (*Controls, n = 20*) and mussels induced to spawn with 1mM serotonin (*n = 40*) three times during the spawning season (July 1991). Spawners were mussels exposed to serotonin that released gametes. Non-spawners were mussels exposed to serotonin that did not spawn. Adjusted means and standard errors are plotted (ANCOVA, mean shell length = 17.5 mm).
<table>
<thead>
<tr>
<th>Date</th>
<th>Serotonin Effect</th>
<th>Spawning Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 July</td>
<td>59%</td>
<td>89%</td>
</tr>
<tr>
<td>24 July</td>
<td>32%</td>
<td>69%</td>
</tr>
<tr>
<td>31 July</td>
<td>50%</td>
<td>16%</td>
</tr>
</tbody>
</table>

Table 3.1: Percentage increase in oxygen consumption of Lake Erie zebra mussels (*Dreissena polymorpha*, n = 40, 12-25 mm) induced to spawn with 1mM Serotonin 3 times during the spawning season (July 1991). Serotonin Effect was the percentage increase in oxygen consumption of Non-spawners mussels (exposed to serotonin, but did not release gametes) over Control mussels (not exposed to serotonin). Spawning Effect is the additional increase in oxygen consumption of Spawners (mussels that released gametes) above the Serotonin Effect.
Table 3.2: Spawning intensity and sex of Lake Erie zebra mussels (*Dreissena polymorpha*, *n = 40, 12-25 mm*) induced to spawn with 1mM Serotonin three times during the spawning season (July 1991). Spawning intensity was qualitatively scored based on degree of cloudiness of water (males) and quantity of eggs (females). Scores ranged from 0 = did not release gametes (sex "Unknown") to 3 = cloudiness of 3% skim milk solution (males) or eggs covering at least 50% of flask bottom (35mm) (females).
Discussion

Spawning is metabolically costly for zebra mussels. Gamete release increases oxygen consumption substantially and is related to overall spawning effort. At its highest value, oxygen consumption increased about 90% in spawning mussels.

The metabolic costs of spawning in zebra mussels are determined by their anatomy. Zebra mussels' gonads are large Y-shaped structures that occupy a large proportion of the soft body when in the final stages of gametogenesis (Ram et al. 1996). Gametes mature in vesicles within the gonad and are transported to the supra-branchial chamber through a narrow ciliated gonoduct. Muscles in or near the gonoduct assist or regulate gamete release and their contractions increase intrachamber pressure to expel gametes out the narrow gonoduct. This process, plus the increase in activity of the cilia on the gill needed to finally eject gametes, increases energy demands. In addition, because zebra mussels are sessile, the gill is the most active structure. Any increase in gill activity, as during spawning, will increase energy demands. Considering gamete release may require as much as a few days and multiple spawning bouts, increased oxygen consumption during spawning could significantly influence short and long term seasonal energy demands.

Zebras mussels are sequential spawners releasing gametes several times over the 6-8 wk spawning season. Lake Erie zebra mussels mature and spawn at least 2 cohorts of gametes during summer (Haag and Garton 1992). The combination of large numbers of gametes, anatomy, and multiple spawning events implies metabolic costs due to spawning could have both short and long term consequences on energy demands of zebra mussels.

Spawning increased oxygen consumption an average of 0.276 J h⁻¹ (0.057-0.39 J h⁻¹). Assuming females release 50% of their 1 million eggs in two spawning events at a rate of 10 eggs sec⁻¹, then each spawning event would last about 14 h. Assuming this egg release rate is near the maximum, that release rate correlates to the maximum increase in oxygen consumption during spawning (0.39 J h⁻¹). A zebra mussel
spawning for 14 h would require 5.46 J to meet oxygen consumption demands alone. During the spawning season (July) a zebra mussel (average shell length = 17.8 mm) uses 13.22 J ind\(^{-1}\) d\(^{-1}\) (3.16 cal ind\(^{-1}\) d\(^{-1}\)) on average to meet daily energy expenditures (calculated from Chapter 1). Based on these calculations, spawning increases that daily energy demand about 40% on spawning days.

Although a 40% increase in energy demands on two spawning days does not alter the seasonal energy budget (Chapter 1), zebra mussels do not feed during spawning (Stanczykowska 1977); thus, all required energy must derive from body reserves. Not surprisingly, zebra mussels lose more body mass during spawning than can be explained by the mass lost via gametes (Nalepa et al. 1993, Chapter 1). Post-spawning body mass contains about 5% in lipid and carbohydrate reserves (Chapter 2). These reserves (8J) are insufficient to meet increased demands of spawning (13.22 J d\(^{-1}\)) thus requiring zebra mussels use other structural components, such as muscle tissue, to meet energy demands.

Energy demands of spawning thus help explain a portion of the additional body mass lost during spawning. If spawning continues for any length of time, with no feeding, zebra Mussel energy reserves must be used to meet energetic demands. However, zebra mussels do not have large energy reserves (Nalepa et al. 1993, Chapter 2) and additional costs of spawning tax already depleted reserves. The additional cost of spawning thus strengthens the argument that reproduction is a stressful time for a zebra mussel.

Energy demands of spawning are an additional stress zebra mussels face at a point in the season when available food is low and temperatures are high (Chapter 1). Low food quality and high temperatures increase mortality and result in a switch in energy allocation strategy investing energy in reproduction in lieu of growth and survival (Chapter 2). When compared to European populations, Lake Erie zebra mussels reproduce earlier, have shorter life spans, and grow as quickly but reach smaller maximum sizes (Mackie and Schloesser 1996). Energy demands of reproduction combined with environmental conditions likely drive high mortality
following spawning, contributing to the shorter life span of zebra mussels, and populations comprised of few large mussels in Lake Erie (Mackie 1992, Berkman et al. unpublished data). Spawning has a significant impact on metabolic costs, increasing overall energy demands during the reproductive period rendering adults vulnerable to post-spawning environmental stressors and may be responsible for differences in current population size structure and energy allocation in Lake Erie zebra mussels compared to European populations.


