Temperature and diet effects on omnivorous fish performance: implications for the latitudinal diversity gradient in herbivorous fishes

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Abstract: Herbivorous fishes show a clear latitudinal diversity gradient, making up a larger proportion of the fish species in a community in tropical waters than in temperate waters. One proposed mechanism that could drive this gradient is a physiological constraint due to temperature. One prediction based on this mechanism is that if herbivorous fishes could shift their diet to animal material, they would be better able to grow, survive, and reproduce in cold waters. We tested this prediction on the omnivore *Girella nigricans* under different temperature and diet regimes using RNA–DNA ratios as an indicator of performance. Fish had increased performance (100%) at low temperatures (12 °C) when their diet was supplemented with animal material. In contrast, at higher temperatures (17, 22, and 27 °C) fish showed no differences between diets. This indicates that omnivorous fishes could increase their performance at low temperatures by consuming more animal matter. This study supports the hypothesis that a relative increase in the nutritional value of plant material at warmer temperatures could drive the latitudinal diversity gradient in herbivorous fishes.

Résumé : Les poissons herbivores se répartissent selon un gradient latitudinal de diversité, dans lequel ils représentent une proportion plus grande des poissons de la communauté dans eaux tropicales que dans les eaux tempérées. Un des mécanismes proposés pour expliquer ce gradient est une contrainte physiologique reliée à la température. Une des prédictions découlant de ce mécanisme est que, si les poissons herbivores pouvaient changer leur régime alimentaire pour augmenter le matériel animal, ils seraient mieux capables de croître, de survivre et de se reproduire dans les eaux froides. En utilisant les rapports ARN–ADN comme indicateurs de performance, nous avons testé cette prédiction chez l'omnivore *Girella nigricans* à différentes températures et sous divers régimes alimentaires. Les poissons ont une performance accrue (100 %) à basse température (12 °C) lorsque leur régime est additionné de matière animale. En revanche, aux températures plus élevées (17, 22 et 27 °C), il n'y a pas de différence chez les poissons, quel que soit le régime alimentaire. Cela indique que les poissons omnivores pourraient améliorer leur performance à basses températures en consommant plus de matière animale. Notre étude appuie l'hypothèse qui veut qu'une augmentation relative de la valeur nutritive de la matière végétale aux températures plus élevées pourrait expliquer le gradient latitudinal de diversité chez les poissons herbivores.

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Introduction

Is poor-quality food better when warm? Is high-quality food better when cold? If so, the ideal diet for an ectotherm could vary with body temperature, potentially explaining broad geographic patterns in species' distributions. Herbivorous ectothermic vertebrates such as fishes and reptiles show striking latitudinal gradients in diversity and abundance that are potentially related to temperature's effect on physiology (Horn 1989; Zimmerman and Tracy 1989). Although multiple mechanisms could act simultaneously to drive these patterns, Floeter et al. (2005) conclude that the most probable mechanism is that herbivorous fishes cannot meet their energetic demands in temperate waters (Gaines and Lubchenco 1982; Horn 1989; Horn and Ojeda 1999). This mechanism, which is based on the interaction of temperature and food quality on fish physiology, may simultaneously explain both the latitudinal diversity gradient in herbivorous fishes and limited evolution of herbivorous fishes in temperate waters. Temperature affects many physiological systems and processes (Jobling 1994); therefore, to determine if a digestive constraint due to temperature limits the geographical distribution of herbivorous fishes requires simultaneously controlling diet across temperature and measuring physiological differences.

Temperature, food quality, and body size are thought to be the most important determinants of food assimilation

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(Kooijman 2000). Tropical fish species have evolved the ability to survive on a low-quality herbivorous diet, whereas temperate and polar species generally have not, despite higher standing stocks of plant material at higher latitudes (Harmelin-Vivien 2002; Floeter et al. 2004). Numerous studies have attempted to determine the simultaneous effects of temperature and diet quality and quantity on growth or condition in fishes (for recent examples, see Mischke et al. 2001; Deng et al. 2002; Johnson et al. 2002). Almost none of this work has focused specifically on herbivorous species. Studies similar to these, if conducted on herbivorous fishes, may help understand how temperature and diet may interact to influence the geographic distributions of trophic groups such as herbivores.

