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Deeper effects of climate change: the influence of temperature and diet quality on fish metabolism

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ABSTRACT

Climate change is predicted to cause an increase in cyanobacterial blooms, creating a food base with lower nutritional quality and thus negatively influencing the health and resiliency of other species in the food web. Fatty acids are used as tracers and health indicators in aquatic systems, providing a means of studying the effects of ecosystem disturbances such as cyanobacterial blooms on food webs. I investigated the influence of shifts in food quality and temperature on the respiratory physiology of the fathead minnow (*Pimephales promelas*) as a case study species. Previous research has addressed segments of this relationship, but the full connection between climate change and fish respiration has not been made in the literature. To bridge this disconnect, I compared the fatty acid composition and resting metabolic rates of fathead minnows held across a range of temperatures (12-28°C) and fed diets with contrasting fatty acid profiles, representing the potential variation in environmental conditions associated with climate change. I found a direct relationship between temperature and resting metabolic rate (i.e., metabolic rates are higher at warmer temperatures), as well as higher metabolic rates in minnows fed a diet with a higher content of essential fatty acids, but there were no significant synergistic or antagonistic effects of the combination of diet and temperature on fish metabolism. The results for fatty acid analysis were difficult to interpret due to lack of statistical power.

INTRODUCTION

Climate change has become an issue receiving much attention in the scientific community as well as the public sphere. The effects most tangible in human society are widely publicized (e.g., rising sea levels, changing weather patterns, and shifting habitat distributions) (Bell et al. 2000; Parmesan and Yohe 2003; Gerlach 2010); however, many of the ecological implications are not as well understood. Climate-induced shifts in an ecosystem can have cascading impacts reaching far beyond the expected outcomes. Understanding these extended impacts is crucial to understanding the greater implications of climate change in an ecosystem.

One of the more visible effects of climate change in aquatic ecosystems is the increased occurrence of cyanobacterial blooms. Society has developed a very negative perception of these rapid increases in cyanobacteria populations due to the formation of thick surface scums as well as the potential for some species to produce toxins, posing a health risk for both human and animal users of the water resource (Anderson et al. 2012). Along with social undesirability, cyanobacterial blooms can have far-reaching ecological effects. Increases in cyanobacterial dominance can significantly alter the autotroph assemblage of a community, both by altering the relative abundance of algal species present and in some cases causing a shift to an alternative stable state in which aquatic macrophytes are eliminated from the system (Scheffer and van Nes 2007). The ramifications of cyanobacterial blooms have been documented in a broad range of taxa, including zooplankton, benthic invertebrates, aquatic macrophytes, and fish (Ghadouani et al. 2003; Oberholster et al. 2009; Scheffer and van Nes 2007).

Changes in fatty acid (FA) composition at the base of the food web constitute one significant mechanism by which cyanobacterial blooms may influence upper trophic levels. Recently, much research has investigated the roles of FA in aquatic ecosystems. Fatty acids are a

class of organic compound with nutritional importance for growth, reproductive success, cell membrane structure and fish overwinter survival (Tyler and Dunn 1974; Love 1980; Watanabe 1985; Pruitt 1990; Verreth et al. 1994). Fatty acids are transferred through food webs, with FA composition being roughly conserved between one trophic level and the next (Petursdottir et al. 2008; Rossi et al. 2008; Taipale et al. 2009). Essential fatty acids (EFA) are those that cannot be synthesized by consumers and must be obtained through their diet. Cyanobacteria contain lower concentrations of many EFA than other phytoplankton taxa (Ahlgren et al. 1992); therefore, they are considered a lower nutritional quality food source for aquatic consumers. Increasing dominance of cyanobacteria in the autotroph assemblage raises concerns of a bottom-up trophic cascade of declining EFA concentrations, potentially leading to compromised condition in upper trophic levels.

One hypothesized cause for this decline in condition is the potential influence of FA on fish metabolism and respiratory physiology. Studies on a range of fish species suggest that certain FA contribute to improved respiratory performance, while others detract from it (Chatelier et al. 2006; McKenzie et al. 2008; Wagner et al. 2004). However, contradictory findings have provided little clear consensus on the benefits or limitations associated with specific FA. The reason for these inconsistencies is unclear, prompting further research into the underlying biochemical mechanisms associated with these metabolic relationships (Vagner et al. 2014). Most studies tend to base these comparisons on measurements of critical swimming speed (U_{crit}) and oxygen uptake (M_{O_2}), although a variety of other metabolic indices can be derived from these measurements or measured separately (e.g., McKenzie 2001). Respiratory performance has inherent importance in the entirety of a fish's physiology. Shifts in FA composition in a food web due to cyanobacterial blooms could potentially affect fish health by

altering their metabolic processes, a pathway that is only recently being explored in trophic ecology.

Rising water temperatures – another projected effect of climate change – may also influence these physiological processes. The effects of temperature on fish metabolic rates are fairly well understood (for a review, see Clarke and Johnston 1999). Increasing temperature will generally increase a fish's metabolic rate; however, each species has a thermal optimum at which it functions best, and increasing temperatures beyond that point will cause metabolic rates to decline (e.g., Pang et al. 2015). Any given species also has a thermal minimum and maximum at either end of the temperature spectrum, beyond which metabolic functions cannot continue. With water temperatures gradually rising as climate change progresses, temperatures may move farther away from species' thermal optima and closer to their thermal maxima, creating unfavorable environmental conditions for many species. The direct effects of temperature on fish metabolism are clear, but indirect influences in other ecosystem processes are less so. In the context of declining EFA in the food web, temperature-induced stress could exacerbate problems associated with a lower quality food source, causing declines in the resilience of fish populations.

I investigated the influence of temperature and food quality on fish metabolism and respiratory physiology, using fathead minnows (*Pimephales promelas*) as a case study species. Despite some inconsistences in the literature, major trends in the effects of FA on respiration tend to persist across a spectrum of taxonomically diverse species (Chatelier et al. 2006; McKenzie 2001); therefore, the results of this study could be relevant in a broader context. The respiration rates and FA composition of minnows were compared in a laboratory-based approximation of the variation in thermal and dietary conditions expected in natural environments. Fish were held at one of five temperatures and fed one of two diets with

contrasting FA compositions for one month, followed by measurement of resting metabolic rate and FA analysis of fish.

METHODS

Study Organism

The fathead minnow is a common minnow species found in a variety of warm water habitats in lakes, ponds and streams throughout much of the continental United States. The fathead minnow is omnivorous, feeding on the most available invertebrates, zooplankton, or algae. It typically lives in schools of familiar individuals, which function as a cohesive unit (Sloman et al. 2006). The fathead minnow is a native species in the United States, but its range has been expanded to many locations through bait bucket releases. In addition to being a frequently used bait fish, fathead minnows are also commonly used in laboratory toxicity testing (Langdon et al. 2006). Adults typically range in size from about 5 to 7.5 cm, so juveniles and smaller adults are ideal sized fish for respiration experiments (i.e., less than 6.5 cm in our respirometers).

