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Effects of fluctuating temperature and food availability on reproduction and lifespan

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ABSTRACT

Experimental studies on energetics and aging often remove two major factors that in part regulate the energy budget in a normal healthy individual: reproduction and fluctuating environmental conditions that challenge homeostasis. Here we use the cyclical parthenogenetic *Daphnia pulex* to evaluate the role of a fluctuating thermal environment on both reproduction and lifespan across six food concentrations. We test the hypotheses that (1) caloric restriction extends lifespan; (2) maximal reproduction will come with a cost of shortened lifespan; and (3) at a given food concentration, relative to a metabolically equivalent constant temperature environment a diel fluctuating thermal environment will alter the allocation of energy to reproduction and lifespan to maintain homeostasis. We did not identify a level of food concentration that extended lifespan in response to caloric restriction, and we found no cost of reproduction in terms of lifespan. Rather, the individuals at the highest food levels generally had the highest reproductive output and the longest lifespans, the individuals at the intermediate food level decreased reproduction and maintained lifespan, and the individuals at the three lower food concentrations had a decrease in reproduction and lifespan as would be predicted with increasing levels of starvation. Fluctuating temperature had no effect on lifespan at any food concentration, but delayed time to reproductive maturity and decreased early reproductive output at all food concentrations. This suggests that a fluctuating temperature regimen activates molecular pathways that alter energy allocation. The costs of fluctuating temperature on reproduction were not consistent across the lifespan. Statistical interactions for age of peak reproduction and lifetime fecundity suggest that senescence of the reproductive system may vary between temperature regimens at the different food concentrations.

1. Introduction

The thermal environment is a determinant of energy utilization. In endotherms this is driven by the need to maintain a constant body temperature for thermal homeostasis (Bicego et al., 2007). While ectotherms do not maintain a constant body temperature, they make behavioral, physiological and cellular adjustments to maintain thermal homeostasis. Further, metabolic rate in ectotherms is largely defined by temperature following the Boltzmann-Arrhenius equation for chemical reaction kinetics, with higher temperatures (within the range over which organisms commonly operate) resulting in higher metabolic rates and typically faster growth to reproduction (Dell et al., 2011; Hochachka and Somero 2002). The interaction between energetics and life history traits is evident from studies showing different diet regimens producing a range of outcomes in traits such as reproduction and lifespan. For example, dietary restriction can extend lifespan in many species (Swindell 2012), whereas increased food availability can result in more and bigger offspring (Warner et al., 2015). This response to energy availability can be described as a form of phenotypic plasticity (Wada and Sewall 2014). Understanding the relationships between the metabolic demands of the thermal environment and their interaction with diet can provide further insight on the determinants of plasticity in reproductive lifespan and total lifespan.

Lifespan studies typically maintain animals in a constant laboratory environment despite most animals living in (and having evolved to live in) fluctuating thermal environments, from diel fluctuations over a 24-h period to seasonal fluctuations across a year (Dell et al., 2011;

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Steinberg 2012). Organisms must regulate their cellular function to adjust to variable environments, as demonstrated by gene expression studies that show altered gene expression in fluctuating versus constant temperature environments (Podrabsky and Somero 2004). Studies that specifically test for the effect of fluctuating temperature environments on life history traits have demonstrated that growth rates and development often do not meet expectations predicted from constant mean temperatures (Kern et al., 2015; Kingsolver et al., 2015; Niehaus et al., 2012). In terms of lifespan, fluctuating temperatures have been reported to either increase, decrease, have no effect, or have sex-specific effects depending the amplitude of the fluctuations and their frequency (Colinet et al., 2015; Economos and Lints 1986; Mironidis and Savopoulou-Soultani 2008). Additionally, in response to thermal fluctuations some animals can increase their thermal tolerance and reduce their metabolic plasticity (Chen and Stillman 2012; Williams et al., 2012). Animals can alter their metabolic molecular networks, and more specifically their responsiveness (plasticity) to the environmental conditions, in order to optimize the animal's physiology under a fluctuating thermal regimen (e.g. acclimation) (Angilletta 2009; Podrabsky and Somero 2004; Seebacher et al., 2009). Altering these networks may have pleiotropic effects across the life history, including the allocation of energy to growth, reproduction, and longevity (Colinet et al., 2015; Kern et al., 2015).

Life history theory and resource allocation models predict trade-offs between early life traits (e.g. growth and early reproduction) and later-life traits such that limiting energy (caloric restriction) will delay reproduction, and prolong reproductive and total lifespan (Shanley and Kirkwood 2000; Stearns 1992). The disposable soma theory of aging predicts trade-offs between early life and late-life traits, such that fast growth and early reproduction would limit investment in cell maintenance resulting in a shortened lifespan (Kirkwood 1993). Using this framework we can make predictions as to the effects of a fluctuating thermal environment on reproduction and lifespan. Here we are testing for an interaction between temperature regimen (fluctuating versus constant) and food concentration on key determinants of fitness: reproduction across the lifespan, and lifespan itself using *Daphnia pulex*.