Plant material is a low-quality food and difficult to assimilate (Horn 1989). The dual challenge of low-quality diet and low assimilation efficiency means that herbivorous fish must feed more often than carnivorous species to meet their energetic demands (Arrington et al. 2002). This is likely possible in temperate shallow-water habitats where plant material is generally more available than animal prey (Gaines and Lubchenco 1982). However, at low temperatures, feeding rate declines (Jobling 1994), and because herbivorous fish require nearly constant food intake to survive (Arrington et al. 2002), they may experience disproportionate metabolic costs at low temperatures compared with carnivores if feeding rates experience greater declines than metabolic rates.

Omnivorous fishes present a unique opportunity to test the hypothesis that temperature limits herbivory. Extension of the hypothesis to omnivory predicts that the relative importance of plant material to an omnivore's diet will decline in cold water. Additionally, increasing diet quality by adding animal matter to the diet of omnivores can have nonadditive effects, where growth rate may increase more than expected by the addition of the animal matter alone due to positive effects on the digestion of algae consumed with the animal matter (Bjorndal 1991).

Growth is commonly used to assess an organism's reaction to environmental conditions; however, estimation of this parameter is difficult over short time intervals characteristic of lab studies (Buckley et al. 1999). We chose RNA–DNA ratios to indicate a short-term change in performance in response to temperature and diet (Buckley et al. 1999; Buckley and Szmant 2004). The RNA–DNA ratio reflects the protein synthesis capacity of a tissue and indicates the nutritional and energetic condition of the organism (Buckley 1984; Stillman et al. 1994; Dahlhoff and Menge 1996). We used RNA–DNA ratios as an index of performance. RNA–DNA ratios have been used in this manner in a wide range of organisms (Bulow 1987; Dahlhoff and Menge 1996; Buckley and Szmant 2004).

Determining if a digestive constraint due to temperature limits the geographical distribution of herbivorous fishes requires controlling for digestive differences across temperature, as temperature also affects many other physiological systems and processes. Therefore, it is necessary to determine if the organism could survive and experience increased performance at colder than normal temperatures if the organism were fed a higher-quality diet. To better understand if temperature's effect on digestion limits the poleward thermal limit of herbivorous fishes, we controlled both diet and temperature in the lab to determine if an increase in diet quality increased an omnivorous fish's performance at cold temperatures. We chose the opaleye, *Girella nigricans* (Ayres), a common member of the rocky reef fish assemblage in Baja California and southern California (Love 1996). This species shows dramatic changes in its ecology at low temperatures. At the northern end of its range, *G. nigricans* declines in abundance at low temperatures (Floeter et al. 2005). Additionally, the feeding behavior of this species changes across its range, with animal material becoming more important as temperature declines (Behrens 2005). Because *G. nigricans* experiences a wide range of temperatures across its range (Norris 1963) and readily accepts a wide range of food items in the lab, it is an ideal organism on which to test the simultaneous effects of diet and temperature on performance. We predicted that fishes fed plant material would show a stronger increase in performance with an increase in temperature than fish fed animal material. Here we find that temperature alters the relative performance of *G. nigricans*, where performance increases with diet quality at low temperatures, but the relationship reverses at high temperatures.

