EFFECTS OF INCREASED TEMPERATURE AND DECREASED FOOD QUALITY ON METABOLISM AND GROWTH OF AN ALGIVOROUS CICHLID, *TROPHEUS DUBOISI*, AND EFFECT OF FOOD HABIT ON THE FIELD METABOLISM OF AFRICAN CICHLIDS

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By

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Lesley Yu-Jung Kim ENTITLED Effect of Increased Temperature and Decreased Food Quality on Metabolism and Growth of an Algivorous cichlid, *Tropheus duboisi*, and Effect of Food Habit on the Field Metabolism of African Cichlids BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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ABSTRACT

Kim, Lesley Yu-Jung. M.S. Department of Biological Sciences, Wright State University, 2014. Effect of increased temperature and decreased food quality on metabolism and growth of an algivorous cichlid, *Tropheus duboisi*, and effect of food habit on the field metabolism of African Cichlids.

The metabolic rate of an organism is influenced by mass, temperature, and diet. Climate change is anticipated to increase ambient temperatures of aquatic systems and decrease the quality of food available to algivorous fish. We conducted a lab experiment and a field study to quantify the influence that temperature and diet have on standard and field metabolic rate of cichlids from Lake Tanganyika. The lab experiment demonstrated the effects of increased temperature and decreased food quality on the relative growth rate (RGR) and standard metabolic rate of an algivorous cichlid, *Tropheus duboisi*. We found that in all temperature treatments fish fed a high quality diet had significantly higher RGR than fish fed a low quality diet and that fish at the highest temperature (32 °C) grew at half of the rate of fish at the lower temperatures (26 °C and 29 °C). Neither food quality nor temperature significantly affected the standard metabolic rate of the fish. I conclude that the effects of decreased food quality on RGR from climate change will be a more immediate stressor than increased temperature on metabolic rate. I also measured the field metabolic rates of seven species of African cichlids from three trophic levels. Both mass-specific metabolic rate and gut-fullness scaled positively with

activity level. The algivores had the highest metabolic rates, which may be a result of their higher levels of activity and increased gut-fullness relative to other trophic levels.

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I. INTRODUCTION

Climate change is anticipated to increase the temperatures of both terrestrial and aquatic environments, altering many physical, chemical, and biological processes (Naithani et al., 2011). Primary effects of climate change include the direct increase in temperature of the ambient environment of organisms (Verburg & Hecky, 2009). Direct effects of this increase in temperature will cause some organisms to experience an increase in metabolism and growth, while indirect effects will cause changes in ecosystem processes.

Most aquatic organisms are poikilothermic, meaning that their body temperature is variable and the rates of their metabolic processes are determined by their ambient temperature. Metabolic rates of ectothermic animals will increase with elevating temperature (Zhoa et al., 2011). Increases in temperature positively affect relative growth rate by increasing the rate of metabolic processes within the animal (Angilletta et al., 2004; Sibly & Atkinson, 1994). In poikilothermic ectotherms such as salmon, trout, and some marine fish, increased temperatures accelerate development by increasing rates of embryogenesis, resulting in larger animals at higher temperatures (Houde, 1989; Austreng et al., 1987). High temperatures can also have a negative effect on growth. Once an organism reaches a temperature higher than its optimal range, the proteins start to denature and enzyme processes are inhibited. Above that optimal range, increasing temperature is a hindrance to growth (Portner et al., 2001).

Whether an increase in metabolic rate is translated into more rapid growth or is physiologically detrimental is dependent upon both the magnitude of environmental temperature increase and the physiology of the animal (Hop & Graham, 1995). There is a temperature point within every animal's tolerance range called pejus temperature. At that point, the functionality of the animal's biological processes begins to be negatively affected by further increasing temperature. The magnitude of the environmental temperature change will determine if pejus temperature is reached for that species of animal. The physiology of the animal will determine how tolerant that species is to the range of exposed temperatures. Stenothermal fishes have narrow temperature optima and may be very sensitive to small increases in temperature. In contrast, eurythermal fishes exhibit maximal growth rates across a relatively wide range of temperatures. For instance, the brackish water killifish, *Fundulus heteroclitus*, survives in environments that can vary between 5-10 °C on a daily basis and between 15-20 °C on a monthly basis (Healy & Schulte, 2012). The effect of the magnitude of the temperature increase depends on the species-specific pejus temperature, and whether that animal will experience those temperatures depends on the environment in which it is located.

Aquatic environments will experience the direct effects of temperature change at a slower rate due to the relatively high specific heat capacity of water. Indirect effects of climate change in aquatic systems include changes in the rates of photosynthesis and variability in the availability of nutrients (Verburg & Hecky, 2009; Corman et al., 2010). Despite increasing temperatures, which have caused an increase in the photosynthesis rates of algae, primary production within Lake Tanganyika has decreased in correlation with warming events (O'Reilly et al., 2003). A decrease in biomass of algae will limit

food availability to herbivores in aquatic systems. Increasing temperatures appear to be stabilizing thermoclines and reducing wind mixing of lake ecosystems, which decreases the likelihood of upwelling events (Naithani et al., 2011). In oligotrophic tropical lakes, such as Lake Tanganyika, these abiotic shifts will result in a decrease in nutrient availability, making nitrogen and phosphorus less readily available for uptake by algae (Schindler & Eby, 1997). The ratio of carbon:nitrogen:phosphorus (C:N:P) in algae may be affected by this decrease in N and P availability, increasing the amount of C and the overall ratio. A high C:N:P ratio is associated with poor quality food and is another indirect effect of climate change (Lichtenbelt, 1992; Schindler & Eby, 1997). Fish will be simultaneously exposed to the indirect and direct effects of climate change. Lower food abundance and lower food quality compounded with an increased demand for food due to an increase in metabolic rate and energy requirements could prove to be too much stress for herbivorous fish to overcome.

ECOLOGICAL IMPLICATIONS

Considering the positive relationship between ambient temperature and fish growth rates, it is important to examine how changing temperatures will affect the energy demands of fish. Higher metabolic rates increase energy demands, but there is a threshold for which energy demands cannot be met and the growth and function of the animal begins to decline. At temperatures above the threshold, proteins in the body may begin to denature and metabolic pathways will begin to break down. The point at which basal metabolism begins to decline with increasing temperature is the pejus temperature. An animal experiencing suboptimal temperatures within the range of its ambient and pejus temperature is capable of modifying its behavior in order to maintain functionality.

However, because the fish in Lake Tanganyika are likely to experience the confounding factors of increased food demand and decreased supply and quality of food, they may not be able to compensate.

The purpose of this experiment is to examine how variation in temperature and food quality will affect the metabolism and growth of cichlid fish endemic to Lake Tanganyika. Nutrient and energy limitations have both been proposed to limit herbivore growth (Schindler & Eby, 1997). However, the amount of carbon used by fish to maintain their metabolism is not typically accounted for when determining carbon demands (Sterner, R. & J. Elser, 2002). By measuring resting routine metabolic rates of postabsoptive fish, a basal demand of carbon can be calculated under varying temperature and food quality conditions. The effects of increasing the current temperature of the lake by 3 °C and 6 °C will be simulated in our lab experiment. The fish will then be fed either a high or low quality diet. This will elucidate which factors have a larger impact on the fish of Lake Tanganyika in the foreseeable future and whether or not the interactions of factors will be significant. An aim of this study is to bracket the pejus temperature of *Tropheus duboisi*. The effect of temperature, food quality, and their interactions on metabolic rate will be analyzed in an attempt to provide insight into which factor limits herbivore growth.

II. METHODS

We measured the standard and digestive metabolic rate, as well as the growth rate of 48 juvenile *Tropheus duboisi*. Fish were separated by mass through the process of binning and then randomly assigned a temperature and food treatment. Fish were acclimated to 26 ° C, 29 °C, or 32 °C. The lowest temperature was chosen because it reflects the current temperature of Lake Tanganyika. The fish were placed in holding aquaria (15 gal) for 6 weeks prior to the experiment to allow adequate time for acclimation to temperatures and food quality treatments. Each temperature treatment was applied to 4 tanks, resulting in a total of 12 tanks. Four fish were placed in each tank and separated by partitioning the tank into 4 quadrants using mesh and placing 1 fish in each quadrant (Fig. 1.1). This allowed for identification and consistent measurement of the fish so that growth of each individual could be monitored throughout the experiment.

The 12 holding tanks were kept at a constant temperature by using water heaters and they were monitored for consistency using i-button temperature recorders (Maxim Integrated, San Jose, CA). The tanks were monitored weekly for pH, conductivity, dissolved oxygen, and ammonia levels. DI water was added to the tanks every two days to compensate for evaporation. Holding aquaria filters and sponges were rinsed every week. Fish were monitored daily for health.

Fish were fed either a high quality diet of commercial fish food consisting of high protein levels, or a low quality diet that was a dilution of the high quality food with algae. The high quality food was commercial Spirulina aquarium flake food (Ocean Star

International, UT, USA) and the low food quality food was a mixture of 20% Spirulina aquarium flake food and 80% lab-grown algae. The low quality food was made by blending the commercial flake food and algae with water. The mixture was then poured into aluminum drying pans and kept in a drying oven at 30 °C for 24 hours or until mixtures were dry. Fish were fed as much food as they would consume in 5 minutes (*ad libitum*) twice per day and the amount of food consumed by each fish was measured by taking the mass of the assigned fish food containers before and after feeding. Growth was monitored by measuring the mass of individual fish once per week and again before beginning a respiration experiment. Respiration was measured twice for each fish; once an hour after feeding with a full gut and once after fasting for two days while in a postabsorptive state. This allowed for calculation of energy used for digestion.

Respiration experiments ran from 29 May to 16 June 2013, averaging 6 fish per day. Three large holding tanks, one per temperature treatment, were used to submerge respirometry chambers during the experiment. The holding tanks kept the temperature of the water inside of the chamber constant during the experiment and also kept oxygen-saturated water in the system while the fish acclimated to the respirometry chamber. Respirometry chambers were made of opaque and clear PVC connected by tubing. The chambers were 30.5 cm long, 6.5 cm in diameter, and approximately 0.53 L (Fig. 1.2). Each chamber was equipped with an optical dissolved oxygen (DO) probe and meter (YSI ProODO, Yellow Springs, OH) that was used to detect changes in oxygen concentration throughout the experiments. Both chambers were placed into the large respiration tank with temperature regulated water and bubble stones (Fig. 1.3). One chamber housed the fish and the other chamber served as a blank for the duration of the

experiment. One DO probe was inserted into each of the chambers through rubber ports and set to record DO levels throughout the experiment. During the experiment, the clear portion of the tube that housed the fish was covered to decrease external visual stimuli that might stress the fish.

At the conclusion of the experiment, respiration data were collected for 44 and a total of 6 individuals from each treatment were sacrificed for further analysis.

CALCULATIONS AND STATISTICAL ANALYSES

Mass-specific intake (MSI) of food for each fish was calculated by taking the sum of the mass of the food consumed by a fish divided by the mass of that fish per week divided by the number of days of the experiment (g food/day/g fish).

$$MSI = \left(\frac{\text{food consumed}_{\text{week 1}}}{\text{mass}_{\text{fish week 1}}} + \frac{\text{food consumed}_{\text{week 2}}}{\text{mass}_{\text{fish week 2}}} + \ldots + \frac{\text{food consumed}_{\text{week 8}}}{\text{mass fish}_{\text{week 8}}} \right) / \text{days}$$

Relative growth rate (RGR) calculation:

$$RGR = \frac{(\ln \text{mass}_{\text{final}}) - (\ln \text{mass}_{\text{initial}})}{(\text{time d})}$$

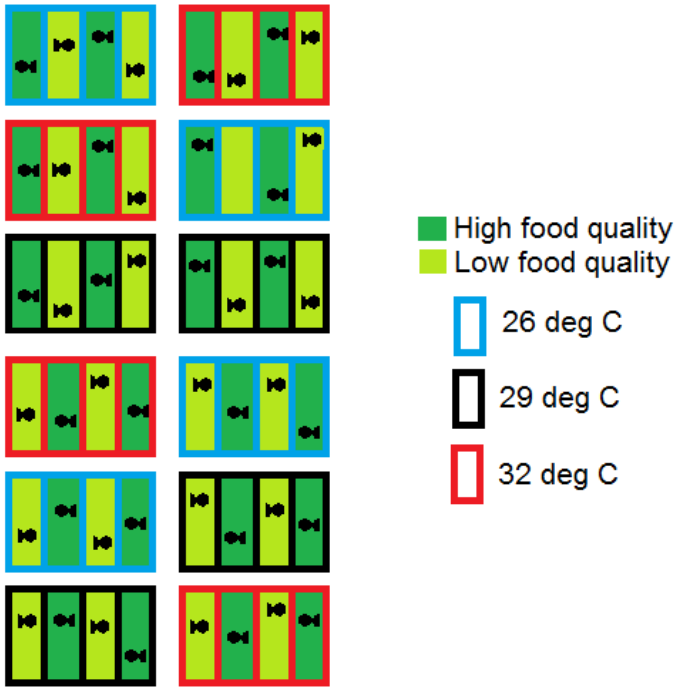
Metabolic rate will be calculated from converting the output of the DO meter (mg O_2/L) into $\dot{V}_{O_2}$ (mg O_2/h) or mass-specific $\dot{V}_{O_2}$ (mg O_2/h/g). The calculated $\dot{V}_{O_2}$ of the blank was subtracted from the calculated $\dot{V}_{O_2}$ of the chamber that housed a fish during the experiment.

$$\dot{V}_{O_2} = \frac{(\text{mg } O_{2\ \text{consumed}}) * (\text{volume}_{\text{chamber}}\ \text{L} - \text{volume}_{\text{fish}}\ \text{L})}{(\text{Time h})}$$

Digestive cost was calculated by subtracting the mass-specific $\dot{V}_{O_2}$ (mg O_2/h/g) of the starved fish from the mass-specific $\dot{V}_{O_2}$ of the same fish measured with a full gut and then dividing by the starved metabolic rate to provide a percentage.

Linear regressions, ANOVAs, and T-tests were run in order to determine correlations and significance levels for the factors of the experiment. ANOVAs using mass-specific intake, RGR, and $\dot{V}_{O_2}$ as response variables with mass, temperature, and food quality as factors. To determine if the average size of the fish in each food quality treatment was influencing $\dot{V}_{O_2}$ results, the residuals of the best fit line for metabolism and mass were plotted for each temperature treatment and then analyzed with an ANOVA. Tukey HSD posthoc tests were run on ANOVA results using a confidence interval of 0.95 to determine significance.

A)



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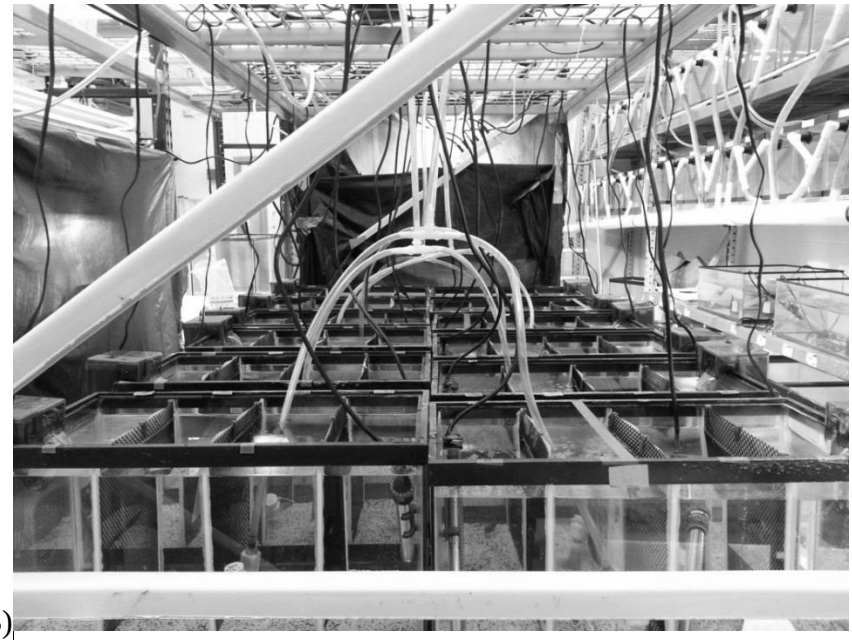


Figure 1.1 A) A diagram of the acclimation holding tank organization and set up. Tank temperatures were either 26 °C, 29 °C, or 32 °C. Food quality treatment assignments were either high or low. All assigned treatments to tanks were random. B) A picture of the lab set up of acclimation holding tanks.