Fish Husbandry

Fathead minnows (n = 150) were purchased at Dockside Outdoor Supply (Colchester, VT) and transported to the Rubenstein Ecosystem Science Lab (RESL). Minnows were divided equally among ten tanks, corresponding to ten different temperature x diet treatments. For a schematic of the experimental setup described below, see Figure 1. Tanks were kept at one of five temperatures (i.e., 12, 16, 20, 24, and 28°C), with two tanks at each temperature. Temperatures were maintained by either heating or chilling a water bath surrounding each pair of

tanks, depending on the treatment's relation to water kept at room temperature (i.e., 22°C). Water in the tanks was isolated from that of the baths; therefore, heat was transferred to or from the tanks via conduction. Warmer temperatures were maintained using three 50 watt aquarium heaters per water bath, with the addition of an insulating layer of reflective bubble wrap around the 28°C water bath to reduce heat loss. These baths were periodically refilled to account for evaporation and maintain the baths' insulating capacity. Colder temperatures were maintained by connecting the water baths to one of two chiller systems, which were kept at 10°C and 19°C, respectively. Water from the 10°C chiller was pumped directly into the bath for the 12°C treatments at a slow enough rate that the ambient air would warm the water to 12°C. From there, the water flowed through an additional bath under a heat lamp before entering the 16°C bath, then returning to the chiller's reservoir. The 19°C chiller pumped directly into the 20°C bath.

Within each of these temperature treatment groups, minnows in one tank were fed a commercial hatchery feed high in essential fatty acids (GEMMA Wean 0.1, manufactured by Skretting), and the other tank received a pet store feed with a lower essential fatty acid content (Omega-One Freeze-Dried Brine Shrimp). Brine shrimp were selected as the low EFA diet due to previous documentation of low n-3 HUFA concentrations in *Artemia* sp. (Conceicao et al. 2010), and GEMMA Wean was selected based on advertisement as a hatchery feed high in n-3 HUFA (S. Backman, personal communication, July 2014). All fish were fed nightly at maintenance rate, as estimated by a bioenergetics model for dace (*Chrosomus spp.*), as that was the closest genus available to compare to fathead minnows (Fish Bioenergetics v. 3.0, University of Wisconsin). The model was run separately for each temperature treatment over thirty days using food energy content directly from Skretting for the GEMMA feed, and the brine shrimp energy content determined by Caudell and Conover (2006). All minnows were fed their

respective diets for one month prior to respiration testing, to allow the fatty acid composition of their tissues to turn over and reflect their diet. One month was determined to be a sufficient feeding period based on the turnover rates observed by Castledine and Buckley (1982) and Robin and Peron (2004).

Tanks were cleaned nightly, using a siphon to remove uneaten food and other waste. Any mortalities were also removed at this time. After cleaning, tanks were refilled with water at approximately the same temperature as their given treatment. A separate reservoir of 28°C water was maintained for the purpose of refilling the warmer tanks. A combination of 28°C and room temperature water was used to refill the 24°C tanks. The colder tanks were refilled with room temperature water run through ice, varying the flow rate through the ice or adding room temperature water to reach the desired temperature. Additional water changes were performed if the water remained cloudy after the initial cleaning.

Respirometry

Resting oxygen consumption of fish was measured using 170 mL swim tunnel respirometers (Loligo Systems, Tjele, Denmark). The following procedures were performed on up to four fish simultaneously, as there were four respirometers available at RESL. Blanks were periodically run to verify that background microbial respiration was not influencing M_{O_2} measurements. Food was withheld for 24 hours prior to respiration testing. Each fish was weighed and placed in the measurement chamber of the respirometer several hours prior to testing, allowing them to acclimate to the new environment and calm down after the stress of being handled. Each fish was tested at the same temperature it had been held at during the previous month. Temperatures were maintained by pumping water through a closed-loop system

controlled by a Temp-4 instrument and its associated software (Loligo Systems, Tjele, Denmark), with the exception of the 28°C trials, in which temperature was controlled by 50 watt aquarium heaters connected to the Temp-4 instrument. A slow flow of water was present to promote water circulation, but not enough to induce fish exertion. To account for this species's nocturnal behavior and minimize disturbance during testing, trials were started in the evening and run overnight for approximately 14 hours. The respirometers' associated software (AutoResp v. 2.1.2, Loligo Systems, Tjele, Denmark) measured each fish's resting rate of oxygen consumption over repeated 1,090 second cycles. Each cycle consisted of a 120 second flushing period to oxygenate the water, a 70 second wait period, and a 900 second M_{O_2} measurement period. All other fish continued to receive their respective diets until the day before respiration testing. Trials for all fish were completed over the course of 20 days.

Following respiration testing, each minnow was anesthetized in an ice bath and euthanized by freezing. In later trials, total length was measured both before and after freezing. For fish from earlier trials without fresh length measurements, fresh length was estimated using a linear regression of fresh and frozen total length ($TL_{fresh} = 4.949 + 0.927*TL_{frozen}$; $n = 14$; $r^2 = 0.97$). Whole fish samples were stored at -20°C during the period of respiration testing, then transferred to -80°C storage until FA analysis could be performed.

Fatty Acid Analysis

Fish Lipid Extraction and Methylation

Lipids were extracted using a modified Folch et al. (1957) method. Pre-weighed whole fish were chopped, then homogenized in 5 mL methanol using an Ultra-Turrax. 5 mL chloroform was added, and the mixture was vortexed, sonicated in a water bath for 15 minutes, and shaken

overnight to obtain full denaturation of the cell membranes. The next morning, 5 mL of 2% sodium chloride solution and 5 mL chloroform were added before shaking for 30 minutes then centrifuging at 4000 rpm for 15 minutes at 8°C. The chloroform layer was dried over anhydrous sodium sulfate and evaporated under a stream of N_2. The lipid extract yielded from this process was weighed, then reconstituted in 300 µL toluene.

Samples were then methylated using a modified method according to Nuernberg et al. (2007). 2 mL of 0.5M sodium methoxide was added to mixture obtained from the previous extraction process. This mixture was shaken for 20 minutes before adding 1 mL of 10% boron trifluoride in methanol. Samples were heated at 60°C for 10 minutes, during which time they were vortexed every 2 minutes. 2 mL saturated potassium bicarbonate and 2 mL n-hexane were added before vortexing for 2 minutes, then centrifuging at 2000 rpm for 5 minutes. The hexane layer was dried over anhydrous sodium sulfate. This extraction process (i.e., addition of n-hexane, vortexing, centrifugation, and drying) was performed two more times, using 1 mL n-hexane during each repetition. The hexane was evaporated under a stream of N_2, yielding the fatty acid methyl esters (FAME). FAME weight was calculated, then a 1% solution of FAME in n-hexane was prepared to be used in gas chromatograph (GC) analysis.