Materials and methods

Sampling and acclimation

We collected opaleye from shallow subtidal habitats in the Santa Barbara harbor (34°24.241′ N, 119°41.519′ W) in southern California. This study population was from the northern region of the species geographic range, where individuals commonly feed primarily on benthic algae (Behrens 2005). Twenty-four juvenile to subadult *G. nigricans* (7.9–15.7 cm standard length (SL)) were captured with hook and line, immediately deposited in separate plastic bags containing fresh seawater, and transported to the laboratory. Fish were housed in one of 24 compartments in 12–38 L aquaria divided by partitions for up to 5 days at ambient temperatures (15 °C) and fed algae (*Ulva* spp.) ad libitum. Once fish began feeding and defecating, we initiated temperature changes in each of four temperature treatment blocks (three aquaria each) of aquaria at a rate of 2 °C·day^{-1} until experimental temperatures were reached. We staggered the initiation of the change of temperature so that all fish would reach the experimental temperature on the same date.

Diet–temperature experiment

Once all temperature blocks reached the appropriate experimental temperatures (12, 17, 22, 27 °C), which encompassed nearly the entire temperature range experienced by *G. nigricans* (11–27 °C; Norris 1963), we measured the standard length and weight of all fish and returned them to the appropriate aquarium compartment. The fish on one side of the aquarium partition were fed algae (*Ulva* spp.) ad libitum, and the fish on the other side were fed algae (*Ulva* spp.) ad libitum plus squid at a rate of 1% of the specimen's body mass per day. We designated these two feeding treatments the herbivory and omnivory treatments, respectively. We monitored daily consumption rates of both fish fed algae and fish fed algae plus squid by determining the difference between the wet masses fed one day and the food still remaining in the next day. After 28 days, we euthanized all specimens with MS-222 (5 g·L^{-1} tricaine methanesulfonate)

and collected white muscle samples. We immediately froze all white muscle samples at –70 °C. To increase sample sizes, we conducted two sequential trials using identical methods.

Sample analysis

To determine RNA–DNA ratios of the white muscle samples, we determined RNA and DNA concentrations in each sample using ethidium bromide fluorescence, following the method of Bentle et al. (1981), as modified by Dahlhoff and Menge (1996). We thawed white muscle samples on ice and weighed and homogenized these samples in 10 volumes of 2 mol·L^{-1} NaCL with a hand-driven glass homogenizer. We incubated 50 µL from each sample in a 1.5 mL solution containing 0.005 mg ethidium bromide and 0.15 mg proteinase K at 37 °C for 60 min. After this initial incubation, we added 1.0 mL buffer (80 mM Tris–HCl, pH 7.5 at 20 °C) and then measured fluorescence at 365 nm excitation and 590 nm emission using a Turner Biosystems TD-100 luminescence spectrofluorometer. We determined fluorescence due to RNA and DNA by sequential digestion of each nucleic acid using 50 µL RNAse I (1.0 mg·mL^{-1}, bovine thymus) followed by a 30-min incubation and DNAse I (0.5 mg·mL^{-1}, bovine pancreas) followed by a 30-min incubation. Then, we estimated RNA and DNA concentrations from a standard curve calculated by measuring the fluorescence of known quantities of RNA and DNA (Sigma calf thymus, 0–4 µg; Sigma calf liver RNA, type IV, 0–8 µg).

Statistical analysis

We performed a split-plot analysis using a generalized linear model (GLM) to determine the effects of diet and temperature on RNA–DNA ratios, including the effects of fish length and trial. We included only the significant interaction terms in the final split-plot model. During the second trial, fishes on the omnivorous diet at the coldest temperature (12 °C) rarely consumed squid, which effectively removed the diet treatment at this temperature. Therefore, we excluded the 12 °C treatment from the second experimental run from the split-plot analysis, but used the data in all other analyses that used percent algal material in the diet as the independent variable. To investigate how diet treatments changed among the temperature treatments (due to feeding differences), we regressed the percent animal material in the diet against temperature. Additionally, we regressed the residual daily consumption of algal material and animal material, after the effect of fish length was removed, against temperature. To determine how RNA–DNA ratios changed as the amount of animal material in the diet increased, we used a GLM to determine the effect of temperature and the amount of animal material in the diet on RNA–DNA ratios, after the effect of fish length was removed. Excluding data from the second trial of the split-plot analysis did not introduce bias into the analysis and did not impact our ability to use the full set of data to test the hypothesis with a second GLM method. After confirming that the data met the assumptions of the statistical models (normality and homogeneity of variances), we performed all analyses on nontransformed data using JMP (SAS Institute Inc. 2002).