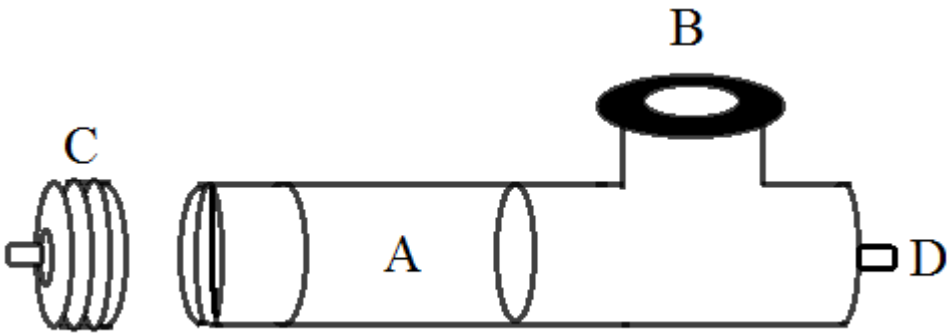


Figure 1.2 Chambers used to run the experiment consisted of A) a clear portion in which the fish was housed during the length of the experiment, B) the rubber DO port that allowed for the ProODO probe to be inserted into the chamber during the experiment, C) the removable end cap that allowed for placement of the fish and the closure of the system, and D) the tube attachment sites that allowed for water to flow through the system during acclimation.

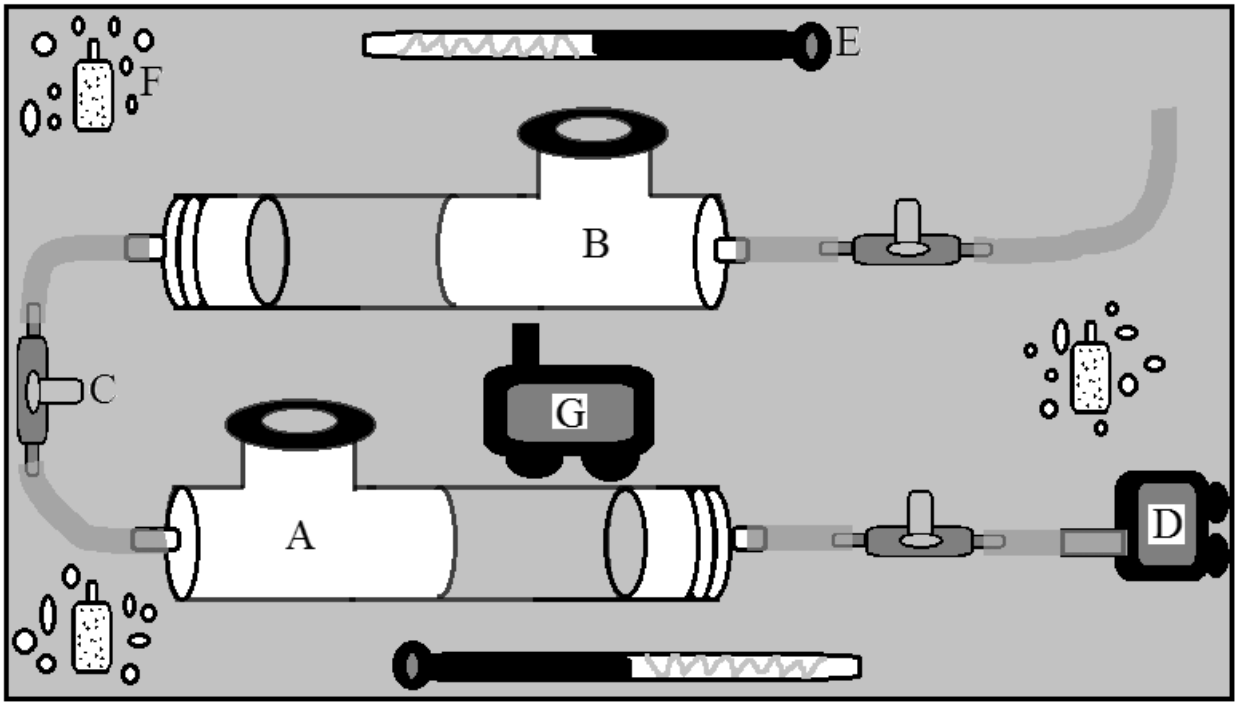


Figure 1.3 The setup for the respiration tanks during the experiment consisted of A) the experimental chamber that housed the fish, B) the empty chamber that served as a blank during the experiment, C) the valves that allowed the system to be closed and opened to the external tank water, D) the pump that pushed tank water into the system and allowed it to flow through when the system was open, E) the water heaters present in order to keep the temperature of the water constant throughout the experiment, F) bubble stones that kept the water oxygen-saturated and mixed, G) a larger pump that kept the water mixed and the temperature and oxygen saturation levels constant throughout the experiment.

III. RESULTS

There were no significant effects of temperature or food quality on mass-specific consumption by the fish (Table 1.1, Fig. 1.4). Both temperature and food quality strongly affected relative growth rate (RGR) (Table 1.2), but there was no interaction between the treatment effects. Fish kept at 32 °C grew at about half the rate of those at 26 °C and 29 °C (Fig. 1.5). Fish at the highest temperature treatment also had the lowest average growth efficiency (Fig. 1.9). Relative growth rate differed between food quality treatments by at least 0.003 g/g/day in all temperature treatments. Dilution of the commercial fish food with 80% Lake Tanganyika algae resulted in a decrease in average RGR of 44%.

Final mass of fish was the best predictor of variation in basal metabolic rate ($\dot{V}_{O_2}$). The slope of ln $\dot{V}_{O_2}$ on ln mass was 0.59. There was no significant effect of either temperature or food quality on the variation in metabolism once the effect of mass was removed (Table 1.5, Fig. 1.7). However, the fish acclimated to 29 °C had the highest average mass-independent metabolic rate, which suggests that the pejus temperature for metabolism is between 26 °C and 32 °C (Fig. 1.7). Average $\dot{V}_{O_2}$ for the high quality 26 °C treatment varied significantly from the high quality 32 °C temperature treatment ($p<0.05$)(Fig. 1.6).

No significant effect of temperature or food quality on digestive cost was observed. However, the interaction between temperature and food quality yielded a marginally significant p-value just above the level of significance ($p=0.09$).

Table 1.1 Analysis of variance for mass specific intake of fish looking at temperature, food quality, and their interactions showed no significant effects of the factors.

ANOVA:Mass-specific intake					
Source of variation	Df	Sum Sq	Mean Sq	F	P
Temperature	2	0.0000535	2.68E-05	0.759	0.474
Food Quality	1	0.0000363	3.63E-05	1.031	0.316
Temperature:Food Quality	2	0.0000321	1.60E-05	0.455	0.638
Residuals	42	0.0014807	3.53E-05		

Table 1.2 Analysis of variance for relative growth rate of fish looking at temperature, food quality, and their interactions showed significant effects for both temperature and food quality independently, but not a significant effect of interactions.

ANOVA:Relative growth rate					
Source of variation	Df	Sum Sq	Mean Sq	F	P
Temperature	2	2.58E-04	1.29E-04	27.607	2.22E-08 ***
Food quality	1	1.53E-04	1.53E-04	32.706	1.01E-06 ***
Temperature:Food Quality	2	8.00E-08	4.00E-08	0.008	0.992
Residuals	42	1.96E-04	4.67E-06		

Signif. codes: ***=0.001, **=0.01, *=0.05

Table 1.3 Analysis of variance for mass specific metabolic rate for all temperatures on high quality diets with mass and temperature having significant effects on metabolic rate.

ANOVA: $\dot{V}_{O_2}$ for High food quality						
Source of variation	Df	Sum Sq	Mean Sq	F	P	
Mass	1	0.06203	0.06203	20.916	0.00043	***
Temp	2	0.02634	0.01317	4.441	0.0321	*
Mass:Temp	2	0.00238	0.00119	0.401	0.67697	
Residuals	14	0.04152	0.00297			

Table 1.4 Analysis of variance for mass specific metabolic rate for all temperatures on low quality diets with no significant effects of factors.

ANOVA: $\dot{V}_{O_2}$ for Low food quality					
Source of variation	Df	Sum Sq	Mean Sq	F	P
Mass	1	0.00687	0.00687	1.079	0.313
Temp	2	0.00946	0.00473	0.743	0.49
Mass:Temp	2	0.01362	0.00681	1.07	0.364
Residuals	18	0.11455	0.00636		

Table 1.5 Analysis of variance for metabolism and mass residuals on temperature and food quality with no significant effects of factors.

ANOVA: $\dot{V}_{O_2}$ residuals					
Source of variation	Df	Sum Sq	Mean Sq	F	P
Temp	1	0.0163	0.01632	0.554	0.461
Food quality	1	0.0051	0.005069	0.172	0.681
Temp:Food quality	1	0.0215	0.021462	0.728	0.399
Residuals	38	1.1202	0.029479		

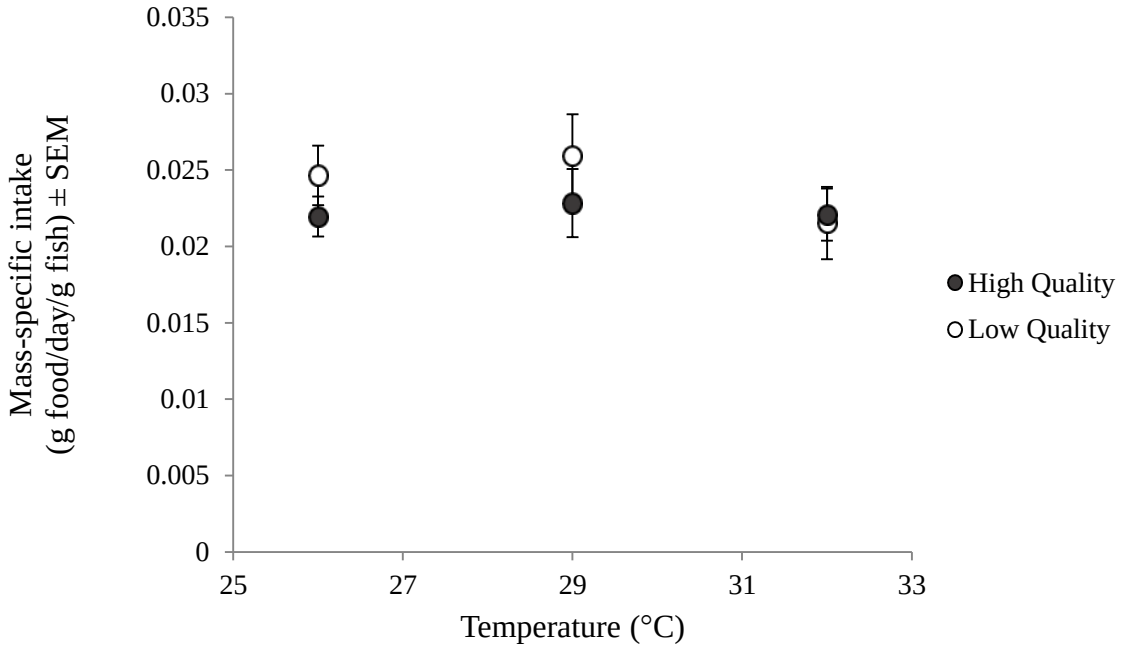


Figure 1.4 Mass specific intake of fish from each temperature and food quality treatment over 10 weeks of acclimation. Average mass specific intake for each temperature and food quality treatment of fish showing no significant differences.

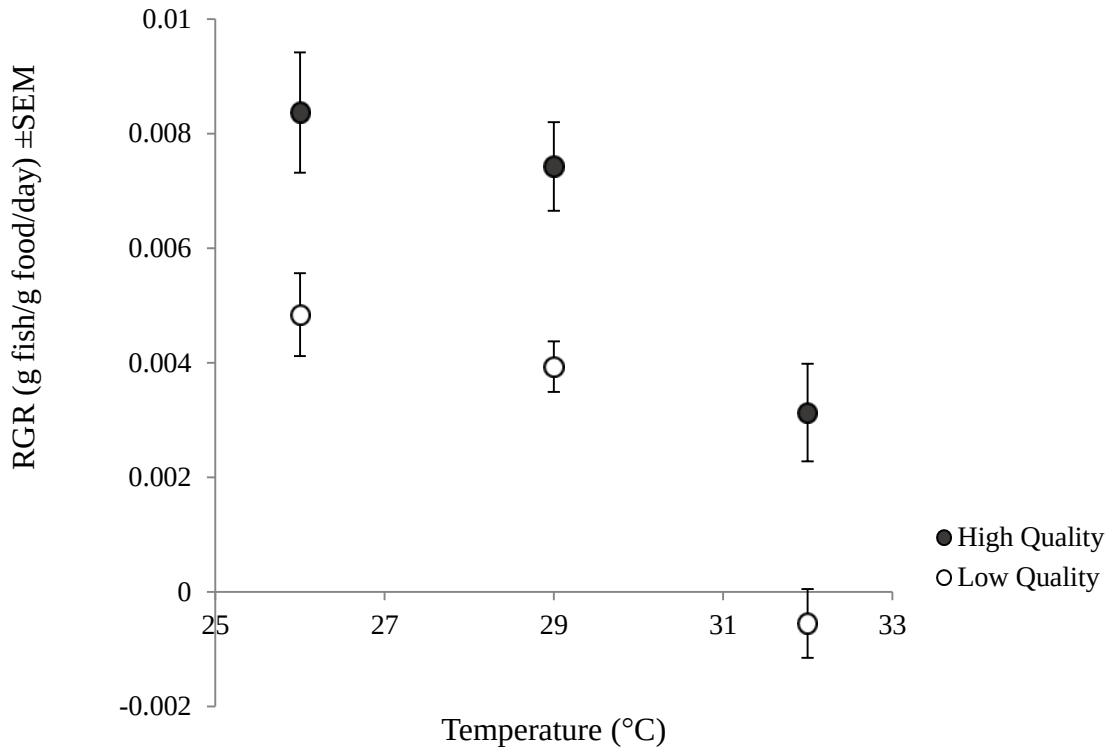


Figure 1.5 Average relative growth rates of *Tropheus duboisi* scale with temperature by food quality.