Direct Transesterification of Feed Lipids

Feed samples were processed using a modified method based on that of Sukhija and Palmquist (1988). 1 mL internal standard (1 mg C13:0 TAG/mL acetone), 2 mL toluene, and 2 mL of 5% sulfuric acid in methanol were added to 500 mg feed sample, and the mixture was incubated overnight at 50°C. After cooling to room temperature, 5 mL of 5% sodium chloride and 2 mL hexane were added. Samples were vortexed for 2 minutes, then centrifuged at 4000 rpm for 2 minutes. The desired hexane layer was removed, and this extraction was repeated two

more times using 1 mL hexane each. 4 mL saturated potassium bicarbonate was added to the hexane solution, which was then vortexed for two minutes and centrifuged at 4000 rpm for 2 minutes. The hexane layer was added to 1 g sodium sulfate, gently shaken, and left to stand for at least 10 minutes. The solution was cleaned over silica gel and charcoal, then evaporated under a stream of N_2. FAME weight was calculated, then a 1% solution of FAME in n-hexane was prepared to be used in GC analysis.

Gas Chromatography

FAME yielded from this process were analyzed using a GC-2010 gas chromatograph (Shimadzu, Kyoto, Japan), and the sample's FA composition was quantified and identified using GCsolution software (v. 2.30.00, Shimadzu, Kyoto, Japan). FA results were expressed as percentages of FA detected with a chain length between 10 and 22 carbon atoms.

Data Analysis

Individual fish from each tank were treated as independent replicates for all analyses. This technically constitutes pseudoreplication; however, it was the most feasible option due to limitations in the experimental infrastructure available. The influence of diet and temperature on M_{O_2} was tested through a full-factorial analysis of covariance (ANCOVA). The residuals from a linear regression of weight versus M_{O_2} were used as the response variable in the ANCOVA to eliminate the influence of weight on the model. M_{O_2} values were log transformed to better fit a linear model.

ANCOVAs were used to test for the influence of dietary treatment, temperature treatment, and the interaction of diet x temperature on minnow FA composition. Separate tests were run for individual FA, classes of FA (i.e., saturated FA [SAFA], monounsaturated FA

[MUFA], polyunsaturated FA [PUFA], EFA, conditional EFA, branched chain FA, omega-3 [n-3], and omega-6 [n-6]), and the ratio of n-3/n-6 FA. The EFA classification included linoleic acid (LA; 18:2 n-6) and alpha-linolenic acid (ALA; 18:3 n-3), while the conditional EFA classification included arachidonic acid (ARA; 20:4 n-6), eicosapentanoic acid (EPA; 20:5 n-3), and docosahexanoic acid (DHA; 22:6 n-3). Student's t-tests were used to test for differences in FA composition between the two experimental diets. Significance was determined at $p \leq 0.05$. Statistical analyses were performed using JMP Pro 11.0 software (SAS Institute Inc., Cary, NC).

RESULTS

Respirometry

Of the 46 minnows surviving the one month feeding period, useable respirometry data were obtained for 36 individuals, ranging from 0 to 11 individuals per temperature x diet treatment group. Mean (±SD) values for total length, weight and oxygen consumption are reported for each treatment group in Table 1. Length did not differ significantly between treatments. ANOVAs of weight versus treatment followed by a Tukey-Kramer HSD test in each dietary treatment group revealed a significant difference between only two temperature treatments within the high EFA group (i.e., weights in the 20°C treatment were significantly greater than those in the 28°C treatment; $p = 0.0341$).

The linear regression of weight versus M_{O_2} yielded a significant negative relationship; i.e., M_{O_2} decreased with an increase in weight ($r^2 = 0.31$, $p = 0.0004$; Figure 2). The residuals obtained from this regression to be used as the response variable in the ANCOVA are displayed in Figure 3.

The full factorial ANCOVA testing the influence of temperature and diet produced a statistically significant model ($r^2 = 0.38$, $p = 0.0015$; Figure 4). Effect tests identified both temperature and diet as significant factors ($p = 0.0133$ and 0.0101, respectively). The interaction between temperature and diet was not found to be significant.

Fatty Acid Analysis

Student's t-tests revealed many statistically signficant differences betweeen the two experimental diets (Table 2). In the broader categories of FA, the low EFA feed contained greater percentages of MUFA and branched chain FA. The high EFA feed had greater percentages of PUFA, EFA, conditional EFA, n-3 and n-6 FA, as well as a higher n-3/n-6 ratio. The low EFA feed had a higher conent of four SAFA (15:0, 17:0, 18:0, and 20:0); six MUFA (16:1 t, 16:1, 17:1, 18:1 9t, 18:1 n-9, and 19:1); five PUFA, including one EFA and one conditional EFA (18:3 n-6, ALA, 20:3 n-6, ARA, and 20:3 n-3); and ten branched chain FA (e.g., methyl-11-methyldodecanoate; see Table 2). The high EFA feed had a higher content of four SAFA (10:0, 14:0, 16:0, and 22:0); seven PUFA, including one EFA and two conditional EFA (LA, 18:2 tt, 20:2 n-6, EPA, 22:2 n-6, 22:5 n-3, and DHA); and one branched chain FA (methyl-18-methylnonadecanoate).

Small sample sizes likely reduced the statistical power of comparisons of FA compostion between minnow treatment groups; however, many significant relationships still emerged in the data (Table 3). Diet was found to have an influence on 18 of the 42 FA detected, as well as the broader categories of SAFA, PUFA, and EFA. Fish in high EFA treatment groups had higher contents of PUFA, EFA, and eight of the individual FA (18:1 9t, LA, methyl-18-methylnonadecanoate, 18:3 n-6, ALA, 20:1 n-12, 20:1 n-9, and 20:2 n-6). Fish in the low EFA

treatment groups had higher contents of SAFA and ten of the individual FA (12:0, 14:0, methyl-12-methyltetradecanoate, 16:1 t, methyl-14-methylhexadecanoate, 17:0, 18:0, ARA, 22:0, and 22:5 n-3).

Temperature had a statistically significant influence on 14 of the 42 FA detected, as well as the broader categories of MUFA, conditional EFA, branched chain FA, n-3 FA, and the n-3/n-6 ratio. Fish FA content decreased with increasing temperature in MUFA, branched chain FA, and ten of the individual FA (12:0, 13:0, methyl-12-methyltridecanoate, methyl-13-methyltetradecanoate, 16:1, methyl-15-methylhexadecanoate, 18:2 tt, 18:3 n-6, ALA, and 20:3 n-3). The opposite trend was observed in conditional EFA, n-3 FA, the n-3/n-6 ratio, and four of the individual FA (18:0, ARA, 22:0, and 22:5 n-3), in which FA content increased with increasing temperature. A similar direct relationship was apparent in relation to DHA, PUFA in the low FA treatment groups, and SAFA, although these correlations were not statistically significant.