Results

Diet and temperature influenced the RNA–DNA ratios of the omnivorous study species (Fig. 1). The effect of diet was dependent on the experimental temperature (Table 1, split-plot analysis of variance (ANOVA), diet × temperature interaction, $F_{[3,16]} = 4.001$, $p = 0.027$). At 12 °C, fish fed an omnivorous diet had higher (100% increase) RNA–DNA ratios than fish fed an herbivorous diet ($F_{[1,16]} = 6.457$, $p = 0.022$; Fig. 1), whereas at higher temperatures (17, 22, and 27 °C), the diet did not affect the RNA–DNA ratio (17 °C, 19% increase, $F_{[1,16]} = 1.637$, $p = 0.219$; 22 °C, 18% decrease, $F_{[1,16]} = 0.816$, $p = 0.380$; 27 °C, 34% decrease, $F_{[1,16]} = 3.072$, $p = 0.100$). At these higher temperatures, there was relatively low power to detect differences between diet treatments (17 °C, power = 0.225; 22 °C, power = 0.136; 27 °C, power = 0.378). A moderate increase in sample size (33%) at 27 °C may have allowed us to detect a significant difference between diet treatments. However, extremely large increases in sample size would have been required to detect the observed differences between the diet treatments at 17 °C (240%) and 22 °C (476%). Therefore, it seems unlikely that low statistical power led to the finding of no difference between diets at the intermediate temperatures (17 °C and 27 °C). Additionally, there were changes in performance with temperature within diet treatments. Fish fed an omnivorous diet showed a significant decline in RNA–DNA ratios at higher temperatures ($F_{[3,18]} = 3.437$, $p = 0.039$; Fig. 1). In contrast, fish fed an herbivorous diet showed a moderate, but nonsignificant, increase in RNA–DNA ratios from 12 °C to the higher temperatures ($F_{[1,15]} = 4.170$, $p = 0.059$; Fig. 1).

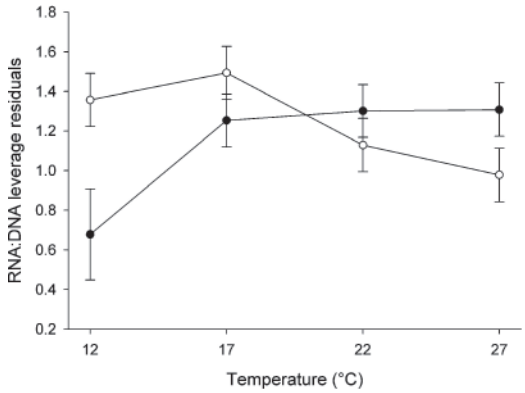
Although fish fed the omnivorous diet received 1% body mass of animal material per day, diet treatments varied among temperature treatments because differences in consumption of both algae and squid among temperatures (Fig. 2). The percent animal material in the diet declined as the experimental temperature increased ($R^2 = 0.506$, $F_{[1,22]} = 22.519$, $p < 0.001$; Fig. 2*a*). After we removed size-related differences in feeding rate, the amount of algae consumed ($R^2 = 0.637$, $F_{[1,21]} = 18.442$, $p < 0.001$; Fig. 2*b*) and animal material consumed ($R^2 = 0.173$, $F_{[1,22]} = 4.604$, $p = 0.043$; Fig. 2*c*) increased with temperature. The decline in the percent animal material was driven by relative differences in the change in the amount of algal and animal material consumed at the various temperatures (Fig. 2).