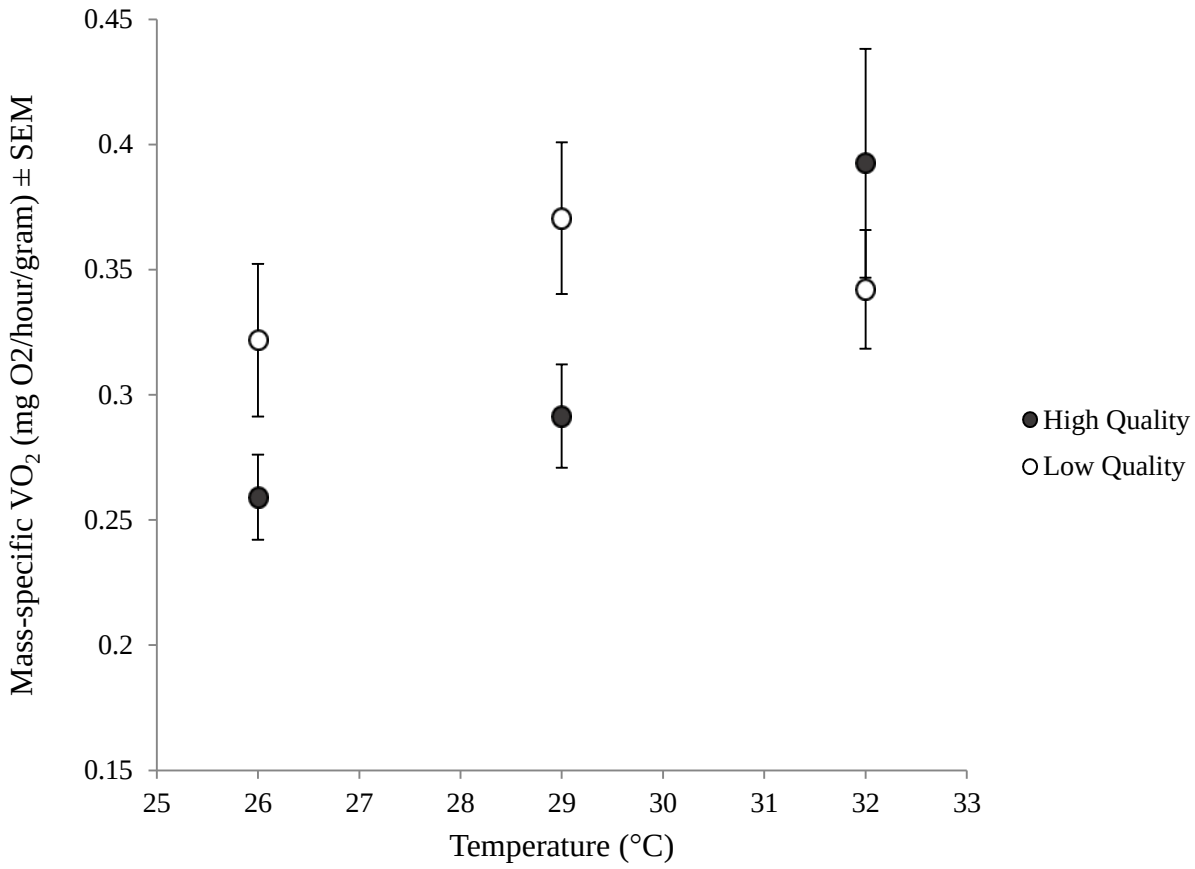


Figure 1.6 Average mass specific $\dot{V}_{O_2}$ and standard errors for each food quality diet scale with temperature.

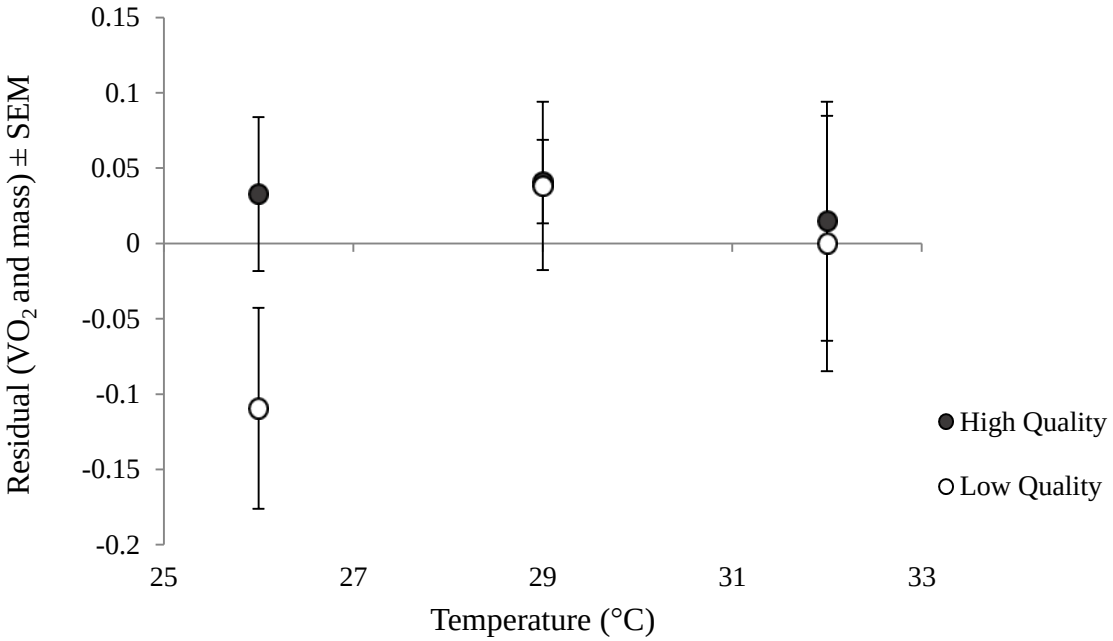


Figure 1.7 Plot of average residuals and standard error of the means for each temperature treatment by food quality.

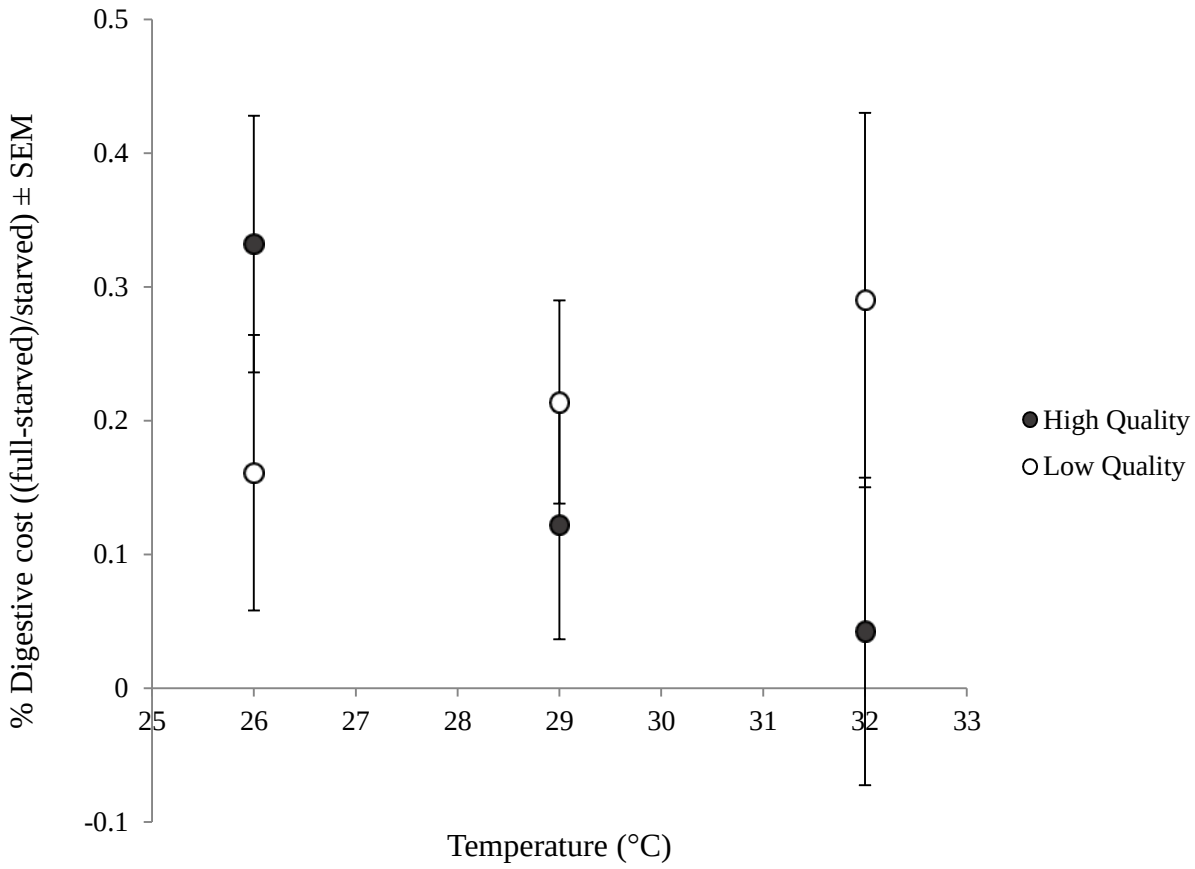


Figure 1.8 Consequences of high and low quality diet acclimation and digestive cost at each temperature treatment.

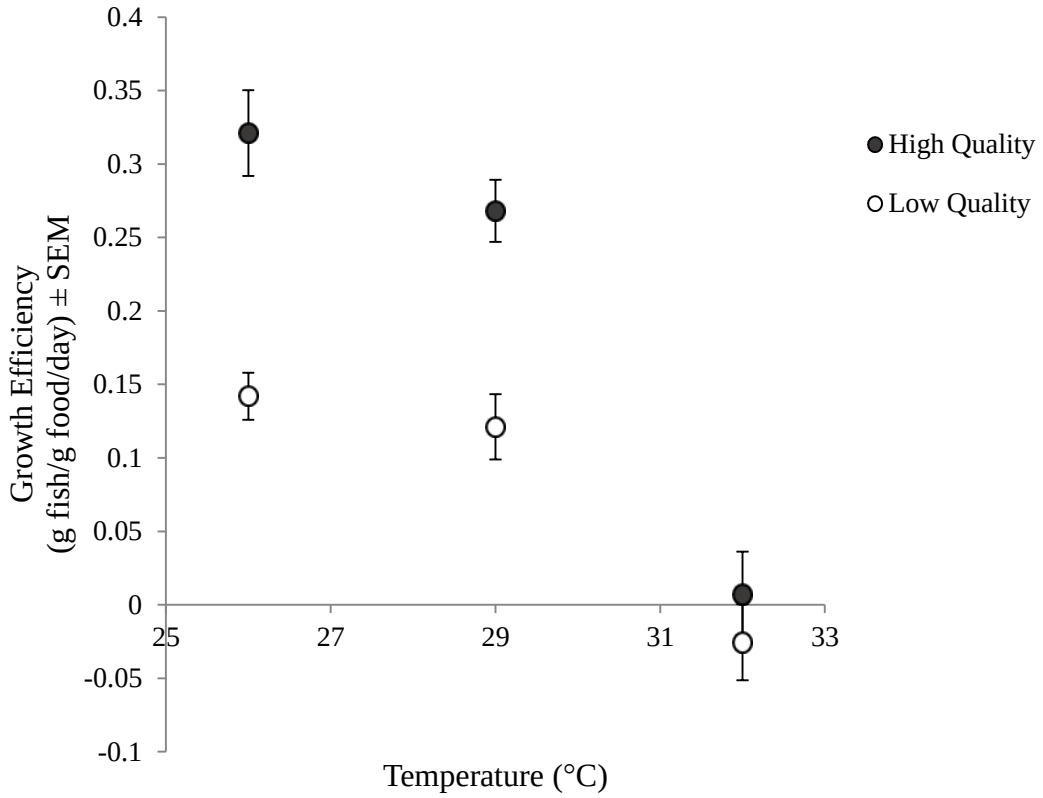


Figure 1.9 Average growth efficiency for fish decrease with increasing temperature for both food quality treatments with growth efficiency at 32 °C being significantly lower than the other two temperature treatments.

IV. DISCUSSION

Both food quality and temperature independently had significant effects on relative growth rate (RGR) but not on measured basal metabolic rate. Fish expend varying amounts of energy towards different activities such as growth and respiration, and each action can have a unique optimal temperature (Fonds et al., 1992). The range of the temperatures used in the experiment appeared to bracket the pejus temperature for *Tropheus duboisi*. Within a given food quality treatment, fish acclimated to 26 °C and 29 °C grew at the same rate. However, RGR was significantly lower ($p < 0.01$) at 32°C (Fig. 1.5), and fish fed a low quality diet at the 32 °C did not grow. This suggests that the combined effects of the higher temperature and the lower food quality were too great for the fish to overcome, and they began to lose biomass. Based on these results, the optimal temperature for growth is above 26 °C and below 32°C. It is clear that the temperature 32 °C is detrimental to the growth of *T. duboisi*.

FOOD QUALITY EFFECT ON RGR

The fish in this experiment were fed *ad libitum*, and fish fed a low quality diet might have compensated by increasing consumption. However, there were no differences in mass-specific intake among treatments (Fig. 1.4). Grasshoppers and lizards increased their food intake and decrease the transit time of food in their system when food quality was lowered (Yang & Joern, 1994; Lichtenbelt, 1992). The variation seen in RGR was not due to differences in mass-specific intake of the fish, but can be attributed solely to the effect of temperature and food quality on growth (Table 1.2).

The lower quality diet resulted in a consistent decrease in average RGR across temperature treatments (Fig. 1.5). Increases in food quality affect relative growth rate by providing the organism with the energy and compounds needed for anabolism. Organisms acclimated to high qualities of food often have a higher growth rate and fecundity than those on lower qualities (Bukovinszky et al., 2012; Cruz-Rivera & Hay, 2000; Kilham et al., 1997). Cichlid fish with a higher amount of protein in their diet (high quality) as compared to diets of algae (low quality), experience a greater increase in mass over time (Tadesse et al., 2003). Our findings also demonstrate that organisms acclimated to a higher quality diet have a higher growth rate. An 80% dilution with algae of our high food quality consistently decreased the relative growth rate of fish by about 44% in all temperature treatments. The magnitude of the effect of the increase in temperature from 29 °C to 32 °C on RGR for both qualities of food was similar to the magnitude of the effect of differences in food quality within treatments. Relative growth rate decreased by 58 % between the 29 °C high quality food and the 32 °C high quality food. The strong effect of food quality on RGR is likely to be even more critical in Lake Tanganyika due to the low quality of the algae available to grazers. As climate change decreases the frequency of physical processes that promote upwelling and the spread of nutrients to near shore ecosystems, herbivore food sources will continue to decline in quality (Naithani et al., 2011).

Changes in food quality in response to climate changes may be more influential than direct negative effects of temperature on RGR. A significant difference between high and low food quality at each temperature was observed (Table 1.3). However, there was no significant difference between the 26 °C and 29 °C temperature treatment for high

or low food quality. A direct effect of climate change is the increasing temperature of the water and the rate of surface water warming in Lake Tanganyika has been documented at 0.1°C per decade. Based on our experiment, even if the lake increased in temperature by 3 °C over the next 300 years, the fish would still not experience a significant decrease in growth due to increased temperature. This indicates that food quality will have a greater effect on the fish living in Lake Tanganyika than temperature will in the foreseeable future. Potential declines in food quality due to lower nutrient availability are likely to decrease the RGR of herbivorous fish in Lake Tanganyika. The food quality used in this experiment was much higher than the food quality that these herbivores have access to in Lake Tanganyika. We expect that the trend of decreasing food quality within the lake will cause RGR to decrease on an even greater scale than observed in our experiment.

METABOLIC RATE

After controlling for the effects of fish mass, the metabolic rates of fish did not differ significantly among temperature or food quality treatments (Tables 1.4 & 1.5). For fish acclimated to high food qualities, mass-specific VO2 increased with each temperature increment and increased significantly between the 26 °C (0.26 mg O_2/h/g) and 32 °C (0.39 mg O_2/h/g) temperature treatments (Fig. 1.6). Ambient temperature dictates the speed of physiological processes in most ectotherms and therefore, influences metabolic rate (Pirozzi & Booth, 2009). Higher temperatures result in higher rates of metabolism in fish (Zhoa et al., 2011; Turker, 2011; Miklos et al., 2003). A significant increase in metabolic rate with temperature for each food quality was expected in our study but only observed in one instance for low food quality between the highest and lowest temperature treatments. It is possible that the high food quality kept differences at

a minimum and supplemented the increased need for energy to support elevated respiration.

The majority of the variation seen in metabolic rate can be attributed to differences in mass of the fish between treatments (Fig. 1.6). We expected to observe an optimum temperature for metabolic rate within our experimental range of temperatures, but did not see a significant difference once data were corrected for mass. Once the optimum temperature for an animal is surpassed, the $\dot{V}_{O_2}$ begins to decline with increasing temperatures (Klok et al., 2004). The shape of the relationship we saw was expected but not significant (Fig. 1.7). One constraint of our experiment was that it was not possible to separate the cost of growth from the cost of basal metabolic rate for these fish. At 32 °C the fish could be allocating energy to maintenance of metabolic pathways and respiration but not growth (Bullock, 1954). The cichlids used in our experiment were eurythermal and had high tolerances for variation in temperature. Designing an experiment that allows for more sensitive measurements of metabolic rate as well as includes temperature treatments that exceed 32 °C may be needed to detect significant changes in metabolic rate for this species of fish.