The interaction of diet and temperature had a significant influence on the ANCOVA models of only 2 of the 42 individual FA (22:0 and 22:5 n-3). In both models, fish in low EFA treatment groups had a lower content of the FA at lower temperatures, and had a higher content of the FA at higher temperatures.

DISCUSSION

I observed a significant negative correlation between weight and M_{O_2} ($r^2 = 0.31$, $p = 0.0004$). In the significant model produced by the ANCOVA ($r^2 = 0.38$, $p = 0.0015$), both temperature and diet were found to exert significant influence on M_{O_2} ($p = 0.0133$ and 0.0101, respectively). The two diets used in this experiment had significantly different FA compositions.

ANCOVAs testing the influence of diet, temperature, and diet x temperature interactions on minnow FA composition revealed many significant correlations between temperature and diet treatments and FA content, although the trends in the data are not consistent between all FA in the fishes' profiles.

The observed influence of weight on M_{O_2} agrees with the literature. Metabolic efficiency generally increases with fish size (for review, see Clarke and Johnston 1999). In the context of this experiment, efficiency is indicated by a decrease in M_{O_2}; therefore, my results reflect this expected trend. Metabolic activity in fish increases with temperature (for review, see Clarke and Johnston 1999). The observed increase in oxygen consumption with temperature across my treatment groups agrees with this, with respiration rates increasing to meet higher metabolic demands.

While dietary treatment had a significant influence on oxygen consumption, it is difficult to determine the specific dietary components responsible for this relationship based on the results of FA analysis. The higher amounts of LA, PUFA and EFA in fish in the high EFA dietary treatment groups and lower amounts of ARA in the low EFA dietary treatment groups correspond to similar trends seen in the feed FA compositions, suggesting that variation seen between fish treatment groups is in fact related to differences in dietary FA. Therefore, the lower M_{O_2} observed in the low EFA treatment groups may be explained by some difference in dietary FA, but a more statistically rigorous study is required to clarify this interaction. Vagner et al. (2014) reported a similar relationship between metabolic efficiency and dietary n-3 HUFA content (i.e., EPA and DHA), in which golden grey mullet fed a diet low in n-3 HUFA attained oxygen at a lower rate. A similar relationship could account for the observed differences in minnow M_{O_2}, but the current results of FA analysis cannot definitively support this.

While significant differences in minnow FA composition were found between treatment groups, it is possible that the relationships between diet, temperature and FA composition are stronger than my results suggest. A substantial possibility of a type II error (i.e., accepting the null hypothesis when it is false) exists based on several aspects of my study. On the most basic level, I ended up with very small sample sizes in almost all treatment groups. Only one treatment (high EFA at 28°C) met the initial goal of analyzing ten fish per treatment group. The small numbers of surviving fish that produced useable data reduced the statistical power of my analyses, thereby contributing to potential type II error. My overall study design further contributed to small sample size by including only one tank per treatment, although this was ignored in my statistical analyses. If each tank was treated as a "true replicate" according to the definition provided by Heffner et al. (1996), the data from all the fish in a tank should be averaged into a single sample to reduce the effects of individual variability and potential social interactions within each group. An ideal study design would included multiple tanks per treatment group; however, limitations in infrastructure precluded this option in my study. Comparisons of FA composition between the two feeds also could have benefited from increased sample sizes.

Although it was not tested, variation in the length of the experimental feeding period may have influenced my results. All fish were initially subjected to the same 30 day feeding period; however, continued feeding over the course of respiration testing increased the feeding period for some treatments by up to 20 days (i.e., a 67% increase in the duration of feeding). While the differences in FA composition between treatment groups were not always significant, there tended to be increasing differentiation between the high EFA and low EFA treatment in each temperature treatment group as the duration of feeding increased. In an improved study design,

the starting dates of different treatment groups would be staggered to avoid the need for prolonged feeding of some groups while waiting for respiration testing to be completed for others.

Complications in food quality and feeding logistics may also have confounded my results. First, notable differences in feed density hindered efforts at equal feeding rates between treatments. Due to the lower density of the low EFA food, a larger volume was required to reach the suggested mass for maintenance feeding rates. A large portion of this was not consumed; therefore, food was provided based on volume rather than mass in subsequent feedings to reduce waste. Although no data are available on consumption rates, it is likely that fish in low EFA treatments were underfed relative to fish in high EFA treatments. This could be overcome by providing smaller amounts at multiple times per day to reach the total daily maintenance rate; however, the nocturnal habits of this species made this an unrealistic option. Second, differences in protein, fiber, or other nutrients between feeds could exert unanticipated influences on metabolic measurements, further confounding my results. Future study designs would be significantly improved by the use of controlled diets, formulated to be identical in everything but their EFA content. In addition to creating feed treatments with distinctly different FA profiles, this would also control the effects of feed density seen in my study.

I initially sought to study a species closer to its thermal maximum to investigate the potential implications of climate-induced warming of waters. However, the differential survival rates of minnows across temperature treatments in my study suggest I may have been observing the species closer to its minimum thermal tolerance rather than its thermal maximum. In my treatment groups, survival rates increased substantially with temperature. Heath et al. (1997) documented a critical minimum temperature of 4.41°C and a critical maximum temperature of

38.55°C for juvenile fathead minnows. These values far exceed the range of my temperature treatments (i.e., 12 - 28°C); however, the cold end of my spectrum is much closer to a thermal extreme than my warmest treatments, which fall around the optimum temperature range for fathead minnows (~26°C; average from studies reviewed by Lechleitner 1992). Minnows also needed to acclimate to more stressful temperatures faster than would occur in a natural environment (i.e., over several hours rather than weeks or months), which may have influenced overall survival in the colder temperature treatments.

My study only addressed environmental influences on the fathead minnow's resting metabolism; it did not look for any changes in active metabolic rate. Critical swimming speed (U_{crit}), or the maximum effort from which a fish can recover, is commonly used as a measure of active metabolism in fish. Expanding this study to include measurement of additional metabolic indices such as U_{crit} would provide information on a broader scope of metabolic activity, allowing for investigation of potential ecological tradeoffs associated with differences in dietary EFA. Differences in fish FA composition can have contrasting effects on different metabolic indices, which results in variable implications regarding a fish's adaptation to the local environment. For example, a fish with a high U_{crit} would be more successful in foraging and predator avoidance, but a higher resting metabolic rate would decrease its tolerance to oxygen-poor conditions. Different metabolic strategies are better suited to different sets of environmental conditions; therefore, factors such as dietary EFA availability could influence a fish's adaptation to the local environment. The capacity to adapt is a key component of the resilience of species confronted with environmental change. Furthering our knowledge of environmental influences

on adaptive capacity will provide insight crucial to understanding the greater implications of climate change in aquatic ecosystems.