Because of feeding differences among individuals within a temperature treatment, we could determine how changes in the percent animal material at a given temperature affected the RNA–DNA ratio. After removing the effects of fish size and time, the effect of the percent animal material in the diet on RNA–DNA ratios changed across temperature treatments (temperature × percent animal material, $F_{[3,40]} = 3.421$, $p = 0.026$; Fig. 3). The slope of the relationship between RNA–DNA ratio and the percent animal material in the diet increased (172%) with declining temperature from the highest temperature (27 °C) to the lowest temperature (12 °C) (Fig. 3).

Discussion

Diet and temperature interacted to determine performance in *G. nigricans*. At low temperatures, *G. nigricans* performed better when fed an omnivorous diet than an herbivorous diet. In contrast, performance under the two diets was

Fig. 1. Mean residual RNA–DNA ratios for the two diet treatments (herbivore, algae ad libitum; omnivore, algae ad libitum plus 1% body mass in squid per day) plotted against temperature. The effect of temperature on RNA–DNA ratio was dependent on diet (diet × temperature interaction, $F_{[3,16]} = 4.001$, $p = 0.027$). Solid circles represent the herbivore treatment; open circles represent the omnivore treatment. Error bars represent ±1 standard error.



not significantly different at higher temperatures. This was at least partially because fish on the omnivorous diet consumed a higher proportion of algae as temperature increased. The pattern became clearer when the actual amount of animal material in the diet was considered. At low temperatures, the relationship between performance and the amount of animal material in the diet was positive. However, at high temperatures, the slope of the relationship reversed, with performance declining with an increase in the amount of animal material in the diet. The low performance of the herbivorous diet in cold temperatures supports the hypothesis that a thermal constraint on digestive physiology limits the distribution of herbivorous fishes to warm waters. The decline in performance at high temperatures suggests a cost to carnivory at high temperatures, which may be related to the extensive evolution of herbivorous fishes in tropical waters. However, it should be noted that this experiment was conducted on a cold-water population and the potential for local adaptation should be considered when extrapolating these results to warm-water populations.

RNA–DNA ratios are commonly used indicators of potential for protein synthesis, growth, and nutritional condition in a wide range of organisms (Frantzis et al. 1992; Dahlhoff and Menge 1996; Caldarone et al. 2003). RNA–DNA ratios vary in response to many environmental and biological conditions, but typically minimum values are greater than 1.0. When the ratio falls below 1.0, it is usually attributed to physiological stress, such as food limitation (Kono et al. 2003) or hypoxia (Aday et al. 2000). The RNA–DNA ratios in this study suggest that *G. nigricans* experienced physiological stress at the lowest and highest experimental temperatures, but the stress was dependent on diet. At 12 °C, the mean ratio for fish fed exclusively algae was 0.68 (standard error (SE) = 0.23). In contrast, those fish at the same temperature with a diet that was supplemented with animal material had a mean ratio of 1.36 (SE = 0.13). Although not statistically different, at the highest temperature (25 °C), fish on the omnivorous diet had a mean ratio of 0.98 (SE = 0.14), whereas those fish on the herbivorous diet had a mean ratio of 1.31 (SE = 0.13). The difference between the two diet treatments indicated that the stress was driven by diet rather than by the thermal tolerances of *G. nigricans*.

Because increased diet quality should be beneficial in nearly all cases, the finding of a negative effect of animal material in the diet at high temperatures on performance was unexpected, especially as carnivorous fishes are common at all latitudes and temperatures. Low temperatures and animal material in the diet can independently suppress gut passage rate (Fänge and Grove 1979; Klumpp and Nichols 1983; Fris and Horn 1993). Diet-related suppression of gut passage rate at high temperatures in herbivores might limit food consumption below minimum levels required to meet their increased metabolic demands at high temperatures. However, further research into the effects of temperature and food quality on digestive physiology is needed to elucidate the cause of this result.