Despite the food quality treatment, the fish in our experiment that were acclimated to 32 °C visibly had lower activity levels, higher rates of operculum movement, and were less resilient to handling stress. These observations support our conclusion that 32 °C is stress-inducing temperature for these fish and beyond the pejus temperature for growth for this species of fish. If pejus temperature had not been reached, such was the case for the fish acclimated to 29 °C, then activity rates, operculum movements, and resilience to stress would not have been noticeably different from those

of fish acclimated to 26 °C (Ananthakrishnan & Kutty, 1974). Fish acclimated to 32 °C were less tolerant to handling stress and 80% of mortality during the experiment was from fish in this temperature treatment.

FOOD QUALITY EFFECTS ON METABOLIC RATE

Although we expected that fish fed a high quality diet would have higher basal metabolic rates, there were no significant differences in metabolic rate between food quality treatments (Fig. 1.6 & 1.7). Cichlid fish studies found that feeding fish a diluted fish meal diet lowered routine metabolic rates (Stadtlander et al., 2012). However, when mass is eliminated as a factor in our data, food quality has no effect on the metabolic rate of the fish (Fig. 1.7). The two food qualities used in this experiment consisted of high levels of protein in order to help offset the effect of the high temperatures used. Even the low quality food was only an 80% dilution of commercial fish food. It is possible that our food qualities were too similar to yield significant results for metabolic rate (Jauncey, 1982).

Animals experience an increased demand of oxygen after feeding associated with digestion called specific dynamic action (SDA) (Lefevre et al., 2012). The time at which the SDA oxygen demand peaks is highly variable between species and is not known for *T. duboisi*. Although we did not know when the SDA peak occurred after feeding, as it was outside of the scope of this research project, each fish was consistently measured for full gut metabolic rate one hour after feeding during our experiment. Interactions between mass and temperature as well as between mass and food quality were marginally significant with p-values just above a significant reading ($p=0.086$ & $p=0.09$ respectively). Re-running the ANOVA excluding mass as a factor, the interaction

between temperature and food quality was again just above a p-value considered to be significant ($p=0.09$).

Though not significant, our results show that for fish acclimated to a low quality diet, there is a trend towards increasing temperature treatment being positively correlated with digestive cost (Fig. 1.8). This could be due to the higher digestibility of the high quality food as compared to the low quality food. The fish that were acclimated to a high quality diet required more energy to digest their food at the lowest temperature treatment and less energy to digest their food at the two highest temperature treatments. Another possible explanation is temperature-dependent differences in assimilation of nutrients from the food (Lichtenbelt, 1992).

FOOD QUALITY AND TEMPERATURE INTERACTIONS

An increase in respiration through a higher metabolic rate at higher temperatures translates to a decrease in relative growth rate (Wang et al., 2013). A 10 °C increase in temperature nearly doubles the rate of biological processes in poikilothermic animals such as fish (Vosloo et al., 2013). Respiration is a fundamental biological process for life, and as temperature increases and metabolism increases, the demand for oxygen increases as well. As temperatures increase and an animal moves towards its upper thermal tolerance range, it begins to allocate more energy to maintain basic physiological processes and less energy to growth and reproduction. Our experiment shows that the allocation of energy has shifted away from growth at higher temperatures and lower food qualities because fish acclimated to a lower quality diet also experienced a lower RGR (Fig. 1.5), while metabolic rates were not affected by temperature or food quality (Fig. 1.7).

CONCLUSIONS

The temperature optimum for growth is lower than the pejus temperature for respiration. This means that the effects of climate change on fish growth will be apparent long before the effects can be seen in measurements of metabolic rate. Growth and reproduction increase fitness of an animal, but do not ensure that the animal's principal requirements for survival are met. Energy allocated to growth is of a lower priority and limited by a lower temperature than energy allocated to metabolic demands. The same can be said for reproduction. It is likely that activities such as growth and reproduction have a lower pejus temperature than metabolic rate and therefore, the effects of increasing temperature and decreasing food quality will be observed in these activities long before they are present in metabolism.

The indirect effect of climate change on food quality in Lake Tanganyika is not very well known. The rate of decline in food quality expected from increasing temperature has yet to be established. Based on our results, a slight decrease in food quality can cause significant decreases in the growth of algivorous fishes. It is clear that more research must be done describing the effects of climate change on lake algae. Once the effects of temperature change on C:N:P ratios and fatty acid contents of primary producers are known, a greater understanding of the effects on the food web and ecosystems will be possible.

V. Introduction

Standard metabolic rates of all organisms scale with mass to approximately the ¾ power (Anderson-Teixeira et al., 2009; Batterham et al., 1997; Franklin et al., 1995). Phylogenetic variation exists in the relationships between mass and metabolism. One source of phylogenetic variation in metabolic rate arises from differences in temperature regulation strategies. Endothermic organisms that maintain a specific body temperature have a higher metabolic rate than ectothermic organisms that have body temperatures regulated by their environmental conditions. Poikilothermic organisms such as fish can be categorized as eurythermal or stenothermal, depending on their tolerance of temperature variation. Eurythermal organisms are capable of surviving in an environment that has a wide variation in temperature range, whereas stenothermal organisms are confined to survival in a narrow temperature range (Wysocki, 2009).

Diet contributes to variation in metabolic rate because it influences digestive physiology (Yang & Joern, 1994). Gut length, gut microbial composition, and organism size differ by trophic level (Riede et al., 2011). Herbivores tend to have longer guts than organisms belonging to other trophic levels (Wagner et al., 2009). This feature aids in the digestion of such compounds as cellulose. The extended gut increases the transit time of the food allowing for a more complete assimilation of available nutrients from plant matter. Herbivores have a variety of adaptations to increase absorption of nutrients from their food, such as having a ruminant gut and microbial symbionts that are capable of breaking down cellulose and other potentially harmful plant secondary compounds (Pope

et al., 2010; VanSoest, 1996). Carnivores have shorter guts because their main source of energy comes from breaking down proteins and other nutrients that are easier to assimilate (Munoz-Garcia & Williams, 2005).

The food habit hypothesis predicts that diet will explain some of the variation in the relationship between mass and basal metabolic rates. Specifically, it predicts that animals with diets with low energy content or low digestibility will have relatively low metabolic rates. This highly debated hypothesis states that quality, availability, and predictability of diet create predictable variation in the mass-independent basal metabolic rates of organisms. The theory posits that organisms consuming a low quality food will develop lower basal metabolic rates over time (Bozinovic & Sabat, 2010). The thought behind the food habit hypothesis is the energy going into the organism is the limiting factor for metabolic rate and therefore organisms that consume lower energy foods will adapt to their diets by lowering their metabolic energy demand (Bozinovic & Sabat, 2010). This phenotypic plasticity, and the mechanisms that potentially drive an organism to lower its energy demand in response to decreased quality or availability of food, are not fully understood (Cruz-Neto & Bozinovic, 2004). The effect of low food availability or high unpredictability will also result in a low mass-independent SMR (Sabat et al., 2009; Bozinovic et al., 2007). The food habit hypothesis offers an explanation for differences in metabolic rates between organisms of different trophic levels that stems from variation in diet.

The food habit hypothesis is most frequently supported by interspecific comparisons (Bozinovic & Sabat, 2010). Among species within Carnivora, mass-specific basal metabolic rate was positively correlated with increasing percentage of meat in the

animal's diet, while carnivores with higher plant content were found to have lower metabolic rates (Munoz-Garcia & Williams, 2005). In contrast, in a study that partially replaced fish meal with red alga Nori, Nile tilapia that were fed lower percentages of fish meal were found to have lower mass-specific routine metabolic rates of those fed higher percentages (Stadtlander et al., 2012). Therefore, the results of an experiment that used individual organisms from one omnivorous species of fish did not support the food habit hypothesis. In rufous-collared sparrows, birds from lower trophic positions that fed on seeds and fruit had higher basal metabolic rates than birds at higher trophic positions that fed on insects (Sabat et al., 2009). Experimental evidence has both supported and refuted the food hypothesis and more experiments are needed to evaluate the validity of this hypothesis.

Diet also influences activity levels and periodicity of activities. The low-quality diet of herbivores requires that they feed frequently to obtain sufficient nutrients and energy. Herbivores continuously forage and are therefore physically active the majority of the day and they maintain a full gut throughout the day (VanSoest, 1996). Carnivores tend to forage sporadically (Armstrong & Schindler, 2011). They will eat and then rest until digestion has been accomplished to a certain point, and then feed again. The high-protein diet of carnivores enables them to consume food at a less frequent rate than does the high-cellulose diet of herbivores (Armstrong & Schindler, 2011). The difference in digestibility of the food source determines the frequency of feeding and varies by trophic level (Cruz-Neto & Bozinovic, 2004). Therefore, the level of activity of herbivores is typically higher than that of carnivores. Cichlid fish are a useful model organism to explore the food habit hypothesis because they have diverse, but highly specialized,

feeding strategies and are closely related, which may minimize the influence of phylogeny. The metabolic rates of cichlids may be influenced by differences in diet and behavior.

We explored the food habit hypothesis by comparing field metabolic rates of seven species of cichlids endemic to Lake Tanganyika. *Tropheus* and *Eretmodus* are algivorous cichlids that live in shallow rocky habitats and sometimes mildly silted regions. The limited surfaces available for algal growth promotes territoriality in these algivorous fish. Aggressive behavior further increases the activity level of these fish. Both genera brood their young in the mouth (Konings, 1998). Fish in the genera *Neolamprologus* and *Altolamprologus* prey on invertebrates such as insect larvae and small crustaceans. They inhabit shallow to deeper rocky regions, and substrate spawn (Konings, 1998). Fish in the genus *Lamprologus* are piscivorous ambush predators. They live, hunt, and breed among shallow rocky beds and tend to be solitary. Fish in the genus *Lepidiolamprologus* are carnivorous predators as well. They are highly aggressive and live in shallow rocky habitat (Konings, 1998). *Perissodus* is a genus of specialized feeders in which adults primarily consume the scales of other fish (Nshombo et al., 1984). They stalk and ambush their prey and can be found floating near the surface of the water or hidden between rocks near the substrate. *Perissodus* can exhibit schooling or solitary behavior (Nshombo et al., 1984).

The purpose of the study was to explore whether variation in diet invokes changes of metabolic rate in cichlid fish. We hypothesized that algivore field metabolic rates would be lower than those of piscivores due to the differences in protein content of food.

VI. Methods

We measured the field metabolic rate of 7 different species of fish from Lake Tanganyika, Tanzania, East Africa, using closed system respirometry. The fish sampled represented 3 different trophic levels: herbivore, invertivore, and piscivore (Table 2.1). We collected fish from 9 sites along the eastern shore of Lake Tanganyika in the Kigoma region of Tanzania (Fig. 2.1). The experiments were run between 2 August and 19 August 2012, and between 0930 and 1800 hours. We usually sampled one site per day.

Incubation chambers were constructed with clear and opaque PVC pipe (total length – 30.5 cm, 6.5 cm outer diameter). A transparent end section (5.5 cm long) housed the fish. A cap at one end of the chamber allowed us to introduce and remove fish from the chamber. The removable end piece screwed into the chamber end that was farthest from the port where dissolved oxygen (DO) was sampled (Fig. 2.2). A YSI ProODO optical dissolved oxygen probe was inserted into the camber through a rubber port and measured DO at 10 second intervals throughout the experiment. Changes in oxygen were measured by the ProODO meter with accuracy up to ±0.1 mg/L or ±1%. We used two chambers with volumes 0.516 L and 0.524 L, and during each experiment, the same chamber that housed the fish also functioned as the blank for that fish.

Fish were caught by a snorkeler using a monofilament barrier net with 2 cm stretch mesh. Each fish was removed from the net immediately upon capture and placed either directly into a chamber or into a submerged mesh bag until a chamber was available. After the fish was placed in the chamber, a DO meter was inserted into the

sampling port. One end of the chamber was capped with mesh (0.6 cm) for 10 minutes to acclimate the fish to the chamber. During the acclimation period, water from the lake circulated into the chamber freely (Fig. 2.3A). Following the acclimation period, the chamber was sealed closed with the end cap, and the dissolved oxygen was measured for either 20 minutes, or until the dissolved oxygen in the chamber dropped below 5 mg/L, whichever came first (Fig. 2.3B). We then removed the fish, flushed and refilled the chamber with lake water, closed the chamber and recorded oxygen in only the water for 10 minutes. The purpose of the blank was to detect changes in oxygen in the water when a fish was not present. Oxygen concentrations in the chambers were automatically logged every 10 seconds during the acclimation period, the experimental period, and for the blank.

All measurements were collected with the entire chamber submerged in the lake, suspended off of the side of the boat at a depth of ~ 0.5 m. This maintained a constant chamber temperature over the measurement period, it limited unnatural external visual stimuli that might stress the fish, and it allowed the natural waves to keep the water inside of the chambers mixed to make the oxygen levels homogenous throughout the chamber. Temperature of the water during experiments ranged from 25.9 °C to 28.0 °C and was an average of 26.6 °C. There was no effect of site on water temperature.

After measuring field metabolism, we recorded the mass, total length, standard length, and volume of the fish. Photographs of the whole fish and the fish's vent were also taken for documentation. All seven fish species were sampled at six of the nine sites. At the remaining three sites, six of the seven fish species were sampled. A total of twelve

individuals from each species were sacrificed to determine the fish's sex, gut fullness, and gut length.

We removed the gastrointestinal system of 12 individuals from each species to assess gut fullness. Guts were separated into parts, consisting of foregut, midgut, and hindgut, then placed into pre-weighed weigh boats and set in a drying oven for at least 48 hours. The dry mass of each gut section was recorded. Total gut mass is the sum of all parts of the gut and gut contents that were extracted, dried, and weighed. We converted total wet weight of the fish (including gut mass) to dry weight by multiplying wet weight by 0.20. We examined the gut contents and assigned ranks, on a scale of 1-10, based on the proportion of the gut that was full, 10 being 100% full.

The activity level of each species was independently assigned by Dr. Peter B. McIntyre (University of Wisconsin, Madison) on a scale of 1-7 with a lower number indicating a lower activity level. Dr. McIntyre based assessment on 10 seasons of fish observational data in Lake Tanganyika.

Methods for Analysis:

I categorized the dissolved oxygen time series for each fish into three experimental regions: the acclimation period, the experimental closed respiration period, and the blank period. I regressed oxygen concentration in the chamber on time (seconds) for each experimental period using the statistical package R. The slope of the experimental closed respiration period represented the rate of oxygen consumption by the fish. The slope was multiplied by the volume of the chamber (minus the volume of the fish) in order to determine the $\dot{V}_{O_2}$ (mg of O_2/ hour) for each fish. The slope of the blank period was used to analyze microbial respiration taking place inside of the respirometer

when the fish was not present. The slope of the blank was subtracted from the experimental closed respirometer period slope. These three treatments were all analyzed for change in DO content over time. The $\dot{V}_{O_2}$ and mass of the fish were $\log_{10}$ transformed. R Statistical Programming © was used to create regressions and residuals from linear models of these data. I analyzed the residuals of those plots to determine if there was a biased distribution of mass-independent metabolic rates between the trophic levels in accordance with what was expected based on the ¾ allometric scaling relationship.

Table 2.1 Trophic level of all fish species used in the experiment, and the number of sites from which data for that species were collected. For each genus, only one fish species was sampled. Therefore, each species is referred to by genus throughout the paper. **Perissodus microlepis* is a specialized piscivore and only consumes the scales of other fish.