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TABLES AND FIGURES

Table 1. Demographics and resting oxygen uptake (M_{O_2}) of fish swum in respirometry trials. Number of surviving fish per treatment group is listed for both pre-respirometry (i.e., the number remaining after the one month acclimation period; n_{pre}) and post-respirometry (i.e., accounting for stress-induced mortalities during respirometry; n_{post}). Total length (mm), weight (g), and M_{O_2} (mg O_2 kg^{-1} hr^{-1}) are reported based on n_{post}. Values that do not share a common superscripted letter are significantly different, as determined by a one-way ANOVA and Tukey-Kramer HSD *post-hoc* test within each dietary treatment group.

Treatment	n_{pre}	n_{post}	TL (± SD)	W (± SD)	M_{O_2} (± SD)
Low EFA					
12°C	0	0	N/A	N/A	N/A
16°C	3	1	57 (0.00)	1.55 (0.00)	298 (0.00)
20°C	2	2	55 (7.21)	1.58 (0.62)	271 (92.40)
24°C	5	4	49 (1.53)	2.06 (0.44)	285 (197.07)
28°C	7	4	51 (3.47)	0.88 (0.23)	578 (255.49)
High EFA					
12°C	1	1	54 (0.00)	1.66 (0.00)^{ab}	182 (0.00)
16°C	2	2	49 (0.00)	0.96 (0.01)^{ab}	318 (82.84)
20°C	7	7	52 (1.73)	1.89 (0.58)^{b}	337 (141.55)
24°C	5	4	53 (4.82)	1.81 (0.38)^{ab}	445 (89.34)
28°C	12	11	52 (4.32)	1.16 (0.45)^{a}	624 (307.21)
Total	46	36	52 (3.73)	1.42 (0.54)	447 (251.29)

Table 2. Mean FA composition of experimental diets (expressed as percentage of total FA).

ND = not detected.

	Low EFA (n = 2)	High EFA (n = 3)
C10:0*	0.01	0.02
C12:0	0.08	0.14
methyl 11- methyldodecanoate*	0.04	ND
methyl 10- methyldodecanoate	0.02	ND
C13:0	1.90	1.81
methyl 12- methyltridecanoate*	0.31	0.02
methyl 11- methyltridecanoate*	0.03	ND
C14:0*	1.63	3.76
methyl 13- methyltetradecanoate*	2.14	0.18
C14:1	0.07	0.05
methyl 12- methyltetradecanoate*	1.20	0.05
C15:0*	0.96	0.61
methyl 14- methylpentadecanoate*	1.26	0.17
methyl 13- methylpentadecanoate*	0.04	ND
C16:0*	12.35	15.40
C16:1t*	1.41	0.41
C16:1*	11.57	5.03
methyl 15- methylhexadecanoate*	1.20	0.43
methyl 14- methylhexadecanoate*	1.43	0.14
C17:0*	2.11	0.77
C17:1*	1.86	0.45
methyl 16- methylheptadecanoate*	0.51	0.28
C18:0*	8.28	5.35
C:18:1 9t*	0.46	0.31
C18:1 n-9*	23.74	15.44
C18:2 9t,12t	0.10	0.12
C19:0	0.29	0.23
C18:2 n-6 (LA)*	6.15	13.93
C18:2tt*	0.14	0.34
C19:1*	0.15	0.11
methyl 18- methylnonadecanoate*	0.04	0.12
C18:3 n-6*	0.67	0.15
C18:3 n-3 (ALA)*	5.83	3.67
C20:0*	0.04	0.02
C20:1 n-12	0.07	3.03
C20:1 n-9	0.14	1.38
C20:2 n-6*	0.03	0.39
C20:3 n-6*	0.20	0.12
C20:4 n-6 (ARA)*	3.69	1.37
C20:3 n-3*	0.68	0.21

Table 2, continued.

	Low EFA	High EFA
C22:0*	0.01	0.32
C20:5 n-3 (EPA)*	7.05	8.93
C22:2 n-6*	ND	0.04
C22:5 n-3*	0.04	1.46
C22:6 n-3 (DHA)*	0.01	13.25
SAFA	27.66	28.43
MUFA*	39.46	26.21
PUFA*	23.91	43.82
EFA*	11.98	17.60
Conditional EFA*	10.75	23.55
Branched chain FA*	8.23	1.39
n-3*	7.78	23.85
n-6*	10.97	16.45
n-3/n-6*	0.91	1.80

*Student's t-test indicated a significant difference between diets ($p \leq 0.05$).

Table 3. Mean FA composition of whole minnows in each diet x temperature treatment group (as percentage of total FA). Numbers in parentheses indicate treatment sample size. ND = not detected. Significance determined using a full-factorial ANCOVA to the influence of diet, temperature, and the interaction of diet x temperature on the percentage of each FA.

	12ºC	16ºC		20ºC		24ºC		28ºC		Significance		
	High FA (1)	Low FA (1)	High FA (2)	Low FA (2)	High FA (7)	Low FA (4)	High FA (4)	Low FA (4)	High FA (11)	Diet	Temperature	Diet x Temperature
C11:0	ND	ND	ND	0.01	0.01	0.01	ND	0.01	ND			
C12:0	0.04	0.04	0.07	0.02	0.05	0.03	0.04	0.01	0.03	*	*	
methyl 11-methyldodecanoate	0.02	0.02	0.03	0.41	0.01	0.01	0.02	ND	0.01			
C13:0	0.04	0.05	0.05	0.01	0.03	0.04	0.03	ND	0.01		**	
methyl 12-methyltridecanoate	0.13	0.16	0.11	0.04	0.04	0.05	0.09	0.03	0.02		**	
C14:0	1.45	1.13	1.60	0.68	1.82	0.75	1.81	0.67	1.61	**		
methyl 13-methyltetradecanoate	0.80	1.20	1.14	0.54	0.37	0.46	0.67	0.44	0.28		**	
C14:1	0.02	0.01	0.03	ND	0.02	0.07	0.03	ND	0.01			
methyl 12-methyltetradecanoate	0.14	0.20	0.20	0.15	0.07	0.10	0.11	0.13	0.06	*		
C15:0	0.75	0.75	0.81	0.51	0.47	0.50	0.55	0.57	0.51			
methyl 14-methylpentadecanoate	0.25	0.42	0.42	0.13	0.19	0.21	0.27	0.23	0.12			
methyl 13-methylpentadecanoate	ND	ND	ND	0.02	ND	ND	0.01	ND	ND			
C16:0	19.04	19.74	19.99	22.17	18.88	17.29	17.29	22.99	22.39			
C16:1t	0.64	0.64	0.77	0.98	0.69	0.83	0.73	0.98	0.83	*		
C16:1	3.64	3.64	5.49	4.51	4.55	3.01	4.64	2.47	3.52		*	
methyl 15-methylhexadecanoate	0.79	2.12	1.83	1.26	0.72	1.00	1.19	0.99	0.63		*	
methyl 14-methylhexadecanoate	0.18	0.35	0.39	0.39	0.17	0.43	0.28	0.40	0.19	*		
C17:0	1.22	1.63	1.78	1.45	0.79	1.41	1.26	1.56	0.93	**		
C17:1	0.48	0.48	0.85	0.72	0.46	0.59	0.58	0.51	0.45			

Table 3, continued.

