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Final Report

***The Role of Temperature in the Latitudinal Diversity Gradient in Herbivorous Fishes: Implications of Global Climate Change* - Proposal Reference Number: 01 T CEQI 08 1080**

Diet survey along a temperature gradient:
During June 2001 through September 2003, I collected 164 opaleye (*Girella nigricans*) zebraperch (*Hermosilla azurea*), halfmoon (*Medialuna californiensis*) and black surfperch (*Embiotoca jacksoni*) specimens from 18 sites along the coast of Baja California and Southern California (27º N – 35º N). Thirteen of these sites are located on the California Channel Islands and five sites are located on the mainland. At each site I collected between four and eleven opaleye specimens. At two sites, I collected between four and six surfperch specimens. The surfperch specimens will be used to compare the isotope signatures of opaleye (an herbivore) to the signatures of a carnivore. At another five sites, other known herbivores (halfmoon and zebraperch) were collected to compare to the isotope signatures of opaleye. I also collected samples of representative algae species and the invertebrate species living in and on the algae, for a total of 128 samples of each group. Stable Isotopes of Nitrogen and Carbon were analyzed at the Marine Science Institute Analytical Lab at UC Santa Barbara. In addition, stomach content analysis has been performed on all specimens to determine the relationship between feeding on a given day, temperature, and the isotope signature that averages over a longer time period (1 month – 1 year).

Initial analyses using the mixing models using Nitrogen and Carbon Isotopes to determine the proportion of assimilated nutrients that come from algae and animal matter have been unsuccessful. The models are producing non-logical numbers as proportions (e.g. negative values and values larger than one). This is because the delta values of the fish tissue have values greater than would be expected based on the assumed fractionation values and the invertebrate delta values. This could be due to several scenarios. First, the food source delta values are determined mainly by indigestible components, such as structural components. Second, microbial fermentation products may play an important role in the nutrition and, therefore affect the delta values of the fish. These scenarios all address ways that the underlying assumptions of the mixing models may have been violated. However, the data have been very helpful identifying the trophic position of the study species and confirming the validity of classifying Girella as an herbivore, rather than an omnivore as some authors have suggested. Additionally, I am still attempting to determine if the stable isotope data from the fish can be corrected for the changes in algal stable isotope ratios to allow further latitudinal and temperature analyses to be conducted.

The gut analysis data from opaleye showed a high degree of variation in the amount of algae in the diet of opaleye, with values ranging from 45 to 100 % algae. The amount of algae in the diet was highest in kelp forests and algal dominated communities and lowest in barrens and the intertidal. This result is intuitively satisfying, in that we would expect that unless fish travel to different community or habitat types to forage, the amount of algae in their diet should to some extent reflect the amount of algae available within the area they reside. The amount of algae in a fish's diet increased slightly with fish length. There is a documented ontogenetic feeding shift at approximately 50 mm in this species, however data from my study show that opaleye as small as 36 mm SL still consume a large amount of algae. Additionally, the amount of

algae in the diet of this species increased with water temperature on the day of collection. If there is a thermal constraint on digestion, these data may indicate that this species is changing its feeding behavior to counteract changes in digestive ability with temperature. If this is the case, using a single species as a model, this indicates that herbivory may not be a physiologically viable option at cold temperatures and that changes to omnivory or carnivory are required to persist in cold water.

Bacterial growth and fermentation at various temperatures:

In the original proposal I planned to establish cultures of cellulose degrading bacteria, and then study the temperature dependence of growth and survival of these bacterial species in experimental cultures. After discussions with microbiologists regarding the feasibility of maintaining representative cultures for these experiments, I determined that another method might be more applicable to the overriding question. This method, based on work by Kihara and Sakata 2002 (Comp. Biochem. Phys. A 132(2): 333-340) utilizes gas production from small-scale batch cultures to determine rates of bacterial fermentation. Additionally, this gas production has been shown to be highly correlated with short chain fatty acid production by the intestinal microflora. Since, these short chain fatty acids are the fermentation products utilized by the fish, this method can assess the effect of temperature on a fish's ability to uptake energy produced via microbial fermentation.

Between August 2002 and December 2003, I performed six experiments using these methods on the dominant herbivorous fish species from southern California (*Girella nigricans*), the northern Gulf of California (*Girella simplicidens*), Moorea (*Acanthurus nigrofuscus*), and southern New Zealand (*Odax pullus*). The first experiment determined which section of the intestine produced the highest fermentation rates. I used information from this study to sample intestinal contents from the proper area of the intestine for a temperature experiment. The latter experiments determined the effect of temperature and substrate on fermentation rates. The experimental design included four to five temperatures (12º, 18º, 25º, 30º, and 35º C (for tropical species only)) and three substrates (glucose, alginic acid, and carboxymethyl cellulose). The results for all species indicate that fermentation rates are dependent on both temperature and substrate. Fermentation rates increased with temperature and were greatest with glucose, intermediate with alginic acid and no fermentation was observed with cellulose as a substrate. In each species there was a minimum temperature below which fermentation was not detectable. Tropical species never experience temperatures below this critical temperature, while temperatures drop below these critical temperatures for at least part of the year for temperate species. *Girella simplicidens* experiences temperatures ranging from 12º to 35º C in the northern Gulf of California, however even during the coldest period of the year fermentation is not detectable at 12º C, the ambient temperature. Therefore, it appears that the microbial communities within the intestines of herbivorous fishes are constrained by temperature, and while they are adapted to the ambient conditions experience by the host, the temperature constraints may lead to a constraint on the energetics, and therefore the distribution, of temperate herbivorous fishes.

Impact of herbivorous fishes on algal assemblages:

This proposed section of the project was not completed due to logistical constraints and the advise of my dissertation committee. During my oral comprehensive exam my committee and I discussed this part of my thesis and determined that while project was very worthwhile, the scope and logical constraints were sufficient enough to make it difficult to complete as a chapter of my dissertation. The results of this project could be very important to predicting the effect of

climate change on temperate reefs as mediated by changes in the distributions of herbivorous fishes and would be an excellent post-doctoral research project. However, we felt that it was most logical to confirm or reject the validity of a thermal constraint on digestion that could limit the distribution of herbivorous fishes before attempting to determine how this mechanism may affect the ecology of temperate reefs.

Since this project was a significant part of the proposal, we determined it was best to transfer the proposed time and effort to thoroughly investigating the thermal effects on intestinal microflora. Initial work on *Girella nigricans* indicated that temperature's effect on fermentation may be very important to these species and it was important to expand this project to investigate both temperate and tropical species and those that experience dramatic annual variation in temperature. Since much of my early work on fermentation dealt with perfecting the method, by increasing the amount of time available for further work on fermentation, I was able to expand the number of species investigated from a single proposed species to four species.