1.1. Daphnia and specific questions

Daphnia pulex is a cyclical parthenogenic microcrustacean that has long been studied as a freshwater ecology model organism and has more recently been recognized by the NIH as a model organism for biomedical research (NIH). They provide an ideal system for studying the effects of temperature regimens on lifespan and reproduction as they: are short lived (<2 months); have direct development after hatching from the egg within the mother's brood pouch; reach reproductive maturity in 1–2 weeks; have discrete reproductive events that can be quantified throughout the lifespan; and have more genes in common with humans than do *Drosophila* or nematodes (Colbourne et al., 2011). Several seminal studies have described physiological effects in *Daphnia* due to temperature, food levels, diet composition, and population of origin, (Chen and Stillman 2012; Dudycha and Lynch 2005; Heugens et al., 2006; Lynch and Ennis 1983; Lynch et al., 1999; MacArthur and Baillie 1929). Most studies have been conducted under constant temperatures and these demonstrate that higher food concentration shortens time to maturation, increases size at maturation, increases proportion of resources allocated to reproduction, and increases fecundity (Dudycha and Lynch 2005; Heugens et al., 2006; Lynch 1989). At higher (non-stress) temperatures, Daphnia grow faster to maturity, but are smaller, and have small early clutches, yet overall fecundity is not reduced (Heugens et al., 2006). Further higher temperatures are more metabolically demanding (Heugens et al., 2006). Under fluctuating thermal conditions Daphnia have increased thermal tolerance, and a depressed metabolic rate when measured at the lowest temperature (Chen and Stillman 2012) and a faster rate of population increase (van As et al., 1980).

More recently *Daphnia* has been recognized as a model for aging (Kim et al., 2014; Murthy and Ram 2015; Kim et al., 2014; Schumpert et al., 2014). Previous studies on the plasticity of lifespan have shown conflicting results as to whether *D. pulex* demonstrates an extension of lifespan in response to dietary restriction (Dudycha 2003; Kim et al., 2014; Latta et al., 2011; Lynch 1989; Lynch and Ennis 1983). Here we build on these studies to assess the effect of a daily fluctuating temperature, compared to a metabolically equivalent constant temperature, on lifespan and reproduction across six food concentrations. First, we ask whether decreasing energy availability, across six food concentrations, results in a reallocation of resources from growth and reproduction to somatic maintenance as determined by an extension of lifespan and a reduction in reproduction (prediction in Fig. 1A). Further, we hypothesize that at maximal food concentration there will be a cost of reproduction in terms of lifespan. Second, we ask whether a fluctuating thermal environment is energetically costly such that the extension of lifespan and reduction in reproduction will occur at a higher food concentration relative to a metabolically equivalent constant temperature (Fig. 1B). In other words, at the same intermediate food intake level, living in a fluctuating environment, even if at the same metabolic mean over a 24-h period, would alter molecular pathways to increase the energy allocated to maintaining homeostasis and less energy to reproduction and lifespan relative to a constant environment. Third, we ask whether the cost of a fluctuating thermal regimen is consistent as a function of food availability (Fig. 1C), indicating the fluctuations alter metabolic pathways to reallocate energy away from reproduction at all food concentrations; alternatively, if the cost of thermal fluctuations is evident at lower food concentrations but virtually eliminated at the higher (ad lib) food concentrations (Fig. 1D) this would indicate that with unlimited food all energy demands can be met and there is no need to reallocate energy away from reproduction.

2. Materials and methods

We used a strain of *Daphnia pulex* received from Dr. J. Shaw's laboratory at Indiana University in June of 2011. Since then, it has been maintained in Dr. J. Gohlke's laboratory at University of Alabama at Birmingham using previously established protocols (Asselman et al., 2013; Shaw et al., 2007). Media consisted of COMBO media (final concentrations: 388.16 μM Boric Acid, 330.87 μM Calcium Chloride Dihydrous, 307.24 μM Magnesium Sulfate Heptahydrous, 99.93 μM Potassium Chloride, 149.98 μM Sodium Bicarbonate, 232.84 μM Sodium Metasilicate Nihydrous). Selenium (final concentration 0.0126 μM) and Animate solution (final concentrations: 3656.52 μM Lithium Chloride, 19.88 μM Potassium Iodine, 155.51 μM Sodium Bromide, 578.90 μM Rubidium Chloride, 562.60 μM Strontium Chloride Hexahydrate) were added fresh during media changes every other day.

2.1. Treatments

A total of 366 individual Daphnia were used in this study. We exposed animals to one of 12 treatments (six food concentrations by two temperature regimens) and tracked individuals for reproductive output over their lifespan and measured total lifespan.

2.1.1. Food treatments

To provide consistency across the experiment, and the potential for repeatability across experiments and labs, we used RGcomplete as the food source (Reed Mariculture Inc.). RGcomplete is a blend of four microalgae (size 1.5–15 μc; *Nannochloropsis oculata*, *Tetraselmis* sp., *Chlorella vulgaris*, and *Schizochytrium* sp.) that has been nutritionally formulated as a food source for rotifers grown for aquaculture. The algae are dead so there are no effects of the temperature regimens on the algal growth that would alter the energy available in each treatment. Since there is no literature on the use of RGcomplete in *Daphnia*, we use this experiment as a means of validating it as a reliable food

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