	12ºC	16ºC		20ºC		24ºC		28ºC		Significance		
	High FA (1)	Low FA (1)	High FA (2)	Low FA (2)	High FA (7)	Low FA (4)	High FA (4)	Low FA (4)	High FA (11)	Diet	Temperature	Diet x Temperature
methyl 16-methylheptadecanoate	ND	ND	0.10	0.16	0.09	0.04	0.12	0.05	0.05			
C18:0	4.05	4.37	3.45	10.11	3.50	7.73	3.81	11.42	6.75	**	**	
C:18:1 9t	0.30	0.27	0.39	0.07	0.71	0.22	0.48	0.15	0.62	**		
C18:1 n-9	34.27	38.31	32.20	26.97	29.62	35.02	31.32	27.35	28.50			
C18:2 9t,12t	0.44	0.42	0.09	0.14	0.23	0.11	0.20	0.15	0.20			
C19:0	0.23	0.37	0.29	0.24	0.19	0.20	0.23	0.23	0.19			
C18:2 n-6 (LA)	16.05	14.23	13.07	4.44	14.70	12.12	15.85	5.06	10.77	**		
C18:2tt	0.21	0.30	0.43	0.28	0.30	0.25	0.34	0.21	0.23		*	
C19:1	0.10	0.03	0.10	0.08	0.08	0.09	0.07	0.07	0.07			
methyl 18-methylnonadecanoate	0.05	0.04	0.04	0.08	0.17	0.08	0.13	0.08	0.16	**		
C18:3 n-6	0.59	0.60	0.42	0.13	0.43	0.26	0.39	0.10	0.22	*	**	
C18:3 n-3 (ALA)	2.25	2.25	3.42	1.51	2.17	1.30	3.34	1.02	1.50	**	*	
C20:0	0.04	0.01	0.02	0.08	0.04	0.03	0.05	0.08	0.06			
C20:1 n-12	0.53	0.71	0.83	0.18	0.82	0.24	0.54	0.28	0.62	*		
C20:1 n-9	1.51	0.85	1.18	0.57	2.10	0.91	1.73	0.65	1.72	**		
C20:2 n-6	0.79	0.42	0.62	0.42	0.67	0.60	0.76	0.41	0.63	**		
C20:3 n-6	0.94	0.64	0.60	0.66	0.61	0.58	0.61	0.69	0.44			
C20:4 n-6 (ARA)	2.06	1.74	2.25	7.17	1.61	4.82	1.65	7.38	2.50	**	*	
C20:3 n-3	0.32	0.17	0.44	0.41	0.29	0.23	0.35	0.25	0.20		*	
C22:0	0.05	0.04	0.04	0.15	0.05	0.14	0.04	0.24	0.07	**	**	**
C20:5 n-3 (EPA)	1.32	0.70	1.90	3.16	3.55	1.94	2.67	2.87	2.84			
C22:5 n-3	0.71	0.22	0.52	0.82	0.97	1.48	0.71	2.38	1.00	**	**	**
C22:6 n-3 (DHA)	3.55	0.71	2.06	7.90	7.79	4.80	5.01	6.91	9.03			
SAFA	26.91	28.15	28.10	35.42	25.81	28.14	25.11	37.78	32.56	*		
MUFA	41.48	44.95	41.82	34.08	39.04	40.97	40.12	32.46	36.34		*	
PUFA	28.64	21.79	25.39	27.20	32.89	28.24	31.49	27.33	29.35	*		
EFA	18.30	16.48	16.49	5.95	16.87	13.42	19.19	6.08	12.27	**		
Conditional EFA	6.93	3.15	6.21	18.23	12.95	11.56	9.33	17.17	14.37		*	

Table 3, continued.

	12°C	16°C		20°C		24°C		28°C		Significance		
	High FA (1)	Low FA (1)	High FA (2)	Low FA (2)	High FA (7)	Low FA (4)	High FA (4)	Low FA (4)	High FA (11)	Diet	Temperature	Diet x Temperature
Branched chain FA	2.37	4.51	4.26	3.17	1.83	2.39	2.89	2.33	1.53		*	
n-3	5.91	1.80	4.92	12.58	12.60	8.46	8.74	12.41	13.07		*	
n-6	21.08	18.33	17.47	13.24	18.55	18.75	19.80	14.00	15.00			
n-3/n-6	0.28	0.10	0.28	0.95	0.68	0.45	0.44	0.89	0.87		*	

*Significant at the p = 0.05 level.
**Significant at the p = 0.01 level.