The optimal temperature for digesting plant material may differ from the optimal temperature for metabolism. Because of their low-quality diet, herbivorous fishes may need to reduce energy loss by selecting low temperatures but may also require high temperatures for efficient digestion and growth. Herbivorous lizards show such a conflict because of their thermal physiology and are able to actively thermoregulate by basking to meet their present physiological needs (Wikelski and Trillmich 1994). Pulgar et al. (2003) found that the southern hemisphere omnivore *Girella laevifrons* selects intermediate temperatures (16–19 °C) on a high-quality diet (bivalves), but selects lower temperatures (10–13 °C) when fed a lower-quality diet (algae). These authors concluded that *G. laevifrons* selects low temperatures to conserve energy when fed a low-quality diet and selects intermediate temperatures when fed a high-quality diet to optimize digestion. In contrast, our findings suggest that fish perform better at high temperatures when fed a low-quality diet. Because most subtidal marine fishes live in a relatively homogeneous thermal environment, few will be able to choose the temperature that best balances the needs for energy conservation (low temperatures) and digestion (high temperatures).

Feeding behavior of fishes can be affected by many factors, including temperature, and is thought to be driven by the nutrient requirements of the fish (Clements and Raubenheimer 2006). Both the pinfish, *Lagodon rhomboides,* and spottail pinfish, *Diplodus holbrooki*, consume a greater proportion of animal material as water temperature declines, and refused to consume algae below 17 °C (Mark Hay, Georgia Institute of Technology, School of Biology, Atlanta, GA 30332, unpublished data). Feeding behavior in *G. nigricans* changes across its range. In the wild, fish eat significantly more animal material in cold than in warm locations (Behrens 2005). This parallels our results from the laboratory for *G. nigricans*. Although the mass of animal material in the diet was fixed in our experiment, the proportion of animal material included by the fish in their diet was highest in the cold-water treatment. The higher RNA–DNA ratio for the omnivorous treatments versus the herbivorous treatments in cold water suggests that this behavioral change is adaptive. RNA–DNA ratios are likely related to fitness as they are related to potential protein synthesis. Protein syn-

Table 1. Split-plot GLM on the effects of temperature, diet, aquarium, trial, time, and fish length on the RNA–DNA ratio of *Girella nigricans*.

Source of variation	df	MS	*F* ratio	*p* value
Temperature	3	0.1954	2.1893	0.1091
Diet	1	0.1032	0.9748	0.3381
Aquarium (within temperature)	19	0.0841	0.7950	0.6865
Trial	1	0.0418	0.3946	0.5388
Fish length	1	0.0799	0.7554	0.3975
Diet × temperature	3	0.4236	4.0013	0.0265
Error	16	0.1058		

Note: Temperature: 12, 17, 22, 27 °C; diet: herbivore, omnivore. We treated aquarium nested within temperature as a random variable.

Fig. 2. Change in components of the omnivore feeding treatment at the experimental temperatures: (*a*) regression of percent animal material in the diet against temperature ($R^2 = 0.506$, $F_{[1,22]} = 22.519$, $p < 0.001$); (*b*) regression of residual wet mass of algal material in the diet against temperature, after the removal of the effect of fish size ($R^2 = 0.637$, $F_{[1,21]} = 18.442$, $p < 0.001$); (*c*) regression of percent animal material in the diet against temperature, after the removal of the effect of fish size ($R^2 = 0.173$, $F_{[1,22]} = 4.604$, $p = 0.043$).