Fish Species	Trophic Level	# of sites
Tropheus brichardi	herbivore	9
Eretmodus cyanostictus	herbivore	9
Neolamprologus brichardi	invertivore	9
Altolamprologus compressiceps	invertivore	8
Lepidiolamprologus elongates	piscivore	9
Lamprologus lemairii	piscivore	9
Perissodus microlepis	scale eater*	7

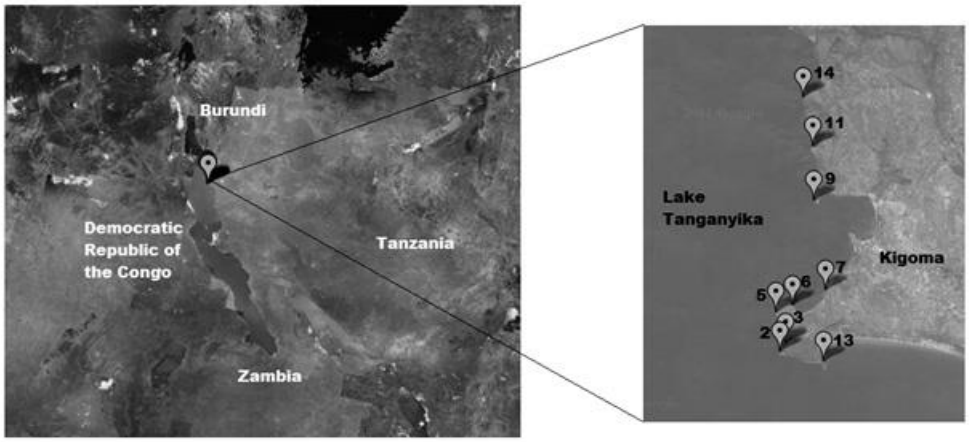


Figure 2.1 Satellite view of Lake Tanganyika (left). Inset shows sample sites north and south of Kigoma Bay, Kigoma Region, Tanzania.

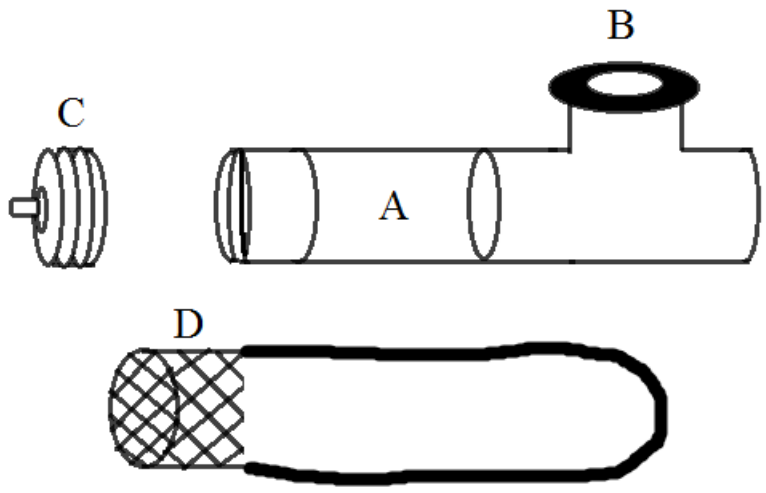
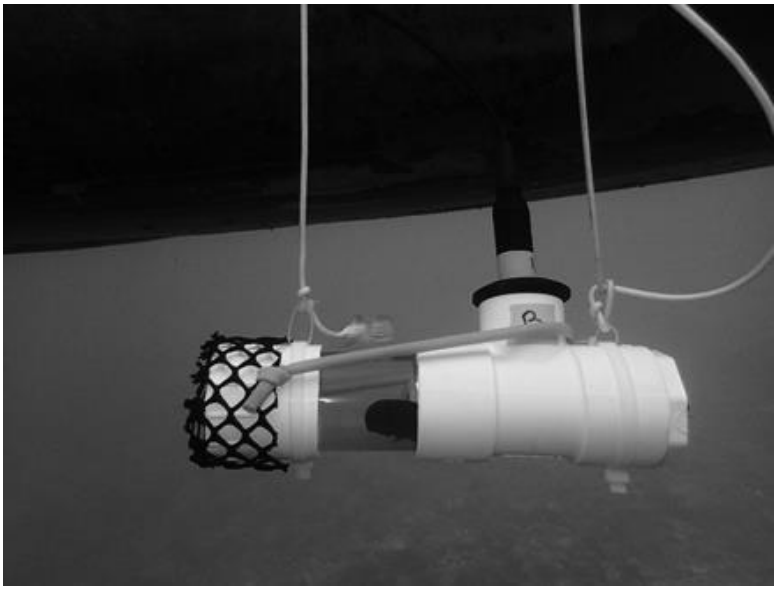


Figure 2.2 The field metabolic chamber consisted of A) a clear portion in which the fish was housed during the length of the experiment, B) the DO port that allowed for the ProODO probe to be inserted into the chamber during the experiment, C) the removable end cap that allowed for placement of the fish and the closure of the system, and D) the mesh cover placed over the open end of the chamber to allow for water exchange with the external environment during acclimation.

A.



B.

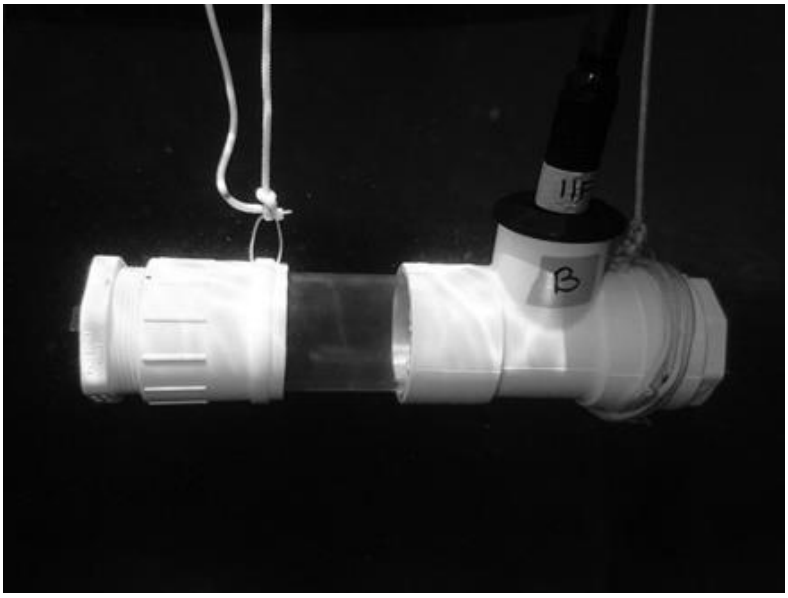


Figure 2.3 A) The open chamber deployed over the side of a boat with a mesh cap on the end to allow exchange of water between the lake and the chamber during the acclimation period. B) The closed chamber deployed in the field with end cap installed for metabolism measurements.

VII. Results

Field metabolic rate ($\dot{V}_{O_2}$) for all species varied significantly from the expected $\dot{V}_{O_2}$–mass relationship for standard metabolic rate predicted by Kleibler's law (Table 2.2, Fig. 2.6). *Tropheus brichardi* had the highest mass-specific $\dot{V}_{O_2}$. For each species in a trophic level, mass-specific metabolic rate increased with increasing activity level (Fig. 2.7).

Tropheus and *Eretmodus* guts consistently had the highest fullness rating among all taxa. Contents consisted primarily of cyanobacteria and diatoms. *Perissodus*, the scale eater, ranked third in gut fullness and no individuals with empty guts were observed. Gut contents of *Perissodus* consisted of nested, stacked fish scales. The two piscivores had the lowest rankings for fullness. When full, piscivore guts consisted of smaller fish and crab exoskeletons. The level of gut fullness for the invertivores was intermediate between *Perissodus* and the other two piscivores. Invertivore guts contained insect larvae such as chironomids. Gut fullness is important to consider because it signifies if energy is being used towards digestion or not. Our algivore species consistently had full guts and were constantly using energy for digestion. In order to correct for digestive cost, we used metabolic data collected on *Tropheus* that were field caught and kept in captivity overnight to empty their guts. A proportion of how much energy was used for digestion was calculated. Correcting for digestive cost lowered the metabolic rate of *Tropheus* from 0.50 mg O_2/h/g to 0.216 mg O_2/h/g (Fig. 2.6).

The regression of $\log_{10}$ mass on $\log_{10}$ metabolic rate had slopes greater than the expected ¾ mass relationship for all trophic levels (Fig. 2.4). The regressions were significantly affected by mass, genus, and trophic level (Tables 2.3 & 2.4). Interactions between mass and trophic level were marginally significant. The trophic levels with the highest slopes were the algivores and scale eaters (Fig. 2.4). These were also the two trophic levels that had the highest activity level and relative fullness (Fig. 2.7 & 2.8). *Tropheus*, *Eretmodus*, and *Perissodus* also had the top 3 gut mass to total fish mass ratios (Fig. 2.9 & 2.12).

Both gut fullness (Fig. 2.8) and gut mass: fish mass (Fig. 2.9) scaled positively with activity level. The two herbivores and the scale-eating piscivore had the highest gut: body mass ratios (Fig. 2.9). *Eretmodus* and *Tropheus* had average gut mass: body mass ratios almost twice as great as *Perissodus.* No individual in these taxa had empty guts whereas most of the piscivores had little to no material in their guts. *Lamprologus* had the largest total mass range (6.83 g-45.52 g) of the seven genera analyzed and 66% of *Lamprologus* had a relative gut fullness < 4 (Fig. 2.10). Within piscivores, fish with full guts had higher mass-specific metabolic rates than those with empty guts (Fig. 2.11). Log_{10} dry weights of guts scaled positively with $\log_{10}$ dry weights of the fish. The slope of the line for algivores was the highest (0.999x) of all 3 trophic levels (Fig. 2.12).

Table 2.2 Analysis of variance of the residuals from the $\log_{10} \dot{V}_{O_2}$ and mass fit to the predicted 3/4 slope line. Species was found to have a significant effect on the residuals.

ANOVA:Residuals	Df	Sum Sq	Mean Sq	F value	Pr(>F)
species	1	1.781	1.7809	12.32	0.000665 ***
Residuals	103	14.882	0.1445		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 2.3 Analysis of covariance of $\log_{10}$ mass and $\log_{10}$ metabolic rate of all fish. Mass and trophic level both had significant effects on the regression slopes. Interactions between mass and trophic level were marginally significant.

ANCOVA	Df	Sum Sq	Mean Sq	F value	Pr(>F)
mass	1	7.601	7.601	352.271	<2e-16***
Trophic level	3	0.221	0.074	3.413	0.0205*
mass:trophic level	3	0.141	0.047	2.18	0.0953.
Residuals	97	2.093	0.022		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 2.4 Analysis of covariance of the $\log_{10}$ mass and $\log_{10}$ metabolic rate of all fish. Mass and genus both had significant effects on the regression slopes. The interactions between mass and genus were not significant.

ANCOVA	Df	Sum Sq	Mean Sq	F value	Pr(>F)
mass	1	7.601	7.601	370.28	< 2e-16***
genus	6	0.491	0.082	3.985	0.00138**
mass:genus	6	0.096	0.016	0.781	0.58687
Residuals	91	1.868	0.021		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

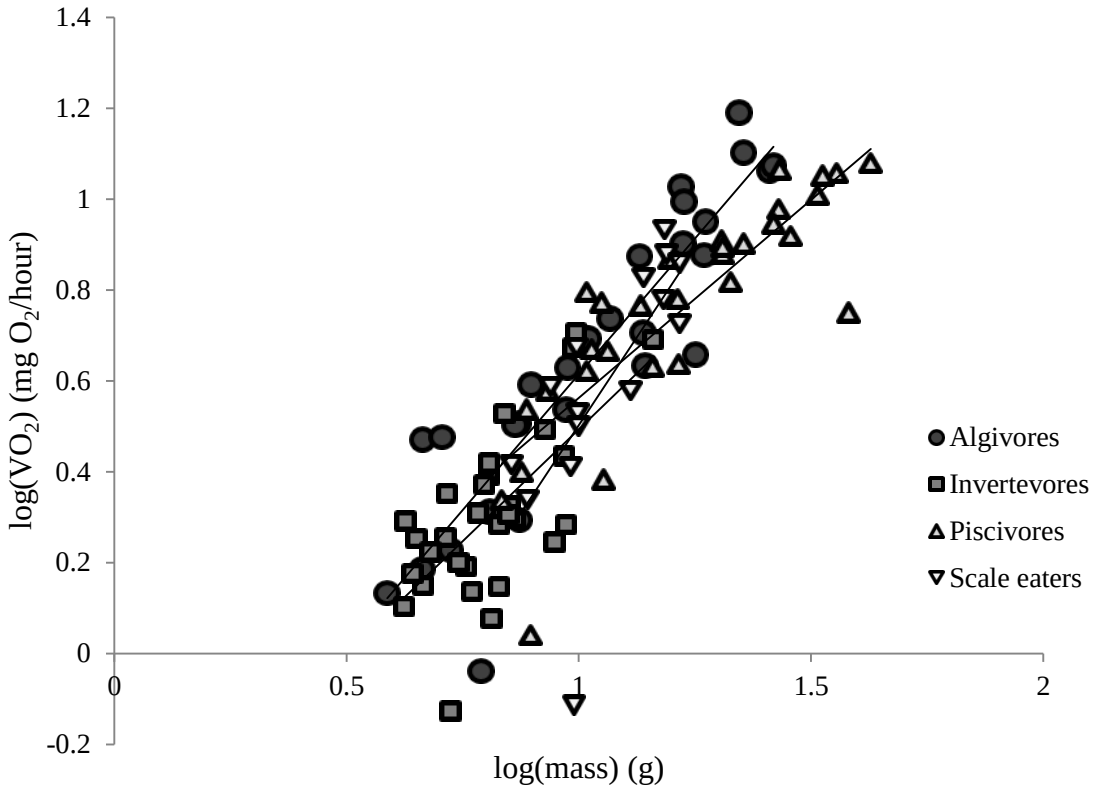


Figure 2.4 The effect of $\log_{10}$ mass on $\log_{10}$ metabolic rate varied by trophic level. All trophic level regressions had a slope greater than the expected ¾ mass relationship. Algivores and scale eaters both had slopes greater than 1. Equations and R^2 values for algivores, invertivores, piscivores, and scale eaters respectively. y=1.1951x – 0.5807, R^2=0.8227; y=0.9854x – 0.4919, R^2=0.4667; y=0.8721x – 0.3099, R^2=0.6993; y=1.5446x – 1.0421, R^2=0.5002.

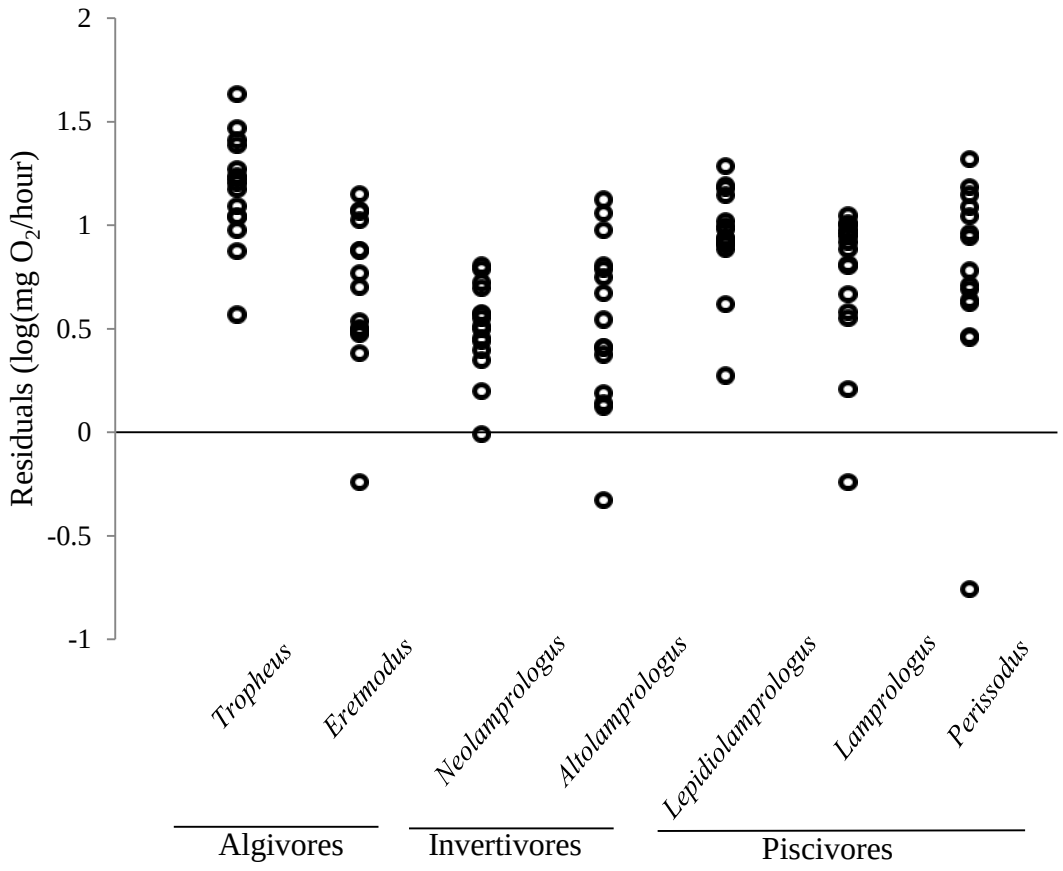


Figure 2.5 The residuals plot made from the $\log_{10} \dot{V}_{O_2}$ and mass fit to a predicted 3/4 slope line and separated into species shows that most organisms were above the prediction line. Taking gut content into account reveals that most individuals with residual values at or below 0 had empty guts.