LIST OF FIGURES

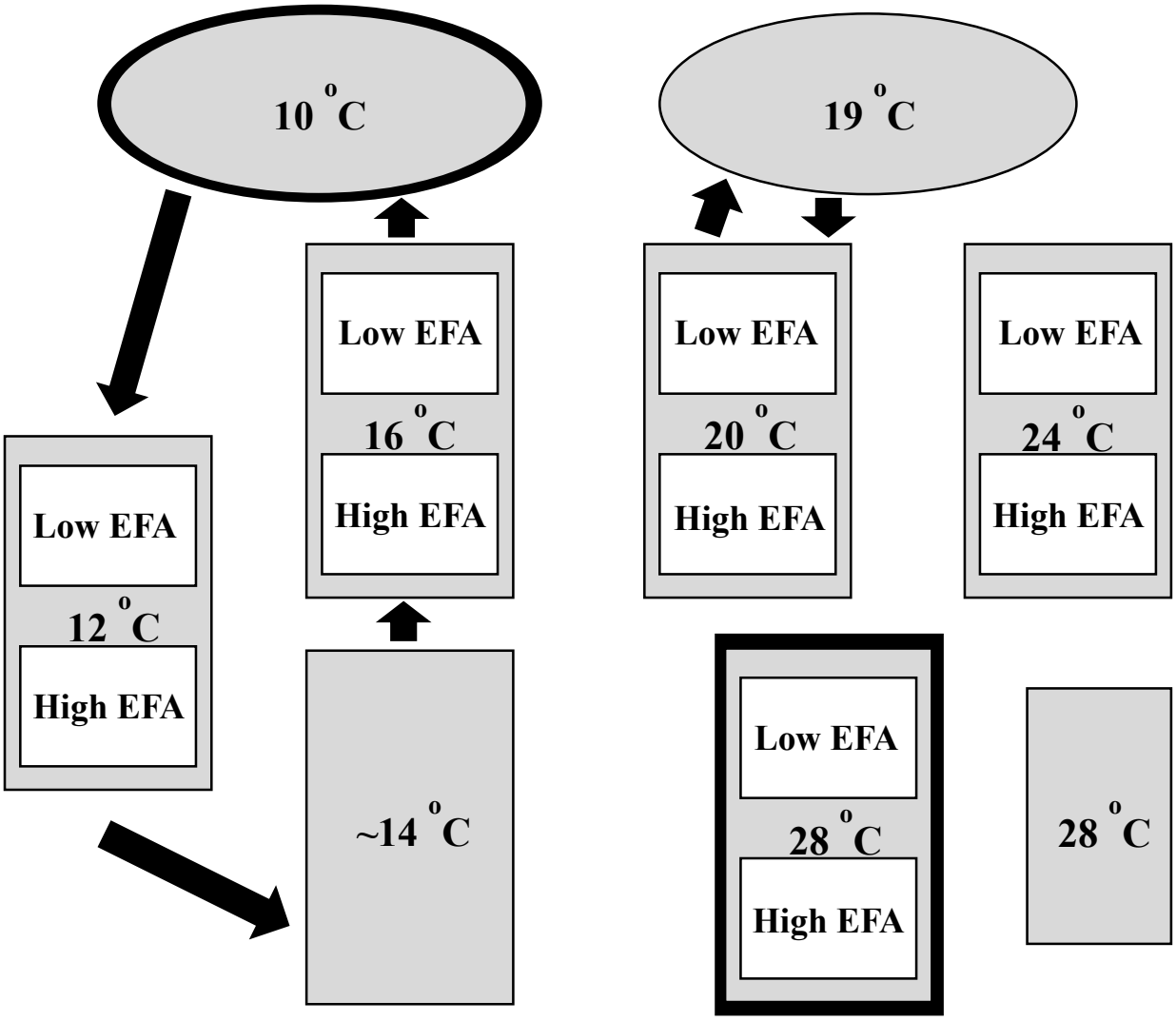

Figure 1. Schematic of the experimental setup. Gray areas represent water baths or reservoirs, with water temperature indicated in each; white areas represent tanks containing minnows. Reservoirs with thick borders were insulated. Arrows indicate the path of water flow through the chiller systems. “Low EFA” and “High EFA” refer to the dietary treatment in each tank. The 14°C reservoir served as a temperature buffer between the 12°C and 16°C baths, and the isolated 28°C reservoir provided water to be used in water changes in the 24°C and 28°C tanks.

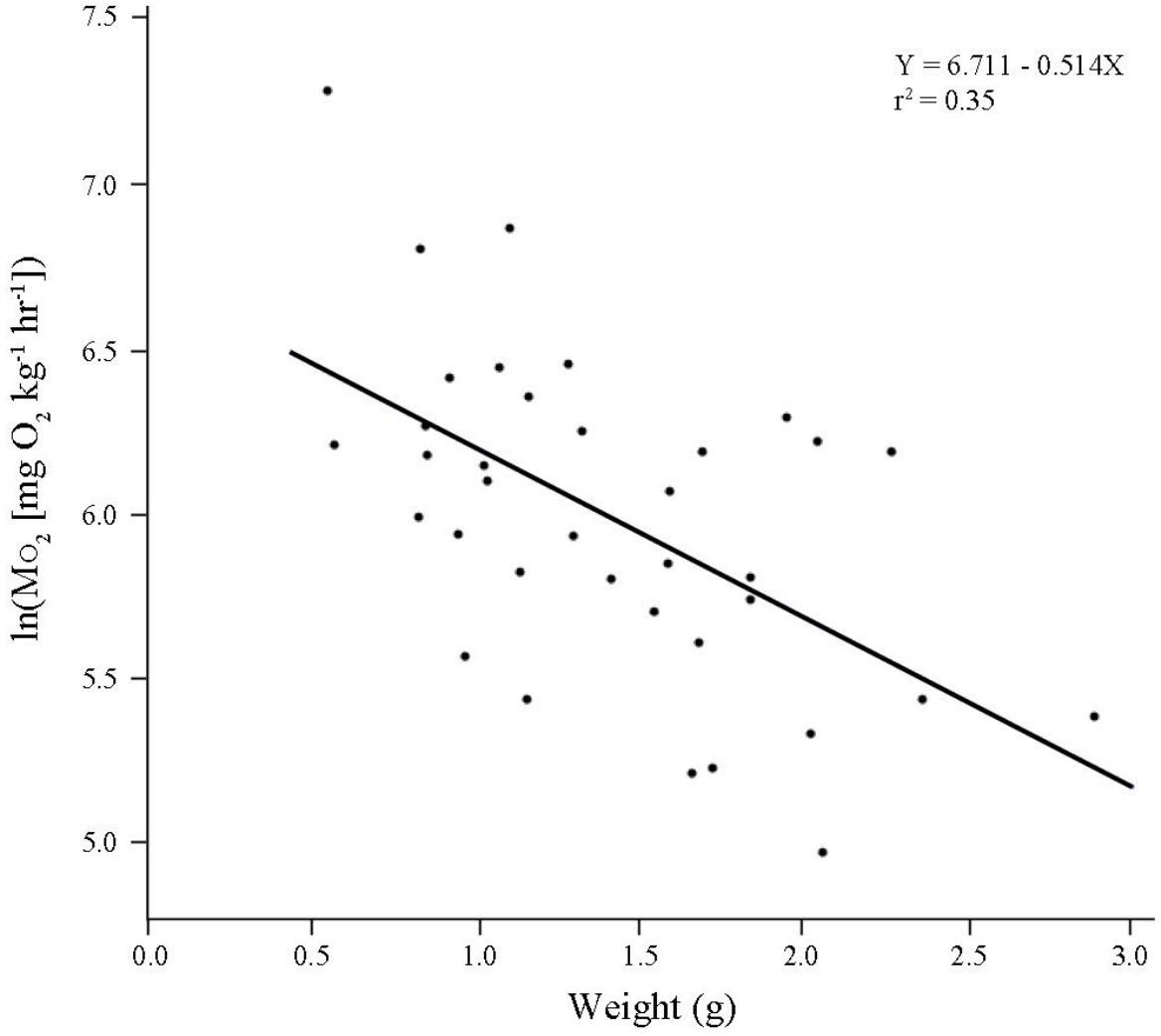

Figure 2. Relationship between minnow ln(M_{O_2}) and weight ($r^2 = 0.31$, $p = 0.0004$).