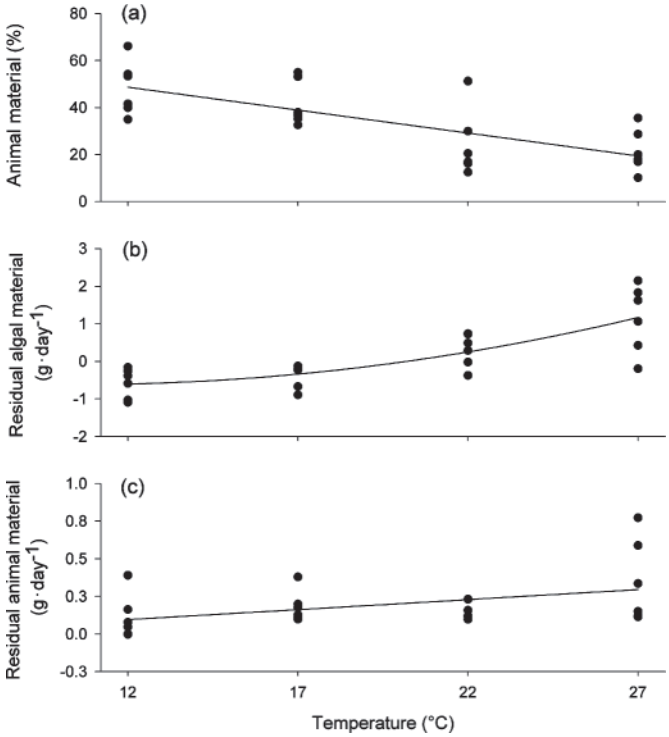
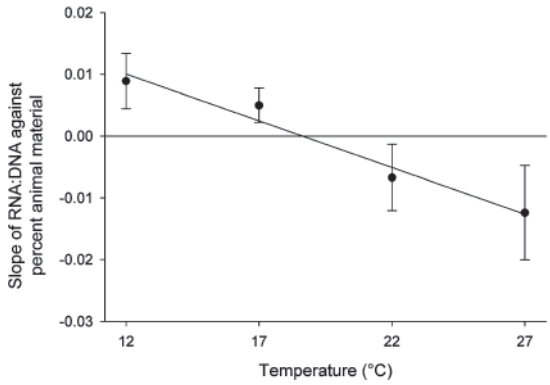


Fig. 3. Slope of individual regression lines of residual RNA–DNA ratios plotted against percent animal material for each of the four temperature treatments (temperature × percent animal material, $F_{[3,40]} = 3.421$, $p = 0.026$). Residual RNA–DNA ratios were used to remove the effect of fish size before initial regression against temperature. Error bars represent ±1 standard error.



thesis is the single most energetically expensive physiological process in fishes (Carter and Houlihan 2001); thus increased RNA–DNA ratios likely indicate increased energy reserves for other processes, e.g., reproduction.

If RNA–DNA ratios can be extrapolated to the fitness of the organism, then our results may show the basis for a selective advantage for herbivorous fishes in the tropics but not in temperate and polar waters. Figure 2 illustrates the potential for temperature-dependent selection, where herbivory should be selected against at low temperatures, whereas carnivory may be selected against at high temperatures (especially if animal material is more difficult to obtain than algal material). This selection against herbivory in temperate and polar waters might explain the relatively low rates of evolution of herbivorous fishes in cold waters and the resulting latitudinal diversity gradient in herbivorous fishes (Gaines and Lubchenco 1982; Horn 1989; Floeter et al. 2005). This seems more parsimonious than the scenario of limited time for evolution and range expansion of herbivorous fishes in cold waters (Mead 1970). The selection against carnivory at high temperatures may further explain the extensive evolution of strictly herbivorous fishes in the tropics and why omnivory would not be favored at all temperatures. This further reduces the reliance on secondary explanations, such as competition, to explain strictly herbivorous fishes in the tropics as competition for food is likely intense in both temperate and tropical systems.

Although the results of the present study show important findings that further our understanding of the potential mechanisms underlying the geographic distribution of herbivory in fishes, they should be extrapolated to all herbivorous fishes with caution. This study was performed on a single species and lacks generality. To better understand if these findings can be generalized to most herbivorous fishes, this type of experiment should be repeated with a range of species from temperate and tropical habitats and with various evolutionary histories.

In conclusion, the effect of temperature on performance of *G. nigricans* is dependent on diet. Fish fed an omnivorous diet had greater performance at low temperatures than those fed an herbivorous diet. In contrast, fish fed an herbivorous diet had greater performance at high temperatures than fish fed an omnivorous diet. These results lend support to the existence of a physiological constraint owing to temperature limiting the evolution of herbivory in cold waters and therefore driving the latitudinal diversity gradient in herbivorous fishes.

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