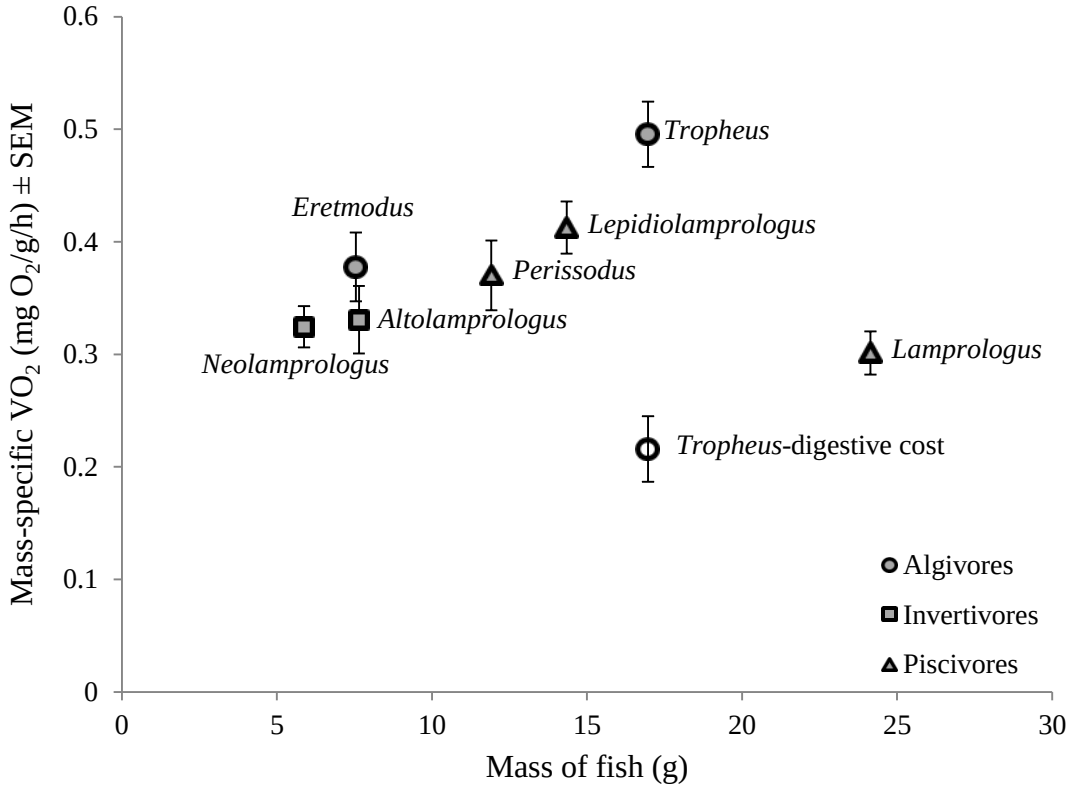


Figure 2.6 Average mass-specific metabolic rate of each species graphed in accordance with mass shows that metabolic rate was generally positively correlated with mass. *Tropheus* is plotted twice because it was adjusted for digestive cost.

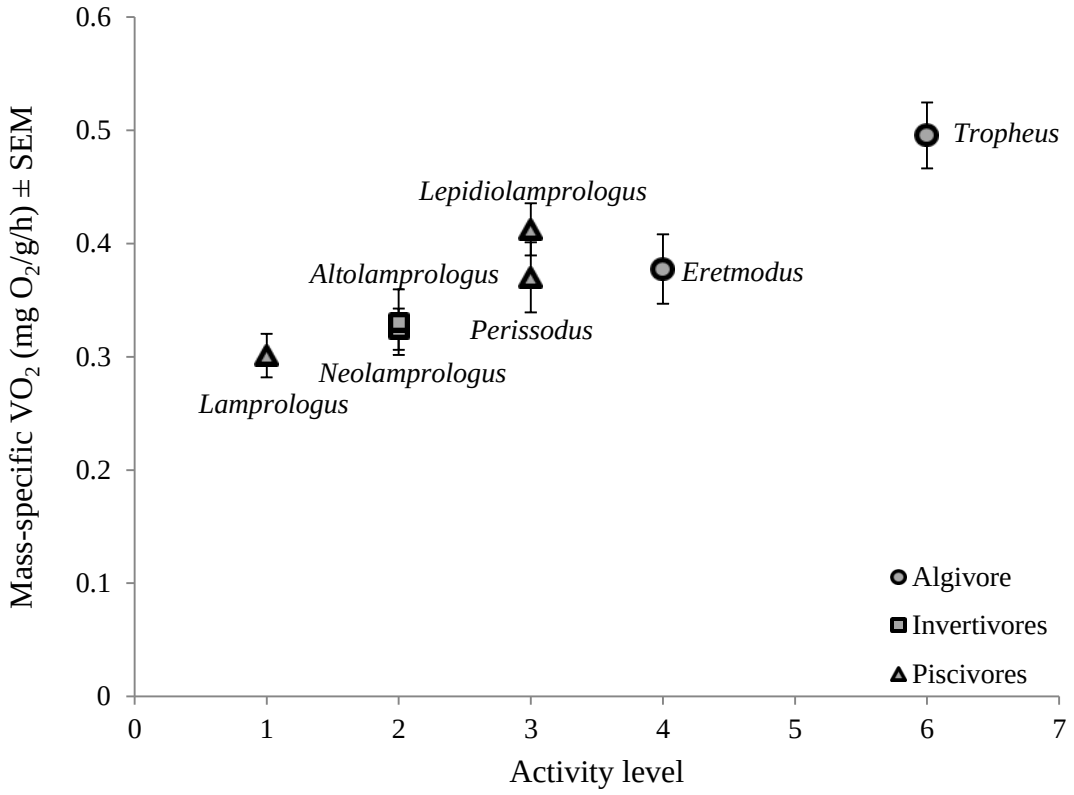


Figure 2.7 Mass-specific metabolism was positively correlated with assigned activity level for all seven species of fish. Activity levels increase from 1 to 7.

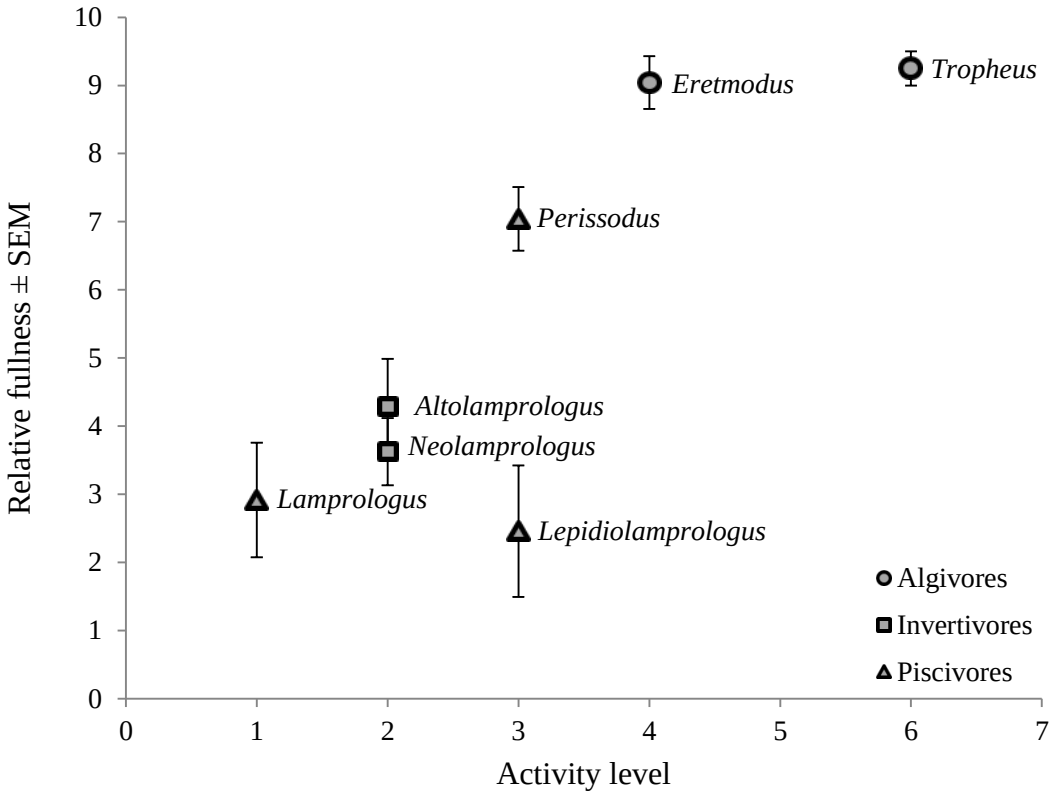


Figure 2.8 Average relative fullness based on stomach content and ranked on a scale of 1-10 and listed by genus (n=12 per species). The piscivores had the highest variability within each species and the herbivores varied the least.

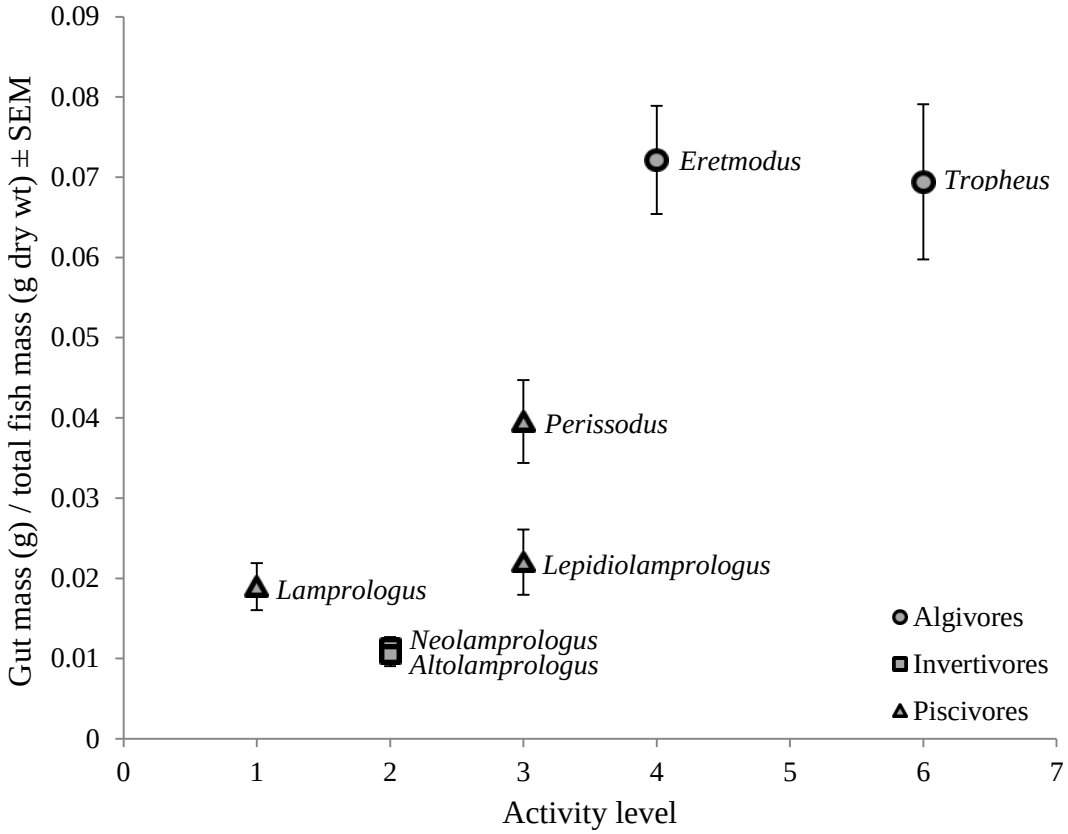


Figure 2.9 Gut mass: total fish mass ratio for each species listed by genus. Gut mass included gut content and gut. The greatest values and variability was seen in the two algivore species.

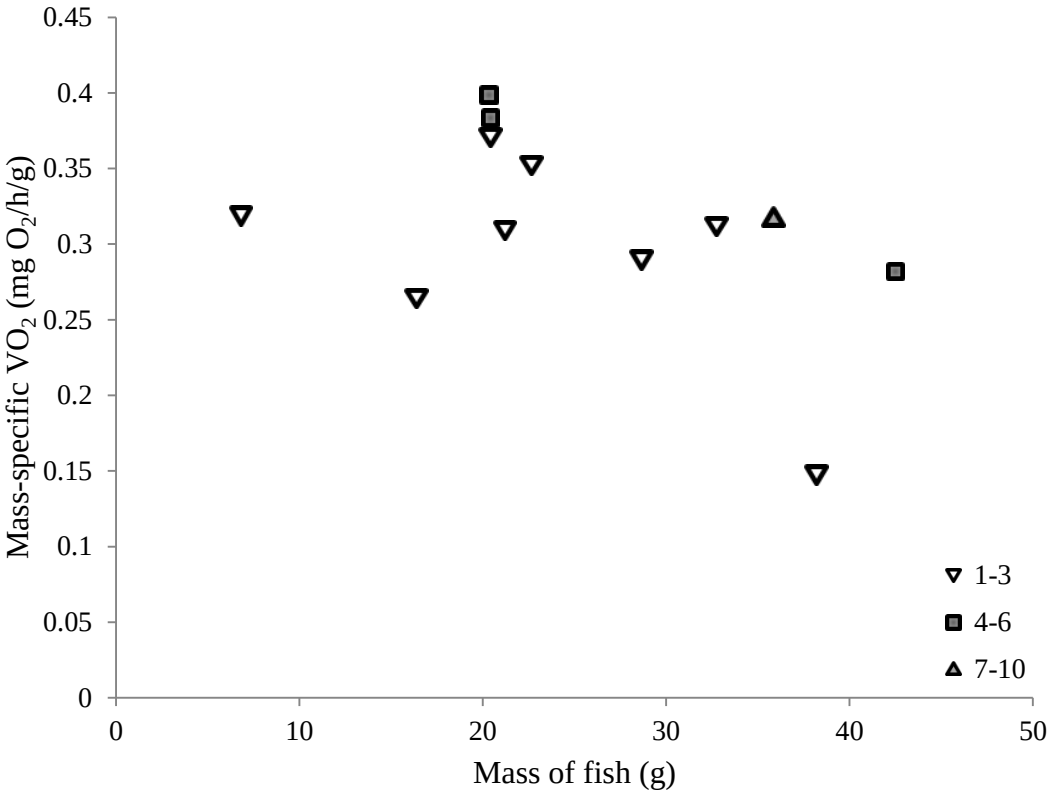


Figure 2.10 The mass-specific metabolic rate of *Lamprologus lemairii* scales negatively with mass. Assigned relative gut fullness was determined by observation (n=12). The majority of the individual fish did not have a gut fullness ranking greater than 3 on a scale of 1-10.