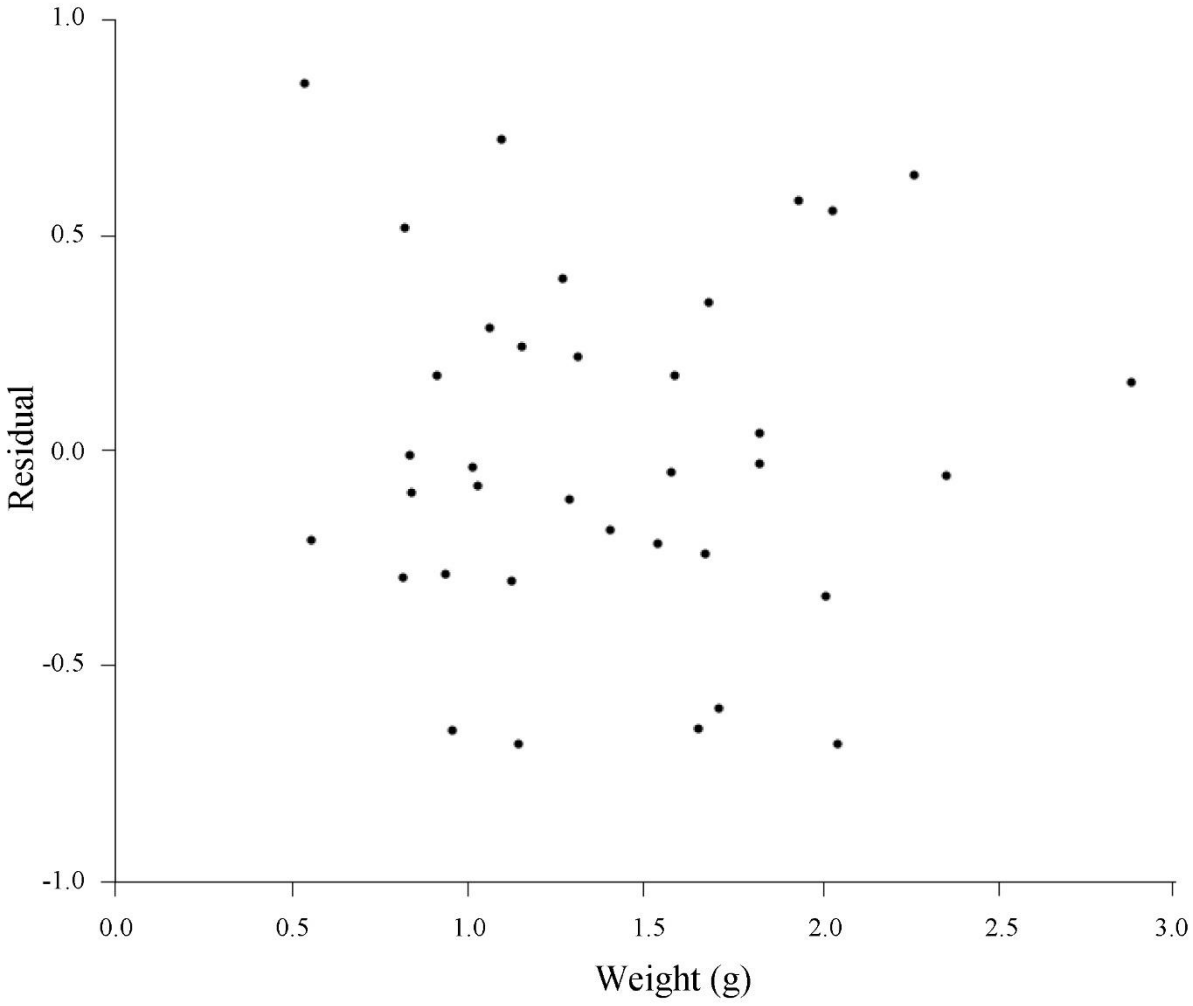

Figure 3. Residuals from the linear regression of ln(M_{O_2}) against weight.

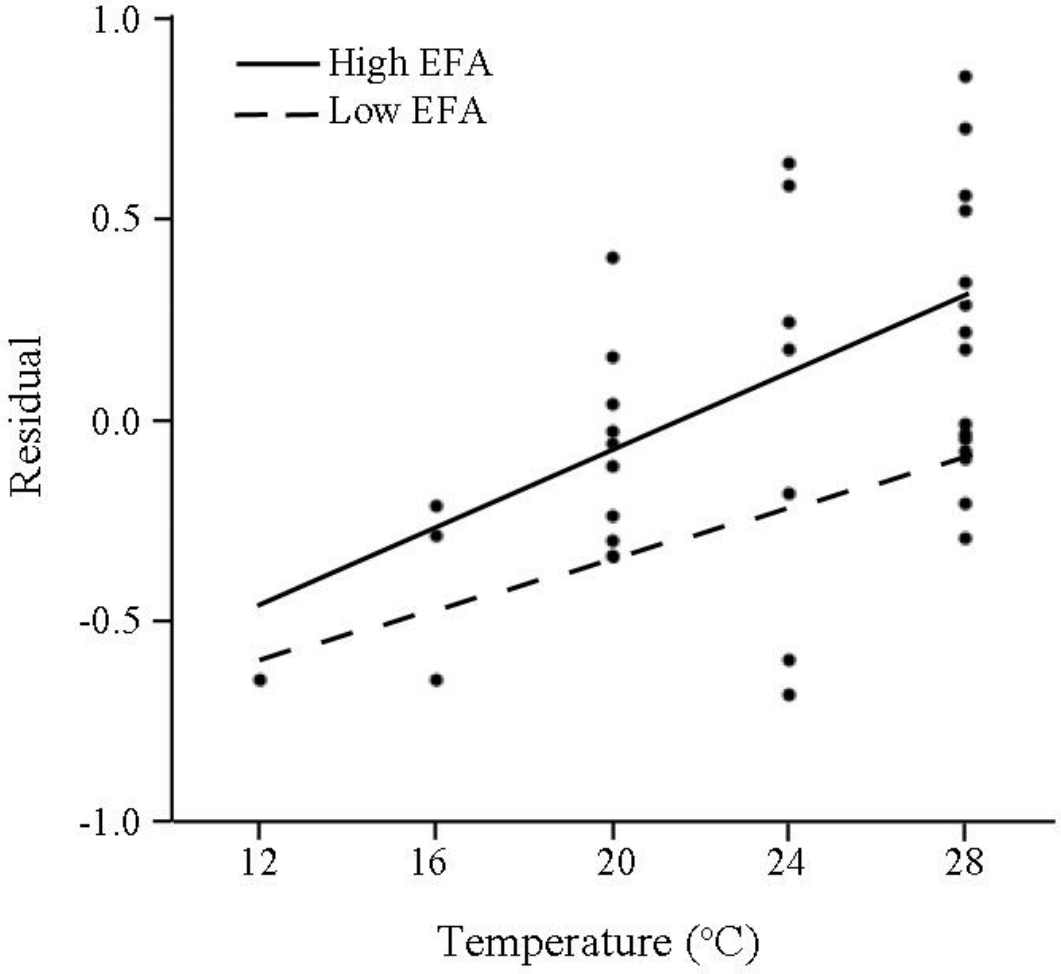

Figure 4. Model of the influence of temperature and diet on M_{O_2} ($r^2 = 0.38$, $p = 0.0015$). M_{O_2} is represented by the residuals from the linear regression of M_{O_2} against weight.