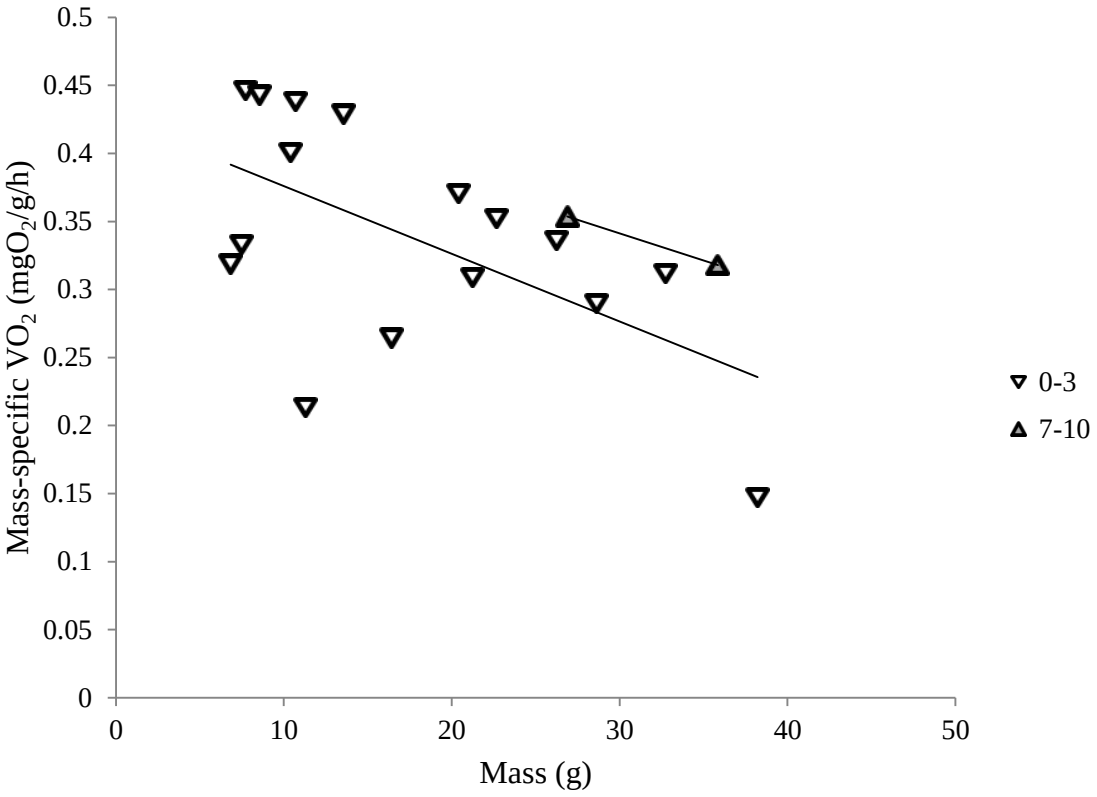


Figure 2.11 Mass-specific metabolic rates of fish with full guts and empty guts from the two piscivore genera *Lamprologus* and *Lepidiolamprologus* as they scale with mass. One individual from each genus was found to have a gut fullness of 7 or greater. The mass-specific metabolic rate for those fish was higher than that of those with gut contents of 3 or less. Equations and R^2 values for 0-3 and 7-10 fullness respectively: y=-0.005x + 0.4257, R^2=0.3329; y=-0.004x + 0.4604.

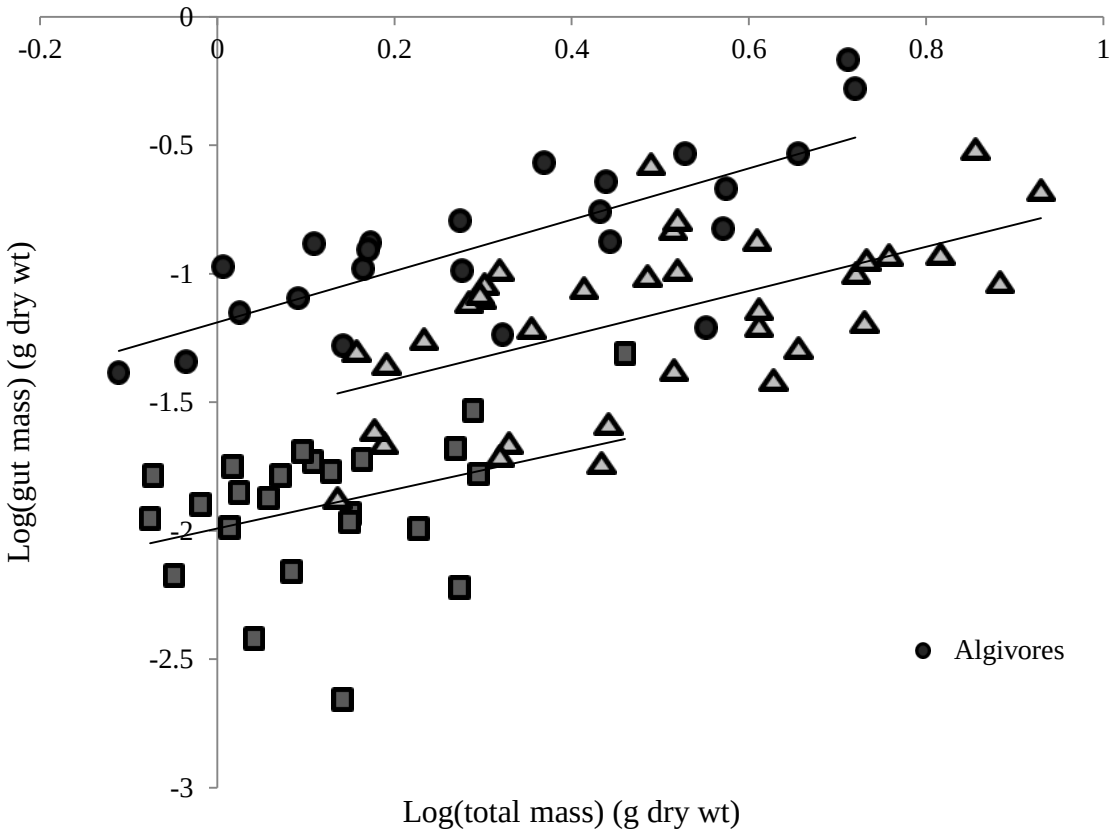


Figure 2.12 Log_{10} dry weights of guts scale positively with log_{10} dry weights of the fish.Algivores have the highest slope and y-intercept of all 3 trophic levels. Equations and R^2 values for algivores, invertivores, and piscivores respectively: y=0.999x + 1.8102, R^2=0.5915; y=0.7589x + 1.0083, R^2=0.1274; y=0.8592x + 1.4171, R^2=0.3378.

VIII. Discussion

Standard metabolism scales predictably with mass and temperature for organisms of the same species. Metabolic ecology provides the basis for our predictions about how metabolism of an organism will scale with mass. Other factors such as activity levels, digestion, and reproduction can influence an organisms' field metabolism as well. The mass-specific field metabolic rate of the seven species used in the study did not correlate negatively with mass as expected (Table 2.2, Fig. 2.6) (Anderson-Teixeira et al., 2009). The positive correlation of mass-specific $\dot{V}_{O_2}$ with mass in these cichlids from Lake Tanganyika factors such as digestive costs and activity are also increasing with mass.

Digestive cost is inherently different between organisms that belong to separate trophic levels because food quality and consumption frequency vary among trophic levels. The food that an organism consumes can influence the body in numerous ways that affect digestive cost. Algivorous fish that consume complex carbohydrates have a higher amylase activity per mg pancreas than do carnivorous fish (Kapoor et al., 1975). Algivorous fish also have higher rates of intestinal glucose absorption than carnivorous species (Buddington et al., 1987). Digestive enzymes present in the bodies of fish shift with dietary changes. Algivorous fish do not use the same digestive enzymes that carnivorous fish do and therefore, an initial difference in digestive cost exists (Louw, 1993). The cost of digestion in algivorous fish can be a high percentage of their total

metabolism. The high temperatures of tropical ecosystems provide an energetic advantage for herbivorous ectotherms as opposed to temperate herbivorous ectotherms, because digestive efficiency increases with temperature. Feeding habit diversity in fish increases as latitude decreases, and algivory in fish is almost exclusively restricted to the tropics (McNab, 2002). This could be in part because herbivorous ectothermic animals, such as lizards, increase digestive efficiency when they are kept at higher temperatures (Harlow et al., 1976). Regardless of taxonomic group, herbivores have the lowest digestive efficiencies of all organisms (McNab, 2002).

Two problems that herbivores face with digestion are copious amounts of fiber that decreases digestibility, and toxic secondary compounds (McNab, 2002). One consequence of these difficulties is the need to maintain a full gut in order to be able to constantly process food and maximize efficiency of digestion. We quantified the gut fullness of the fish used in the field metabolism observation of this study. Dissection of the algivorous fish revealed that their gut fullness was almost always at or above 9 on the scale (1-10) (Fig. 2.8). As a consequence of maintaining full guts, these algivorous fish rarely leave a state of specific dynamic action (SDA), an increase in oxygen demands due to the digestion of food (Lefevre et al., 2012). This may result in higher field metabolic rates for algivores (Fig. 2.8). We know that digestion has an energy cost associated with it and accounting for digestive cost decreased the mass-specific $\dot{V}_{O_2}$ of *Tropheus* by about 40% (Fig. 2.6). *Tiliapia rendallia* has been reported as having an increase in energy usage of 0.88 kJ in association with digestive cost per meal with meal size being 6.8% of the fish's body mass (Secor, 2008). Therefore gut fullness of algivorous fish may be a

contributing factor to the observed higher field metabolic rates compared to invertivores and piscivores.

Carnivores have the highest digestive efficiency and nutrient assimilation of all organisms regardless of taxon (McNab, 2002). Although intestinal length increases with the mass with a species, between trophic levels, carnivores possess the shortest guts (Sibly, 1981). Compared to herbivores, carnivores' food sources are much easier to digest. However, carnivores must have enzymes present in their gastrointestinal tract that can digest proteins, which include the most diverse types of chemical bonds (Hill et al., 2008). Due to the increased digestive efficiency of carnivores, they do not maintain full guts. Of the 24 piscivore fish sampled, only 8% of fish were found to have a gut fullness at or above a level 7. Sixty seven percent of piscivores a gut fullness ≤ 3 (Fig. 2.11). The vast majority of piscivorous fish sampled had empty or near-empty guts. Piscivorous fish with gut fullness levels ≥ 7 (n=2), had metabolic rates were higher than those of fish with empty guts of the same mass. The gut fullness of the invertivore fish did not appear to influence metabolic rate, while none of the scale-eaters had empty or near-empty guts. Comparing field metabolic rates of fish from different trophic levels does not control for digestive cost. Essentially, our observations compare the metabolic rates of algivores with full guts to piscivores with empty guts. If we were to remove digestive cost as a factor, we expect that the metabolic rate of individual algivorous organisms would decrease and that metabolic rate would scale with mass in closer accordance to the 3/4 power rule (Fig. 2.4).

Activity levels, and therefore behavior, have a substantial influence over field metabolic rate. Observations of lacertid lizards that have the same food source but exploit

different foraging methods showed that the widely foraging lizard that actively foraged had significantly higher field metabolic rates than the sit-and-wait lizard (Nagy et al., 1984). The algivore species in our study actively forage throughout the day, while the piscivore species spent more time waiting to ambush their prey (personal observations). These differences in foraging behavior alone, could contribute to the increased field metabolism of the algivore fish species we measured either because the fish algivores had an elevated standard metabolic rate relative to the other trophic levels or because fish with inherently higher activity levels may have been more active in the respirometery chamber than more sedentary species. An effect of lifestyle, including behavior and activity levels, on metabolism has been documented in teleost fish. Killen et al. (2010) found that differences in lifestyle such as the habitat of the fish (pelagic, benthopelagic, benthic, or bathyal) and the activity of the fish affect resting metabolic rates. The differences in habitat and predator-prey interactions affect the types of muscles in the fish, which translates into a difference in metabolism even when the fish is in a resting state. Increased activity rates of fish in nature can result in the trend we saw relating mass-specific metabolic rates and activity level ranking (Fig. 2.7). A naturally more active fish may expend more energy resting or moving around in the respiration chamber than other fish. However, if over generations, increased activity levels have caused

increases in metabolic rates, accounting for digestive cost would not lower the $\dot{V}_{O_2}$ of *Tropheus* to the point that it would fit the expected relationship (Fig. 2.6). It is difficult to separate out these inter-related factors, but it is my conclusion that both gut fullness and activity level, contribute to the higher algivore metabolic rates observed.

Relationships exists between digestive cost, gut fullness, and activity level but independently, they do not have equal influence over field metabolic rate. Gut fullness, digestive cost, and activity level of the fish could explain why algivores had higher field metabolic rates than piscivores and invertivores in our study (Fig. 2.6). A driving force behind the increased activity level of the algivore species we observed could be the low food quality available to them in the lake. The higher activity rankings as well as the fullest guts of the algivorous fish is consistent with the prediction that the fish are compensating for low food quality through increased foraging activity. Other strategies for adapting to eating low quality food include longer guts to extend the amount of time that the food is digested, increased intake and transit time, and symbiotic relationships with microfauna that take up residence in the digestive tracts of some organisms (Wagner et al., 2009; Munoz-Garcia & Williams, 2005, Choat & Clements, 1998). Similarly to algivores, *Perissodus* continually had high levels of gut contents and can be seen as having the third highest ratio of gut mass to fish mass and activity level (Fig. 2.8).

Although field metabolism scaled positively with mass in all seven genera, no genus exhibited an increase in metabolism with mass that corresponded to the ¾ power law (Fig. 2.4). All slopes were >¾ and slopes for algivore and scale eater species were >1. These elevated metabolic rates in larger fish may be due to our inability to remove digestive cost and activity level from field metabolic rate measurements. The taxa with slopes >1 were algivores and scale eaters. These species also had the highest gut fullness and highest activity levels of the observed taxa (Fig. 2.7 & 2.8). Fish with naturally higher activity levels in nature may be prone to more frequent spontaneous activity

during the experiment, leading to increased metabolic rates. In our study, the fish that had the highest activity level rankings also had the highest metabolic rates (Fig. 2.7).

Our results are not consistent with the food habit hypothesis. Fish with less protein in their diet had higher field metabolic rates than those with more protein. This is due to fundamental differences in behavior and physiology of the organisms from different trophic guilds that have underlying influences on their standard metabolic rates. These results could also be representative of the food habit hypothesis as it applies specifically to ectothermic organisms. Most of the support for the food habit hypothesis comes from mammals and endothermic animals (Munoz-Garcia & Williams, 2005; Cruz-Neto & Bozinovic, 2004). Experiments with poikilothermic ectotherms, Nile tilapia, have shown that increasing the amount of protein in the diet of fish leads to a decrease in metabolic rate (Stadtlander et al., 2012). It is possible that the inverse relationship exists for poikilothermic animals as opposed to endotherms, resulting in lower metabolic rates with higher protein intake. It is also possible that our results report a trend that exists only in the field metabolic rate of ectotherms. Standard metabolic rates of lizards have been shown to correlate with food habit after correction for mass, where herbivores have the lowest standard metabolism (Pough, 1983). Trophic level, and therefore food habit, may not be a highly influential factor when determining standard and resting metabolic rate, but it may be highly influential when determining field metabolic rate and activity level.

IX. APPENDIX A

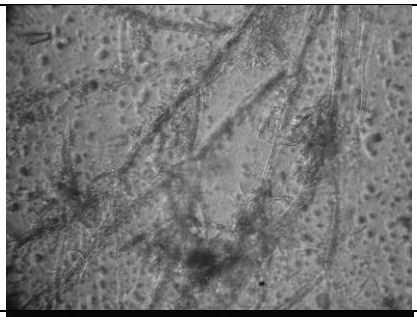
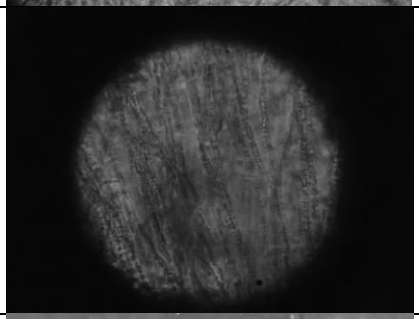
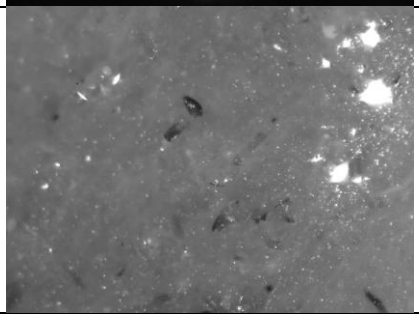
All individual fish used to measure relative growth rates for the lab experiment. Fish were divided by their temperature and food quality assignments for acclimation.

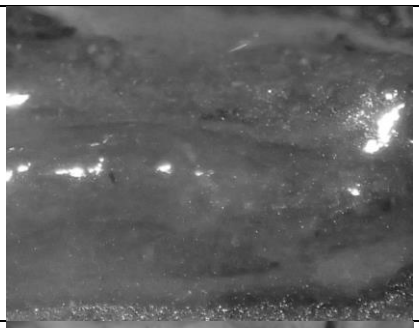
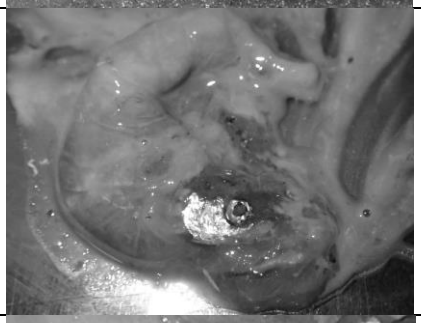
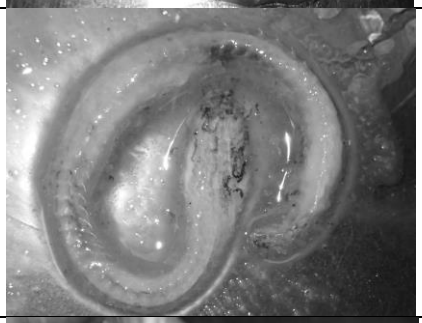
fish ID	tank	food quality	temp °C	fish mass initial	fish mass final	days acclimated at fish mass final
1	3A	Low	26	2.305	2.825	66
3	3A	Low	26	1.493	2.108	66
5	3B	Low	26	3.271	4.055	66
7	3B	Low	26	1.693	1.996	66
9	3D	Low	26	1.352	2.122	66
11	3D	Low	26	2.668	3.319	66
13	3C	Low	26	0.947	1.595	66
15	3C	Low	26	1.951	2.906	66
2	3A	High	26	2.973	4.347	66
4	3A	High	26	1.378	2.911	66
6	3B	High	26	1.125	1.505	66
8	3B	High	26	1.53	2.083	66
10	3D	High	26	3.685	6.684	66
12	3D	High	26	1.29	2.356	66
14	3C	High	26	2.629	5.754	66
16	3C	High	26	1.829	3.482	66
17	1A	Low	32	2.36	2.297	66
19	1A	Low	32	1.785	1.998	66
21	1B	Low	32	3.811	4.03	66
23	1B	Low	32	1.438	1.199	66
25	1D	Low	32	1.635	1.662	66
27	1D	Low	32	3.039	3.124	66
29	1C	Low	32	1.274	1.058	66
31	1C	Low	32	1.924	1.759	56
18	1A	High	32	1.433	1.585	66
20	1A	High	32	0.835	1.07	56
22	1B	High	32	1.575	1.798	49
24	1B	High	32	2.309	2.7	49

26	1D	High	32	3.195	3.755	66
28	1D	High	32	1.531	1.455	66
30	1C	High	32	1.242	1.709	42
32	1C	High	32	2.851	3.541	56
33	2A	Low	29	1.786	2.155	66
35	2A	Low	29	2.131	2.889	66
37	2B	Low	29	0.986	1.249	66
39	2B	Low	29	1.655	2.35	66
41	2D	Low	29	2.533	2.865	66
43	2D	Low	29	1.276	1.69	66
45	2C	Low	29	1.248	1.773	66
47	2C	Low	29	3.899	4.814	66
34	2A	High	29	1.748	2.828	66
36	2A	High	29	2.609	5.175	66
38	2B	High	29	0.486	0.605	35
40	2B	High	29	3.715	5.115	66
42	2D	High	29	1.462	2.448	66
44	2D	High	29	1.333	2.312	66
46	2C	High	29	2.45	3.234	66
48	2C	High	29	1.6	2.994	66

X. APPENDIX B

Pictures of the stomach contents of fish from each trophic level. Individuals were collected from the field and dissected in the lab.

Fish Species	Fish Letter ID	Trophic Level	Stomach contents
Tropheus brichardi	BW	algivore	
Eretmodus cyanostictus	AT	algivore	
Neolamprologus brichardi	BJ	invertivore	

Altolamprologus compressiceps	AC	invertivore		
Lepidiolamprologus elongates	BM	piscivore		
Lamprologus lemairii	CC	piscivore		
Perissodus microlepis	BB	piscivore (scale eater)		

XI. REFERENCES

Ananthakrishnan KR and Kutty MN. 1974. Mortality and breathing rate at high ambient-temperatures in cichlid fish, tilapia-mossambica peters. Indian J Exp Biol 12(1):55-9.

Anderson-Teixeira K, Savage V, Allen A, Gillooly J. 2009. Allometry and metabolic scaling in ecology. Encyclopedia of Life Sciences 1:10.

Angilletta M, Steury T, Sears M. 2004. Temperature, growth rate, and body size in ectotherms: Fitting pieces of a life-history puzzle. Integr Comp Biol 44(6):498-509.

Armstrong, J.B, and Schindler D.E. 2011. Excess digestive capacity in predators reflects a life of feast and famine. Nature 476:84-87.

Austreng E, Storebakken T, Asgard T. 1987. Growth-rate estimates for cultured Atlantic salmon and rainbow-trout. Aquaculture 60(2):157-60.

Batterham A, Tolfrey K, George K. 1997. Nevill's explanation of kleiber's 0.75 mass exponent: An artifact of collinearity problems in least squares models? J Appl Physiol 82(2):693-7.

Bozinovic F and Sabat P. 2010. On the intraspecific variability in basal metabolism and the food habits hypothesis in birds. Curr Zool 56(6):759-66.

Bozinovic F, Munoz JL, Cruz-Neto AP. 2007. Intraspecific variability in the basal metabolic rate: testing the food habits hypothesis. Physiol Biochem Zool 80(4):452-460.

Buddington R, Chen J, Diamond J. 1987. Genetic and phenotypic adaptation of intestinal nutrient transport to diet in fish. Journal of Physiology 393:261-281.

Bukovinszky T, Verschoor AM, Helmsing NR, Bezemer TM, Bakker ES, Vos M, Domis LNdS. 2012. The good, the bad and the plenty: Interactive effects of food quality and quantity on the growth of different daphnia species. PLoS One 7(9):e42966.

Bullock TH. 1955. Compensation for temperature in the metabolism and activity of poikilotherms. Biol Rev Camb Philos Soc 30(3):311-42.

Choat J and Clements K. 1998. Vertebrate herbivores in marine and terrestrial environments: A nutritional ecology perspective. Annu Rev Ecol Syst 29:375-403.

Corman JR, McIntyre PB, Kuboja B, Mbemba W, Fink D, Wheeler CW, Gans C, Michel E, Flecker AS. 2010. Upwelling couples chemical and biological dynamics across the littoral and pelagic zones of Lake Tanganyika, East Africa. Limnol Oceanogr 55(1):214-24.

Cruz-Neto A and Bozinovic F. 2004. The relationship between diet quality and basal metabolic rate in endotherms: Insights from intraspecific analysis. Physiol Biochem Zool 77(6):877-89.

Cruz-Rivera E and Hay M. 2000. Can quantity replace quality? Food choice, compensatory feeding, and fitness of marine mesograzers. Ecology 81(1):201-19.

Fonds M, Cronie R, Vethaak A, Vanderpuyl P. 1992. Metabolism, food-consumption and growth of plaice (pleuronectes-platessa) and flounder (platichthys-flesus) in relation to fish size and temperature. Neth J Sea Res 29(1-3):127-43.

Franklin C, Johnston I, Crockford T, Kamunde C. 1995. Scaling of oxygen-consumption of Lake Magadi tilapia, a fish living at 37-degrees-C. J Fish Biol 46(5):829-34.

Harlow HJ, Hillman SS, Hoffman M. 1976. The effect of temperature on digestive efficiency in the herbivorous lizard, *Dipsosaurus dorsalis.* Journal of Comparitive Physiology 111(1):1-6.

Healy TM and Schulte PM. 2012. Thermal acclimation is not necessary to maintain a wide thermal breadth of aerobic scope in the common killifish (fundulus heteroclitus). Physiol Biochem Zool 85(2):107-19.

Hill R, Wyse G, Anderson M. Nutrition, feeding, and digestion. In Animal physiology. Sinauer Associates Inc. 2008.

Hop H and Graham M. 1995. Respiration of juvenile arctic cod (boreogadus-saida) - effects of acclimation, temperature, and food-intake. Polar Biol 15(5):359-67.

Houde E. 1989. Comparative growth, mortality, and energetics of marine fish larvae - temperature and implied latitudinal effects. Fish Bull 87(3):471-95.

Jauncey K. 1982. The effects of varying dietary-protein level on the growth, food conversion, protein-utilization and body-composition of juvenile tilapias (sarotherodon-mossambicus). Aquaculture 27(1):43-54.

Kapoor B, Smit H, Verighina I. 1975. The alimentary canal and digestion in teleosts. Advances in marine biology 13:109-239.

Kilham S, Kreeger D, Goulden C, Lynn S. 1997. Effects of algal food quality on fecundity and population growth rates of daphnia. Freshwat Biol 38(3):639-47.

Killen SS, Atkinson D, Glazier DS. 2010. The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. Ecol Lett 13(2):184-93.

Klok C, Sinclair B, Chown S. 2004. Upper thermal tolerance and oxygen limitation in terrestrial arthropods. J Exp Biol 207(13):2361-70.

Konings, Ad. Tanganyika cichlids in their natural habitat. Hollywood Import & Export Inc. 1998.

Lefevre S, Do Thi Thanh Huong, Nguyen Thanh Phuong, Wang T, Bayley M. 2012. Effects of hypoxia on the partitioning of oxygen uptake and the rise in metabolism during digestion in the air-breathing fish channa striata. Aquaculture 364:137-42.

Lichtenbelt W. 1992. Digestion in an ectothermic herbivore, the green iguana (iguana-iguana) - effect of food composition and body-temperature. Physiol Zool 65(3):649-73.

Louw, G. Physiological animal ecology. Longman Scientific and technical. 1993.

McNab B. The physiological ecoogy of vertebrates: a view from energetics. Cornell University press. 2002.

Miklos P, Katzman S, Cech J. 2003. Effect of temperature on oxygen consumption of the leopard shark, triakis semifasciata. Environ Biol Fishes 66(1):15-8.

Munoz-Garcia A and Williams J. 2005. Basal metabolic rate in carnivores is associated with diet after controlling for phylogeny. Physiol Biochem Zool 78(6):1039-56.

Nagy K, Huey R, Bennet A. 1984. Field energetics and foraging mode of Kalahari lacertid lizards. Ecology 65(2):588-596.

Naithani J, Plisnier P, Deleersnijder E. 2011. Possible effects of global climate change on the ecosystem of Lake Tanganyika. Hydrobiologia 671(1):147-63.

Nshombo M, Yanagisawa Y, Nagoshi M. 1984. Scale-eating in Perissodus microlepis (Chichlidae) and change of its food habits with growth. Japanese Journal of Ichthyology 32(1):60-73.

O'Reilly CM, Alin SR, Plisnier PD, Cohen AS, McKee BA. 2003. Climate change decreases aquatic ecosystem productivity of Lake Tanganyika, Africa. Nature 424(6950):766-8.

Pirozzi I and Booth MA. 2009. The routine metabolic rate of mulloway (argyrosomus japonicus: Sciaenidae) and yellowtail kingfish (seriola lalandi: Carangidae) acclimated to six different temperatures. Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology 152(4):586-92.

Pope PB, Denman SE, Jones M, Tringe SG, Barry K, Malfatti SA, McHardy AC, Cheng J-, Hugenholtz P, McSweeney CS. 2010. Adaptation to herbivory by the tammar wallaby includes bacterial and glycoside hydrolase profiles different from other herbivores. Proc Natl Acad Sci USA 107(33):14793-8.

Portner H. 2002. Climate variations and the physiological basis of temperature dependent biogeography: Systemic to molecular hierarchy of thermal tolerance in animals. Comp Biochem Physiol A-Mol Integr Physiol 132(4):739-61.

Pough F. Amphibians and reptiles as low energy systems. In Behavioral energetics: vertebrates costs of survival. Ohio State University press. 1983.

Riede J, Brose U, Ebenman B, Jacob U, Thompson R, Townsend C, Jonsson T. 2011. Stepping in Elton's footprints: a general scaling model for body masses and trophic levels across ecosystems. Ecology Letters 14(2):169-178.

Sabat P, Cavieres G, Veloso C, Canals M, Bozinovic F. 2009. Intraspecific basal metabolic rate varies with trophic level in rufous-collared sparrows. Comp Biochem Physiol A-Mol Integr Physiol 154(4):502-7.

Schindler D and Eby L. 1997. Stoichiometry of fishes and their prey: Implications for nutrient recycling. Ecology 78(6):1816-31.

Secor S. 2008. Specific dynamic action: a review of the postprandial metabolic response. J Comp Physiol B 179:1-56.

Sibly R. Stragegies of digestion and defecation. In Physiological ecology: an evolutionary approach to resource use. Blackwell, Oxford. 1981.

Sibly R and Atkinson D. 1994. How rearing temperature affects optimal adult size in ectotherms. Funct Ecol 8(4):486-93.

Stadtlander T, Khalil WKB, Focken U, Becker K. 2013. Effects of low and medium levels of red alga nori (porphyra yezoensis ueda) in the diets on growth, feed utilization and metabolism in intensively fed nile tilapia, oreochromis niloticus (L.). Aquacult Nutr 19(1):64-73.

Sterner RW, and Elser JJ. Ecological stoichiometry: The biology of elements from molecules to the biosphere. Princeton University Press. 2002.

Tadesse Z, Boberg M, Sonesten L, Ahlgren G. 2003. Effects of algal diets and temperature on the growth and fatty acid content of the cichlid fish oreochromis niloticus L. - A laboratory study. Aquat Ecol 37(2):169-82.

Turker H. 2011. The effect of water temperature on standard and routine metabolic rate in two different sizes of nile tilapia. Kafkas Universitesi Veteriner Fakultesi Dergisi 17(4):575-80.

VanSoest P. 1996. Allometry and ecology of feeding behavior and digestive capacity in herbivores: A review. Zoo Biol 15(5):455-79.

Verburg P and Hecky RE. 2009. The physics of the warming of lake tanganyika by climate change. Limnol Oceanogr 54(6):2418-30.

Vosloo D, Vosloo A, Morillion EJ, Samuels JN, Sommer P. 2013. Metabolic readjustment in juvenile south african abalone (haliotis midae) acclimated to combinations of temperature and dissolved oxygen levels. J Therm Biol 38(7):458-66.

Wagner CE, McIntyre PB, Buels KS, Gilbert DM, Michel E. 2009. Diet predicts intestine length in Lake Tanganyika's cichlid fishes. Funct Ecol 23(6):1122-31.

Wang Q, Wang W, Huang Q, Zhang Y, Luo Y. 2012. Effect of meal size on the specific dynamic action of the juvenile snakehead (channa argus). Comp Biochem Physiol A-Mol Integr Physiol 161(4):401-5.

Wysocki LE, Montey K, Popper AN. 2009. The influence of ambient temperature and thermal acclimation on hearing in a eurythermal and a stenothermal otophysan fish. J Exp Biol 212(19):3091-9.

Yang Y and Joern A. 1994. Gut size changes in relation to variable food quality and body-size in grasshoppers. Funct Ecol 8(1):36-45.

Zhao Z, Dong S, Wang F, Tian X, Gao Q. 2011. Respiratory response of grass carp (ctenopharyngodon idellus) to temperature changes. Aquaculture 322:128.